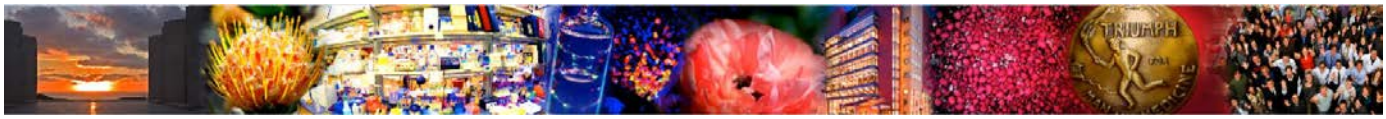
DIABETES RESEARCH CENTERS



2013 Meeting of the Diabetes Centers' Directors

May 29, 2013

Hyatt Regency Bethesda

Bethesda, MD

DIABETES RESEARCH CENTERS

2013 Meeting of the Diabetes Centers' Directors

Table of Contents

c. Boston Area
d. Columbia University
e. JHU-UMD
f. Joslin Diabetes Center
g. University of Alabama at Birmingham
h. UCSD/UCLA
i. UCSF
j. University of Chicago
k. University of Michigan
l. University of Pennsylvania
m. University of Washington
n. Vanderbilt University
o. Washington University in St. Louis
p. Yale University

17. Map of Downtown Bethesda

Agenda
2013 Diabetes Center Directors' Meeting
Wednesday, May 29, 2013

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

7:30 – 8:00 am	Registration
8:00 – 8:10 am	Welcome and opening remarks (Dr. Griffin Rodgers)
8:10 – 9:00 am	The view from NIDDK:
	• Updates (J. Hyde)
	• Perspectives & Opportunities (J. Fradkin)
	-NIH Big Data to Knowledge BD2K (R. Margolis)
9:00 – 9:15 am	Report from the Diabetes Centers Executive Committee (J. Schaffer)
9:15 – 9:45 am	Funding Opportunities: American Diabetes Association (T. Darsow)
9:45 - 10:00 am	NIDDK Summer Medical Student Program: report (A. Powers; S. Kahn)
10:00 – 10:15 am	NIH Common Fund's Metabolomics Program (A. Castle)
10:15 – 10:30 am	Break
10:30 – 10:45 am	DRC Biomedical Research Cores in the 21st Century (J. Hyde)
10:45 – 11:15 am	Roles of Institutional vs. Diabetes-Centric Cores (D. Accili)
11:15 – 11:45 am	Shared Core Facilities (DRCs and NORCs) (J. Schaffer)
11:45 – 12:00 pm	General Discussion
12:00 – 1:00 pm	Lunch (on your own)
1:00 – 1:30 pm	Opportunities for National/Regional Shared Cores for DRCs (A. Powers; S. Kahn)
1:30 – 1:50 pm	Regional Shared Core/Resources (M. Lazar; G. King)
1:50 – 2:10 pm	Expansion of P&F Programs to Partnering Institutions (J. Schaffer; M. Myers)
2:10 – 2:30 pm	General Discussion
2:45 – 3:00 pm	Diabetes Research Centers: up-coming RFA (J. Hyde)
3:00 – 3:15 pm	Submitting Complex Electronic Applications (J. Hyde)
3:15 – 3:30 pm	Diabetes Research Centers website: updates/changes (J. Hyde)
3:30 – 3:45 pm	Wrap-up, final comments & adjourn

DIABETES RESEARCH CENTERS

2013 Meeting of the Diabetes Centers Directors

May 29, 2013

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

PARTICIPANTS LIST

Kristin M. Abraham, Ph.D.
Senior Advisor
Model Systems and Resources
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 795
6707 Democracy Boulevard, MSC 5460
Bethesda, MD 20892-5460
Telephone: (301) 451-8048
Email: abrahamk@mail.nih.gov

Domenico Accili, M.D.
Director
Columbia Diabetes Research Center
Russell Berrie Foundation Professor of Diabetes
Professor of Medicine
Diabetes and Endocrinology Research Center
Columbia University Medical Center
Russ Berrie Pavilion, Suite 238
1150 Saint Nicholas Avenue at 168th Street
New York, NY 10032
Telephone: (212) 851-5330
Email: da230@columbia.edu

Joseph Avruch, M.D.
Professor
Department of Medicine
Harvard Medical School
Chief, Diabetes Unit
Massachusetts General Hospital
185 Cambridge Street, Mailstop: CPZN6408
Boston, MA 02114
Telephone: (617) 726-6909
Email: avruch@molbio.mgh.harvard.edu

Graeme Bell, Ph.D., M.Sc.
Louis Block Professor
Departments of Human Genetics and Medicine The University of Chicago
5841 S. Maryland Avenue, MC 1027
Chicago, IL 60637
Telephone: (773) 702-9116
Email: g-bell@uchicago.edu

Christin Carter-Su, Ph.D.
Henry Sewall Collegiate Professor in Physiology
Professor of Molecular and Integrative Physiology
Associate Director and Director Grants Program
Michigan Diabetes Research and Training Center
University of Michigan Medical School
6811D Medical Science II
Ann Arbor, MI 48109-5622
Telephone: (734) 763-2561
Fax: (734) 647-9523
Email: cartersu@med.umich.edu

Arthur L. Castle, Ph.D.
Program Director
Metabolomics and Informatics
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 791
6707 Democracy Boulevard, MSC 5460
Bethesda, MD 20892-5460
Telephone: (301) 594-7719
Email: castlea@nidd.nih.gov

Lawrence C.B. Chan, M.D.
Betty Rutherford Chair for Diabetes Research
Professor of Medicine and Molecular
and Cellular Biology
Chief
Division of Diabetes, Endocrinology,
and Metabolism
Department of Medicine
Director, Diabetes and Endocrinology Research
Center
Baylor College of Medicine
1 Baylor Plaza, MS185
Houston, TX 77030
Telephone: (713) 798-4478
Fax: (713) 798-8764
Email: lchan@bcm.edu

Streamson Chua, Jr., M.D., Ph.D.
Professor
Departments of Medicine
and Neuroscience
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus
Forchheimer Building, Room 511
1300 Morris Park Avenue
Bronx, NY 10461
Telephone: (718) 430-2986
Email: streamson.chua@einstein.yu.edu

Roger D. Cone, Ph.D.
Joe C. Davis Chair of Biomedical Science
Professor and Chair
Department of Molecular Physiology
and Biophysics
Vanderbilt University School of Medicine
702 Light Hall
2215 Garland Avenue
Nashville, TN 37232-0615
Telephone: (615) 936-7085
Email: roger.cone@vanderbilt.Edu

Tamara Darsow, Ph.D.
Vice President, Research Programs
American Diabetes Association
1701 N. Beauregard Street
Alexandria, VA 22311
Telephone: (703) 299-2020
Fax: (703) 549-1715
Email: tdarsow@diabetes.org

Aneleen Dizon, M.P.A.
Administrator
Diabetes Research Center
Nathan Shock Aging Center
Division of Endocrinology
Department of Medicine
Albert Einstein College of Medicine
Belfer 701
1300 Morris Park Avenue
Bronx, NY 10461
Telephone: (718) 430-2908
Email: aneleen.dizon@einstein.yu.edu

Norman Fleischer, M.D., F.A.C.P.
Jeanne and Jacob Barkey Professor of Medicine
Director, Division of Endocrinology
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461
Telephone: (718) 430-2908
Fax: (718) 430-8557
Email: norman.fleischer@einstein.yu.edu

Jose C. Florez, M.D., Ph.D.
Associate Professor of Medicine
Harvard Medical School
Assistant in Medicine
Endocrine Division
Diabetes Unit
Center for Human Genetic Research
Massachusetts General Hospital
Simches Research Building, CPZN 5.250
185 Cambridge Street
Boston, MA 02114
Telephone: (617) 643-3308
Fax: (617) 643-6630
Email: jcflorez@partners.org

Judith Fradkin, M.D.
Director
Division of Diabetes, Endocrinology,
and Metabolic Diseases
National Institute of Diabetes and Digestive
and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 683
6707 Democracy Boulevard, MSC 2560
Bethesda, MD 20892-2560
Telephone: (301) 496-7349
Fax: (301) 480-3503
Email: judith.fradkin@nih.gov

W. Timothy Garvey, M.D.
Butterworth Professor and Chair
Department of Nutrition Sciences
Director, UAB Diabetes Research Center
GRECC Investigator and Staff Physician
Birmingham VA Medical Center
University of Alabama at Birmingham
Webb 232
1720 Second Avenue South
Birmingham, AL 35294-3360
Telephone: (205) 996-7433
Email: garveyt@uab.edu

Michael German, M.D.
Clinical Director
Diabetes and Endocrinology Research Center
University of California, San Francisco Diabetes Center
35 Medical Center Way, RMB 1025
San Francisco, CA 94143-0669
Telephone: (415) 476-9262
Fax: (415) 514-2346
Email: mgerman@diabetes.ucsf.edu

Gregory Germino, M.D.
Deputy Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 31, Room 9A52
31 Center Drive, MSC 2560
Bethesda, MD 20892-2560
Telephone: (301) 496-5877
Fax: (301) 402- 2125
Email: gregory.germino@nih.gov

James F. Hyde, Ph.D.
Senior Advisor
Career Development ("K" awards)
Neurobiology of Obesity,and Diabetes Research Centers Programs
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 789
6707 Democracy Boulevard, MSC 5460
Bethesda, MD 20892-5460
Telephone: (301) 594-7692
Fax: (301) 480-0475
Email: james.hyde@nih.gov

Steven E. Kahn, M.B, Ch.B.
Professor of Medicine
Division of Metabolism, Endocrinology, and Nutrition
University of Washington
VA Puget Sound Health Care System (151)
1660 S. Columbian Way
Seattle, WA 98018
Telephone: (206) 277-5515
Email: skahn@u.washington.edu

George L. King, M.D.
Director of Research and Head
Section on Vascular Cell Biology
Joslin Diabetes Center
Mezzanine Floor, 4504
1 Joslin Place
Boston, MA 02215
Telephone: (617) 309-2662
Email: george.king@joslin.harvard.edu

Mitchell A. Lazar, M.D., Ph.D.
Chief
Division of Endocrinology, Diabetes, and Metabolism
Director, Institute for Diabetes, Obesity, and Metabolism
Sylvan Eisman Professor of Medicine
University of Pennsylvania Perelman School of Medicine
12-102 Smilow Center for Translational Research
3400 Civic Center Boulevard
Philadelphia, PA 19104
Telephone: (215) 898-0198
Email: lazar@mail.med.upenn.edu

Rudy Leibel M.D.
Christopher J. Murphy Memorial Professor
of Diabetes Research
Professor of Pediatrics and Medicine
Director, Division of Molecular Genetics
Co-Director, Naomi Berrie Diabetes Center
Co-Director, Diabetes and Endocrinology Research
Center
Co-Director, New York Obesity Research Center
Medicine at the Diabetes and Endocrinology
Research Center
Columbia University Medical Center
Russ Berrie Pavilion, Suite 238
1150 Saint Nicholas Avenue at 168th Street
New York, NY 10032
Telephone: (212) 851-5315
Email: rl232@columbia.edu

Ronald Margolis, Ph.D.
Senior Advisor for Molecular Endocrinology
Division of Diabetes, Endocrinology and
Metabolic Diseases
National Institute of Diabetes and Digestive and
Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 693
6707 Democracy Boulevard, MSC 5460
Bethesda, MD 20892-5460
Telephone: (301) 594-8819
Email: rm76f@nih.gov

Owen P. McGuinness, Ph.D.
Associate Director
Diabetes Research Center
Director, Metabolic Pathophysiology Core of
Vanderbilt Mouse Metabolic Phenotyping Center
Director, Hormone Assay and Analytical Services
Core
Professor, Department of Molecular Physiology
and Biophysics
Vanderbilt University School of Medicine
702 Light Hall
Nashville, TN 37232-0615
Telephone: (615) 343-4473
Fax: (615) 322-7236
Email: owen.mcguinness@vanderbilt.edu

Martin G. Myers, Jr., M.D., Ph.D.
Marilyn H. Vincent Professor of Diabetes Research
Director, Michigan Diabetes Research
and Training Center
University of Michigan
6317 Brehm Tower
1000 Wall Street
Ann Arbor, MI 48105
Telephone: (734) 647-9515
Fax: (734) 232-8175
Email: mgmyers@med.umich.edu

Ali Naji, M.D., Ph.D.
Professor of Surgery
Penn Transplant Institute
University of Pennsylvania Medical Center
1 Founders Building
3400 Spruce Street
Philadelphia, PA 19104
Telephone: (215) 662-2066
Fax: (215) 615-4900
Email: ali.naji@uphs.upenn.edu

Jerrold M. Olefsky, M.D.
Distinguished Professor of Medicine
Associate Dean for Scientific Affairs
Department of Medicine
University of California, San Diego
Stein Clinical Research Building, Room 228
9500 Gilman Drive
La Jolla, CA 92093-0673
Telephone: (858) 534-6651
Fax: (858) 534-6653
Email: jolefsky@ucsd.edu

Alvin C. Powers, M.D.
Chief
Division of Diabetes, Endocrinology,
and Metabolism
Joe C. Davis Chair of Biomedical Sciences
Director, Vanderbilt Diabetes Center
Professor of Medicine and Molecular Physiology and
Biophysics
Vanderbilt University School of Medicine
8435E MRB4 (Langford)
2213 Garland Avenue
Nashville, TN 37232-0202
Telephone: (615) 496-3422
Email: al.powers@vanderbilt.edu

Griffin P. Rodgers, M.D., M.A.C.P.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 31, Room 9A52
31 Center Drive, MSC 2560
Bethesda, MD 20892-2560
Telephone: (301) 496-5741
Fax: (301) 402-2125
Email: griffin.rodgers@nih.gov

Jean Schaffer, M.D.
Virginia Minnich Distinguished Professor of Medicine
Director, Diabetic Cardiovascular Disease Center and Diabetes Research Center
Washington University School of Medicine
660 S. Euclid Avenue, Box 8086
St. Louis, MO 63110
Telephone: (314) 362-8717
Email: jschaff@wustl.edu

Clay F. Semenkovich, M.D.
Herbert S. Gasser Professor
Chief, Division of Endocrinology, Metabolism, and Lipid Research
Co-Director, Diabetes Research Center
Washington University School of Medicine
Campus Box 8127
660 S. Euclid Avenue
St. Louis, MO 63110
Telephone: (314) 362-7617
Fax: (314) 362-7641
Email: csemenko@dom.wustl.edu

Robert Sherwin, M.D.
Director
Yale Diabetes Center
Department of Internal Medicine
Yale University School of Medicine
P.O. Box 208020
300 Cedar Street
New Haven, CT 06519
Telephone: (203) 785-4183
Email: robert.sherwin@yale.edu

Steven Shoelson, M.D., Ph.D.
Head
Section of Pathophysiology and Molecular Pharmacology
Joslin Diabetes Center
1 Joslin Place, Room 640
Boston, MA 02215
Telephone: (617) 309-2528
Fax: (617) 309-2407
Email: steven.shoelson@joslin.harvard.edu

Gerald Shulman, M.D., Ph.D.
Associate Director
Yale Diabetes Endocrine Research Center
Department of Internal Medicine
Yale University School of Medicine
P.O. Box 208020
300 Cedar Street
New Haven, CT 06519
Telephone: (203) 785-5447
Email: gerald.shulman@yale.edu

Philip Smith, Ph.D.
Deputy Director
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 689
6707 Democracy Boulevard, MSC 5460
Bethesda, MD 20892-5460
Telephone: (301) 594-8816
Fax: (301) 480-3503
Email: philip.smith@nih.gov

Dr. Ming-Jer Tsai, Ph.D.
Charles C. Bell Research Professor
Program in Developmental Biology
Department of Molecular and Cellular Biology
Baylor College of Medicine
1 Baylor Plaza, MS130
Houston, TX 77030
Telephone: (713) 798-6253
Email: mtsai@bcm.edu

Fredric E. Wondisford, M.D.
Professor of Medicine, Pediatrics,
and Physiology
Director, Metabolism Division
Director, The Johns Hopkins University Diabetes
Institute
The Johns Hopkins University School
of Medicine
CMSC10-113
600 N. Wolfe Street
Baltimore, MD 21287
Telephone: (410) 502-5761
Fax: (410) 502-5779
Email: fwondisford@jhmi.edu

Hsin-Chieh "Jessica" Yeh, Ph.D.
Assistant Professor of Medicine
and Epidemiology
Director, Epidemiology SubCore
The Johns Hopkins University-University
of Maryland Diabetes Research Center
2024 E. Monument Street, Suite 2-600
Baltimore, MD 21205
Telephone: (410) 614-4316
Email: hyeh1@jhmi.edu

Contractor Support:

Angela Meadows
The Scientific Consulting Group, Inc.
656 Quince Orchard Road, Suite 210
Gaithersburg, MD 20878
Telephone: (301) 670-4990
Fax: (301) 670-3815
Email: ameadows@scgcorp.com

Armond Middleton
The Scientific Consulting Group, Inc.
656 Quince Orchard Road, Suite 210
Gaithersburg, MD 20878
Telephone: (301) 670-4990
Fax: (301) 670-3815
Email: arm1956@juno.com

DIABETES RESEARCH CENTERS

UPCOMING NIH/NIDDK MEETINGS & WORKSHOPS

DMICC Workshop on Research Supported by the Special Statutory Funding Program for Type 1 Diabetes Research — June 6-7, 2013
NIH Neuroscience Center (NSC)
Conference Room B1/B2

NIDDK-NCI Workshop on Pancreatitis-Diabetes-Pancreatic Cancer — June 12-13, 2013
Lister Hill Auditorium, NIH Campus
http://www2.niddk.nih.gov/News/Calendar/PDPC2013.htm

Diabetic Wound Healing -Translation and Clinical Opportunities — October 9, 2013
NIH Neuroscience Center

Exploring the Function(s) of Brown and Beige Fat in Humans — October 15-16, 2013
Lister Hill Auditorium, NIH Campus
http://www2.niddk.nih.gov/News/Calendar/HumanBAT2013

Clinical Research Strategies for Fructose Metabolism — November 13-14, 2013
Building 31C room 10, NIH Campus
http://www2.niddk.nih.gov/News/Calendar/FructoseMetab2012.htm

DIABETES RESEARCH CENTERS

Ongoing Program Announcements

PAR-10-197: NIDDK Multi-Center Clinical Study Implementation Planning Grants (U34)

PAR-11-157: NIDDK Multi-Center Clinical Study Cooperative Agreement (U01)

PAR-11-221: Collaborative Interdisciplinary Team Science in NIDDK Research Areas (R24)

PAR-11-306: NIDDK Central Repositories Non-renewable Sample Access (X01)

PAR-12-172: Translational Research to Improve Obesity and Diabetes Outcomes (R18)

PAR-12-173: Planning Grants for Translational Research to Improve Obesity and Diabetes Outcomes (R34)

PAR-12-265: Ancillary Studies to Major Ongoing Clinical Research Studies to Advance Areas of Scientific Interest within the Mission of the NIDDK (R01)

PAR-13-013: Research Using Biosamples from Selected Type 1 Diabetes Clinical Studies (DP3)

PAR-13-028: Research Using Subjects From Selected Type 1 Diabetes Clinical Studies (Living Biobank) (DP3)

PAR-13-047: Bioengineering Interdisciplinary Training for Diabetes Research (T32)

PAR-13-074: Small Grants for New Investigators to Promote Diversity in Health-Related Research (R03)

PAR-13-101: High-End Instrumentation Grant Program (S10)

PAR-13-114: Improvement of Animal Models for Stem Cell-Based Regenerative Medicine (R01)

PAR-13-228: Biomarkers for Diabetes, Digestive, Kidney and Urologic Diseases Using Biosamples from the NIDDK Repository (R01)

PAR-13-231: Phenotyping Embryonic Lethal Knockout Mice (R01)

National Institute of Diabetes and Digestive and Kidney Diseases

2013 Diabetes Center Directors' Meeting

Bethesda, MD

National Institute of Diabetes and Digestive and Kidney Diseases

2012-13 Diabetes Centers Executive Committee

- **Jean Schaffer, Washington University, Chair**
- Mimmo Accili, Columbia University
- Larry Chan, Baylor College of Medicine
- Martin Myers, University of Michigan
- 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

2013 Diabetes Center Directors' Meeting

- Meeting Book:

http://diabetescenters.org/niddk-directors-meeting

- Centers Information:

  Includes Research Publications: submitted by each Diabetes Center; all have been added to the Featured Publications portion of the Diabetes Centers website; rotated on an annual basis

2013 Diabetes Center Directors' Meeting

- Meeting Book:
  - Agenda
  - Up-coming meetings of interest
  - Upcoming Funding Opportunities (notification of additional opportunities will be sent via e-mail when published in NIH Guide)
  - Featured Publications from Centers
  - Additional materials for presentations at the meeting

2013 Diabetes Center Directors' Meeting

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

National Institute of
Diabetes and Digestive
and Kidney Diseases

NIDDK

Perspectives and Opportunities

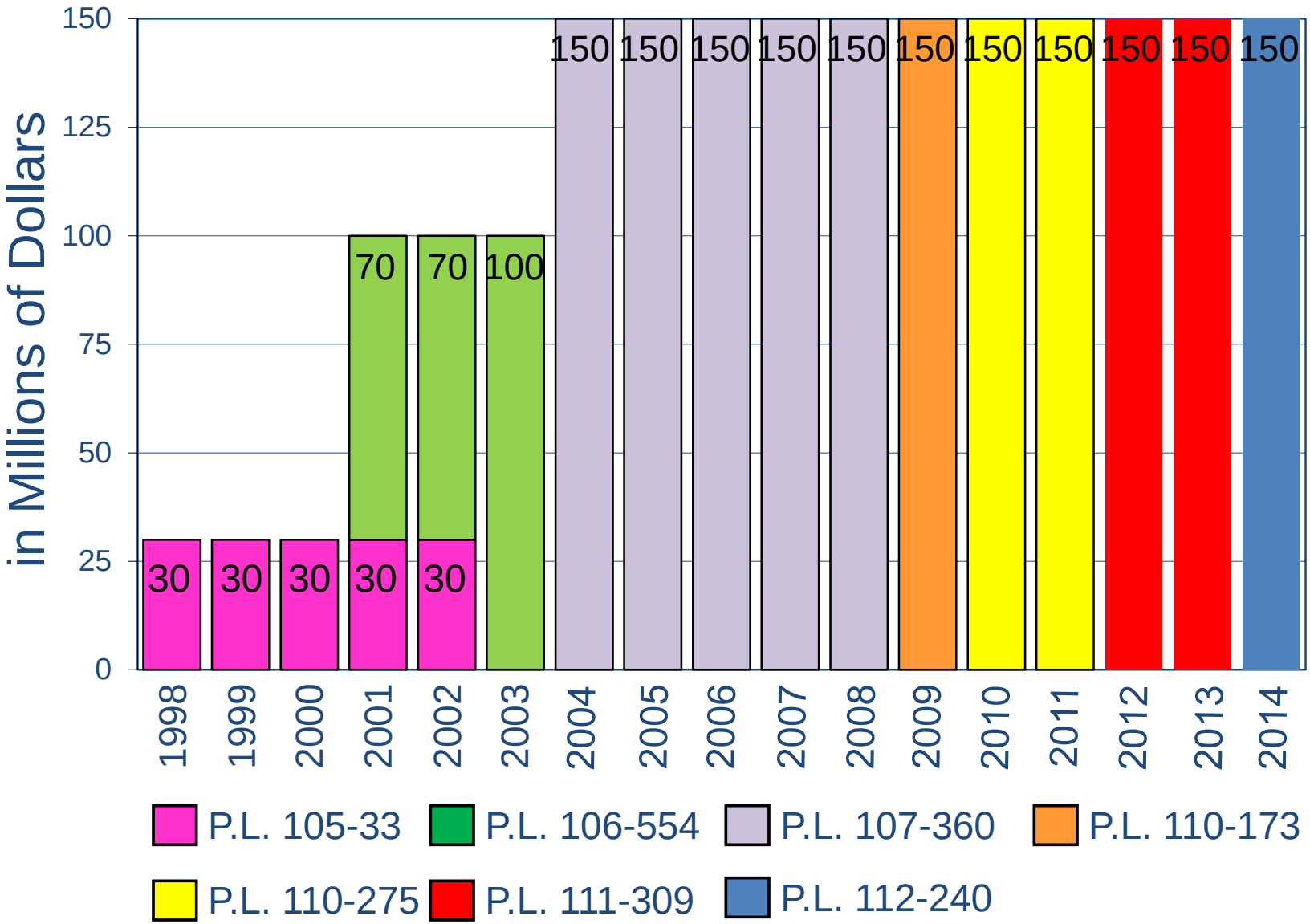
Judith Fradkin, M.D.

Director, Division of Diabetes, Endocrinology, and Metabolic Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

May 29, 2013

Total Funding: $2.04 Billion over 17 years



Planning Meeting for Research Supported by the SDP
June 6-7, 2013

- Coordinated by the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC)
- DMICC members invited to
  - Submit proposals for use of the new funds
  - Suggest panel members

Planning Meeting for Research Supported by the SDP
June 6-7, 2013

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

- New research initiatives to be supported in FY14 and/or FY15 (should SDP be continued)
- Continuations/expansions of ongoing programs (*e.g.*, programs starting new funding periods in FY14 or FY15)

Planning Meeting for Research Supported by the SDP
Panel Members

- *Autoimmunity*: Dr. Diane Mathis, Dr. John Looney, Dr. Jeff Bluestone
- *Transplantation*: Dr. Ronald Gill, Dr. Megan Sykes
- *Clinical*: Dr. Robert Sherwin, Dr. John Buse
- *Behavior*: Dr. Georgeanna Klingensmith, Dr. Timothy Wysocki
- *Biostatistics*: Dr. Mark Espeland, Dr. James Neaton
- *Beta cell biology*: Dr. Domenico Accili
- *Genetics*: Dr. Rudy Leibel
- *Complications*: Dr. Ann Marie Schmidt, Dr. Eva Feldman, Dr. Matthew Breyer, Dr. Robert Eckel, Dr. John Penn
- *Artificial pancreas*: Dr. Ed Damiano
- *Patient perspective*: Ms. Judy Hunt

Note – Panel members have expertise in multiple areas.

Non-traditional Grant Funding Mechanisms

Type 1 Diabetes Targeted Research Award (DP3)

- supports investigator-initiated research projects
- up to 5 years of research costs are paid in the first FY

High Impact Research and Research Infrastructure Cooperative Agreement Programs—Multi-Year Funding (UC4)

- supports cooperative agreement research projects
- up to 5 years of research costs are paid in the first FY

Moving Forward….

Fostering Collaborations

- across disciplines
- across consortia

Empowering researchers

- human islets
- living biobank

Pragmatic clinical research

- artificial pancreas
- behavioral research

Translational Research

Human Islet Research Network

New Directions…

NIDDK is proposing to build a new team science platform to help organize translational research related to the loss of beta cell mass in T1D:

The Human Islet Research Network (HIRN)

Human Islet Research Network

The Vision Behind HIRN

Mission:

To understand how **human beta cells** are lost, and to find innovative strategies to protect and/or replace functional beta cell mass in T1D.

Organization:

A **modular research network** of small consortia, each focused on a specific biological challenge, that can evolve over time in response to emerging scientific opportunities, and changes in the level or origin of funding.

Instruments to Pursue Goals and Priorities

- Staff outreach
- Workshops
- Special Emphasis Funds
- Large grants (P01, R24, U34/U01)
- Funding Opportunity Announcements

TrialNet Clinical Centers Re-competition

Completion of ongoing trials requires new enrollment and retention and follow-up of current participants:

- Fund a larger number of Clinical Centers (up to 20 in North America) at a lower per Center cost
- Level of support based on past/current productivity and with geographic consideration

Joint Statement of Collaboration on Diabetes Research

June 12, 2012

In Washington, D.C., U.S. Department of Health and Human Services Secretary Kathleen Sebelius (center) and India's Honorable Ghulam Nabi Azad, Minister of Health and Family Welfare (second from right), sign the Joint Statement on Collaboration on Diabetes Research. Witnesses were Dr. Griffin P. Rodgers (left), Dr. V.M. Katoch (second from left), and the Honorable Krishna Tirath, India's Minister of State for Women and Child Development (right).

Indo-US Workshop on Innovative Approaches and Technologies for Diabetes Prevention and Management

India International Centre
Delhi, India

February 4-6, 2013

NIH National Institute of Diabetes and Digestive and Kidney Diseases

Workshop Scientific Topics

- Innovative Technologies for Management and Prevention
- Development of Diabetes (Pathogenesis and Pathophysiology)
- Diabetes in Youth
- Prevention and Management of Diabetes and its Complications

Small Business Research

- Small Business Innovation Research (SBIR)
  - Set-aside increased from 2.6% in FY12 to 2.7% in FY13
  - Will continue to increase by 0.1% each FY until it reaches 3.2% for FY2017
- Small Business Technology Transfer (STTR)
  - Set-aside increased to 0.35% in FY12 and FY13
  - Will increase to 0.4% for 2014 and 2015, and to 0.45% for 2016 and thereafter

How can we most productively use these funds?

Source: http://grants.nih.gov/grants/funding/sbir/funding.htm

NIH Common Fund

- Annual Budget ~$550M
- Maximum of 10 years support
- Supports programs that meet the following criteria:
  - Transform the way research is done
  - Synergistically promote and advance the individual missions of NIH Institutes to promote health
  - Sufficiently complex that it requires coordination among Institutes
  - Outcome can be accomplished within the period of funding from the Common Fund
  - Something that no other entity is likely to do
- Solicits ideas for new programs each year

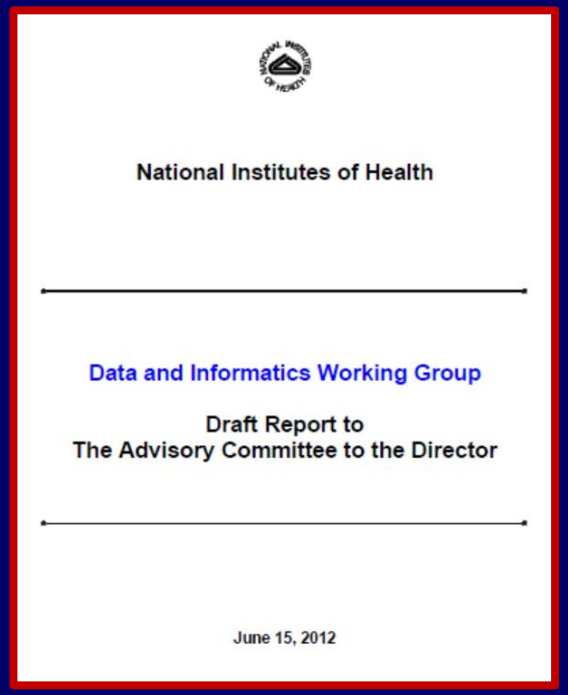
Common Fund Programs with High Relevance to NIDDK and Significant Involvement by NIDDK Program Staff

- KOMP (mouse knockout project)
- Molecular Libraries Program
- Epigenomics Program
- Microbiome Program
- Metabolomics Program
- High Risk/High Reward Program
  - Pioneer Awards
  - Early Innovator Awards
  - Transformative R01s
  - Early Independence Awards

Creation of NIDDK Common Fund Working Group

- Seek broad input from research community
- Develop program concepts in best interest of NIDDK
- Provide adequate time to develop concepts
- Useful for regular initiative development process

National Institute of
Diabetes and Digestive
and Kidney Diseases



National Institutes of Health

Data and Informatics Working Group

Draft Report to
The Advisory Committee to the Director

June 15, 2012

NIH Biomedical Big Data Initiative: Implementation Plan

Big Data

- Loosely-defined term used to describe data sets so large and complex that they become awkward to work with using on-hand database management tools
  - Data-enabled=data-intensive=data-driven=big data
- Difficulties include capture, storage, search, sharing, analysis, and visualization
- Large datasets from:
  - Complex physics experiments, astronomical surveys, climate studies, consumer data, social media, finance, business **AND** genomics, proteomics, chemical biology, clinical investigations
- For our use: "studies that seek to understand biological processes through data-intensive techniques" *Kolker, et al., Omics 16:138, 2012*

“Big Data”: Implications

- Era of massive data sets
  - http://www.nature.com/nature/journal/v455/n7209/index.html
- Recent explosion of biomedical data
  - Genome sequence data
  - Public health databases
- Need for new and better ways to make the most of this data
  - Speed discovery and innovation
  - Ultimately lead to improvements in the nation’s health and economy

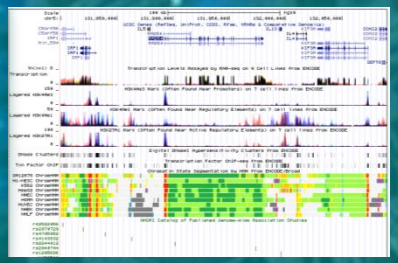
What's in a Name?

- Big Data
- Bioinformatics
- Computational Biology
- Biomedical Informatics
- Information Science
- Biostatistics
- Quantitative Biology
- **Data Science**

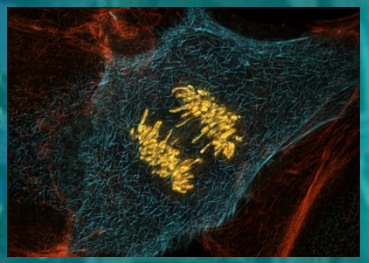
Historical Background

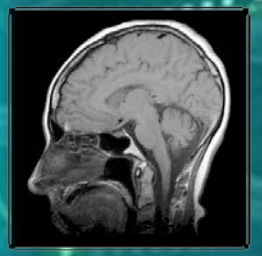
- Data and Informatics Working Group (DIWG) of the Advisory Committee to the NIH Director
  - David DeMets & Larry Tabak, Co-Chairs
  - Report provided to NIH in June 2012
- Framework proposal developed by trans-NIH working group
  - Presented by Eric Green at ICD Leadership Forum in September 2012
- ICDs enthusiastically supported moving forward on a major initiative jointly supported by Common Fund and ICs

Myriad Data Types



‘Omic





Imaging

Phenotypic

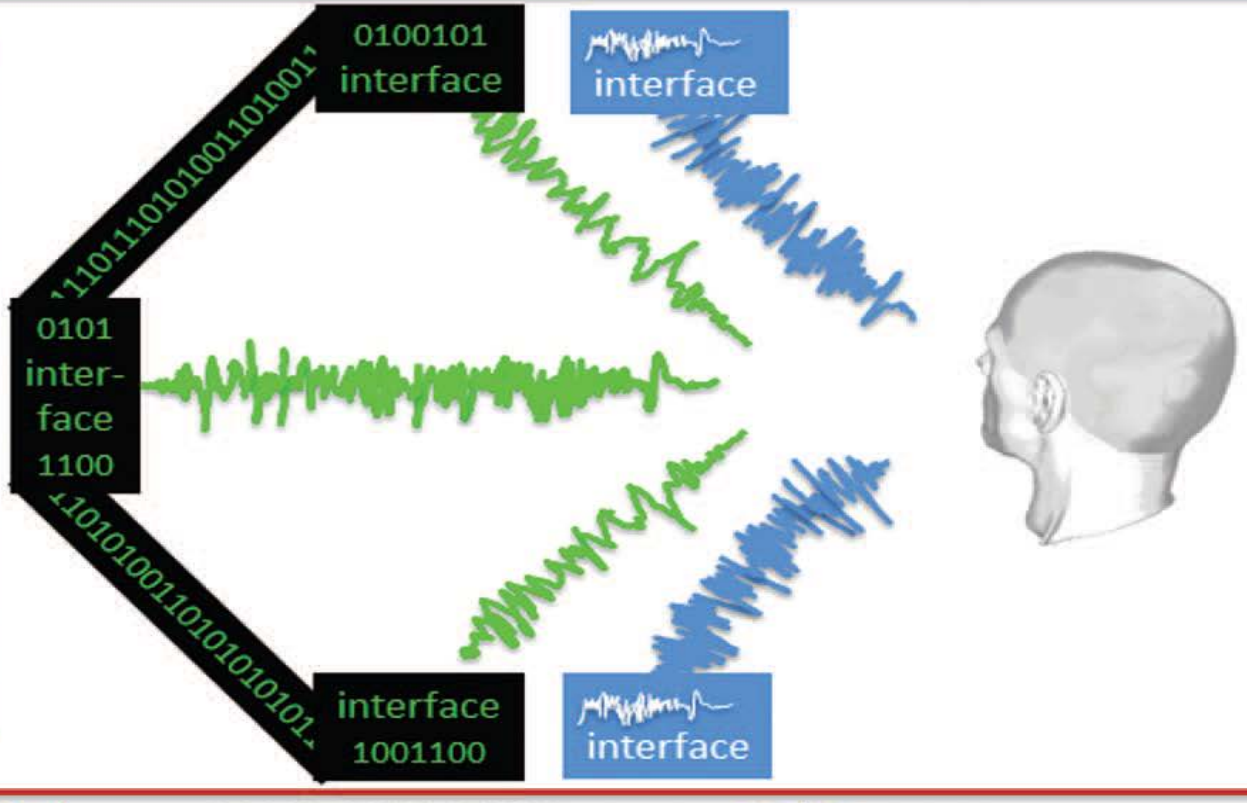
Clinical

STORAGE

digital	analog
PC hard-disk | DVD & Blu-Ray | Digital tape | Server & Mainframe hard-disk | CDs & Minidisks | Other hard-disks (portable) | Portable media player | Memory cards | Mobile phones & PDA | Videogames others | Floppy disks | Digital camera & camcorders internal | ChipCards |	Video analog | Photo print | Audio cassette | Photo negative | Cine movie film | Vinyl LP | TV episodes film | X-Rays | TV movie film | Newsprint | Other paper & print | Books |

COMPUTATION

	digital
General-purpose	Personal computers | Videogame consoles | Servers & Mainframe | Supercomputers | Pocket calculators | Mobile phones/ PDA |
Application-specific	Digital Signal Processors *CD, DVD & PVR, cameras & camcorders, modems & setup boxes, GPS, portable media, printer & fax, radio, fixed phone, mobile phone* | Microcontrollers | Graphic Processing |

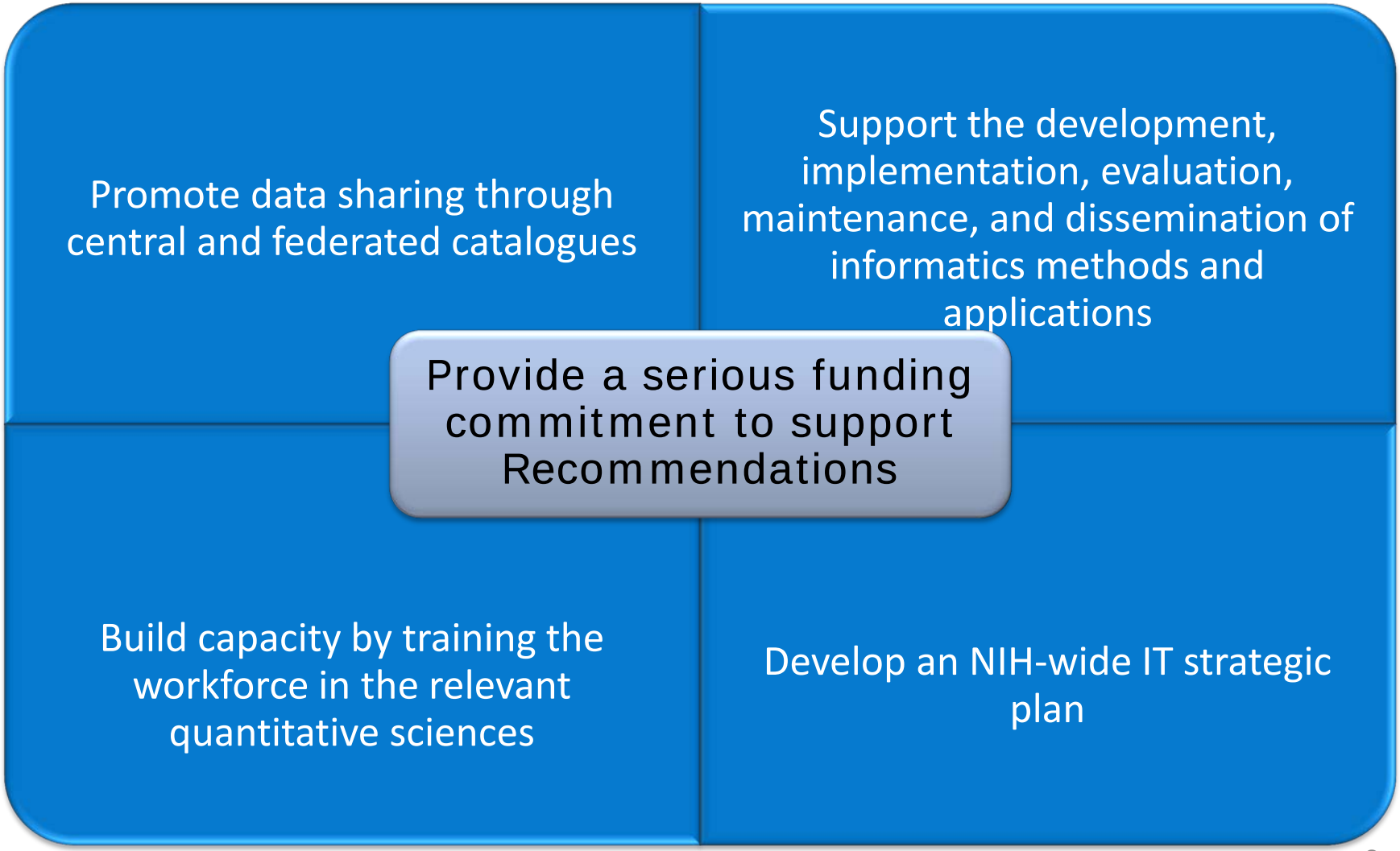


COMMUNICATION

	digital	analog
Broad-casting	TV-terrestrial | TV-cable | TV-satellite | Radio | Personal navigation GPS |	TV-terrestrial | TV-cable | TV-satellite | Radio | Newspapers | Paper advertisement |
Telecom	Fixed (voice) phone | Internet | Mobile (data) phone | Mobile (voice) phone |	Fixed (voice) phone | Mobile (voice) phone | Paper postal letters |

DWIG Report: Summary of Recommendations

http://acd.od.nih.gov/06142012_DIWG_ExecSummary.pdf



Six Big Problems to Solve

1. Locating the data
2. Getting access to the data
3. Extending policies and practices for data sharing
4. Organizing, managing, and processing biomedical Big Data
5. Developing new methods for analyzing biomedical Big Data
6. Training researchers who can use biomedical Big Data effectively

Proposal: Four Programmatic Areas

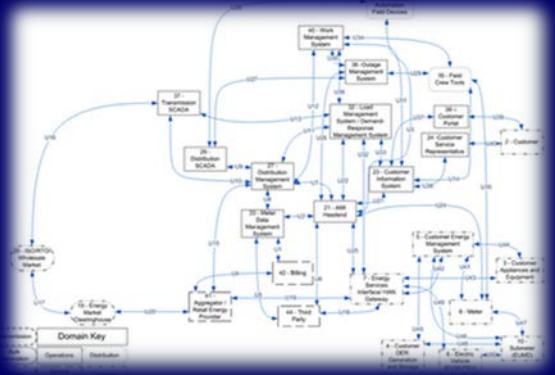
I. Facilitating Broad Use of Biomedical Big Data

II. Developing and Disseminating Analysis Methods and Software for Biomedical Big Data

III. Enhancing Training for Biomedical Big Data

IV. Establishing Centers of Excellence for Biomedical Big Data

I. Facilitating Broad Use of Biomedical Big Data



1. Create resources and policies to encourage data and software sharing
2. Create easy-to-use catalogs that allow researchers to find and cite datasets and software, and track their use
3. Establish community-driven frameworks for developing and using standards for data and metadata

II. Developing and Disseminating Analysis Methods and Software for Biomedical Big Data

1. Develop approaches for managing and processing biomedical Big Data
2. Support methods and software development for analyzing and integrating biomedical Big Data

III. Enhancing Training for Biomedical Big Data

1. Increase funding for training in biomedical Big Data areas
2. Enhance the review of training applications in biomedical Big Data
3. Support curriculum development for biomedical Big Data training

IV. Establishing Centers of Excellence for Biomedical Big Data

Investigator-Initiated Centers

- Imaging protocols
- Rare drug reactions, diseases, and variants
- Microbiome data
- Diagnosing neurological disorders
- Obesity research

Centers Addressing Identified Needs

- Data catalog and citation mechanisms
- Capitalizing on EHRs
- Data portals
- Privacy and imaging data
- Data aggregation
- ‘HumanBase’

Implementation over 7 years

- Trans-NIH but jump-started by the Common Fund
- Significant community involvement via workshops and RFIs
- FOAs designed to implement the four parts of BD2K over a period of two years
- External evaluation panel
- Associate Director for Data Science

Big Data Activities in FY13-14

- **Organize NIH Scientific Data Council**
  - **Recruit Associate Director for Data Science**

- **Workshops and RFIs on:**
  - **Sharing clinical data for research use**
  - **Data and software catalogs: August 21-22**
  - **Optimal approaches to data/metadata standard development**
  - **Needs for software development**
  - **Training needs: RFI NOT-HG-13-003**
  - **NIH-initiated Centers of Excellence: FOA pending**

- **Develop new NIH policies/review procedures for:**
  - **Encouraging data and software sharing**
  - **Use of research data in the cloud**

DIABETES RESEARCH CENTERS

Diabetes Centers EXECUTIVE COMMITTEE

2013 Roster

Jean Schaffer, Washington University in St. Louis (Chair)

Mimmo Accili, Columbia University

Larry Chan, Baylor College of Medicine

Martin Myers, University of Michigan

Jerry Olefsky, University of California, San Diego

Jeff Pessin, Albert Einstein College of Medicine

Jerry Palmer, University of Washington
NIDDK Advisory Council liaison

DIABETES CENTERS EXECUTIVE COMMITTEE REPORT 2012-2013

During 2012-2013 the Diabetes Center Executive Committee included the following Center directors:

Center Director	Program	Lines of communication
Jeff Pessin	Einstein	BADERC, Joslin
Martin Myers	Michigan	Chicago, Vanderbilt
Hassy Cohen/Jerry Olefsky	UCSD/UCLA	Colorado, UCSF
Jerry Palmer	U Washington	DK National Advisory Council
Jean Schaffer (chair)	Washington U	UAB, Penn
Mimmo Accili	Columbia	Yale, DK National Advisory Council
Larry Chan	Baylor	JHU/UMD

Conference calls were held on a monthly basis and covered the following topics:

Review of the last Director's Meeting in November 2011: Overall the meeting was considered a success. Interest was expressed in presentations by leaders of individual centers to share best practices and exchange information regarding center changes as they move into new cycles of funding.

Recent Diabetes Center RFAs and review cycles: New opportunities in the RFA for regional/national cores and expansion of Pilot & Feasibility were pursued by some applicants, and these components were generally well received. Effective with the next RFA, applications will be electronic. For future cycles, incorporation of program guidelines into the RFA document (rather than as separate document) could help clarify.

Diabetes Centers Website: Centers are encouraged to use the Pilot & Feasibility (P&F) Reviewer Database—each center can now download its data, edit off-line, and submit in order to update. To streamline renewals, through the website, NIDDK is also creating a tracking system for awarded P&F Grants Outcomes with an on-line table in which to track needed data. This is expected to go live shortly so that centers will be able to enter data online or into downloaded Excel files that can be submitted to NIDDK.

Medical Student Summer Program: The logistics and experience of the program and upcoming dates for 2013 were reviewed.

NIDDK Report on Center Site Visits: Posting of the report regarding the center site visits was briefly discussed.

Criteria for center membership: The definition of Center Research Base varies across the funded centers. Additionally, there is variability across centers in the criteria used to determine eligibility for use of center cores. The committee recommended these topics as areas in which sharing of best practices could lead to program improvements and greater clarity for program guidelines.

Outreach/Expansion of Center Program: NIDDK would like for the Centers to expand delivery of services to diabetes researchers more broadly (e.g., regionally, nationally), a goal that may be facilitated in part by increasing efficiencies. It will be important to balance this goal with provision of services to researchers at the institutions that host the Centers, in view of the depth of these diabetes research communities and in view of limited resources. Another potential concern is the degree to which this emphasis could move focus away from support of outstanding diabetes science to the development of small business operations at the host institution. There may be process models to best achieve this goal that are specifically well-suited, depending on institution. This topic was recommended for discussion at a future annual meeting.

2013 Annual meeting: The agenda for the annual meeting was discussed.

Executive Committee Membership: The Committee's goal is to improve communication between Center leadership and program staff at NIDDK. We welcome participation and suggestions of topics for discussion. Center Directors interested in serving should contact Jim Hyde.

Executive Committee Report 2012-2013

Center Director	Program	Lines of communication
Jeff Pessin	Einstein	BADERC, Joslin
Martin Myers	Michigan	Chicago, Vanderbilt
Hassy Cohen/Jerry Olefsky	UCSD/UCLA	Colorado, UCSF
Jerry Palmer	U Washington	DK National Advisory Council
Jean Schaffer (chair)	Washington U	UAB, Penn
Mimmo Accili	Columbia	Yale, DK National Advisory Council
Larry Chan	Baylor	JHU/UMD

Executive Committee Report 2012-2013

- Review of the last Director's Meeting 11/2011
- Recent Diabetes Center RFAs and review cycles
- Diabetes Centers Website
- Medical Student Summer Program
- NIDDK Report on center site visits
- Criteria for center membership
- Outreach/Expansion of Center Program
- 2013 annual meeting
- Executive Committee Membership

American Diabetes Association Research Funding Opportunities

Tamara Darsow, PhD
Vice President, Research Programs
American Diabetes Association

American Diabetes Association®

American Diabetes Association Mission

To prevent and cure diabetes and to improve the lives of all people affected by diabetes

Professional Resources

Scientific Sessions

Professional education

Peer-reviewed journals

DiabetesPro

Medical Information

Clinical Practice Recommendations (treatment guidelines)

Medical publications

RESEARCH

Direct research funding

Advocacy

Research support

Diabetes prevention and care

Legal advocacy and support

Legislative action

Communities

Community health education programs

Center for Information & Community Support

Forecast magazine

Diabetes.org

American Diabetes Association®

Commitment to Research

The American Diabetes Association has been supporting diabetes research in the academic community for more than half a century, significantly contributing to the landscape of diabetes research

Since Program inception in 1952:

- Nearly 4,000 research projects have been funded
- More than $640 million has been invested in diabetes research

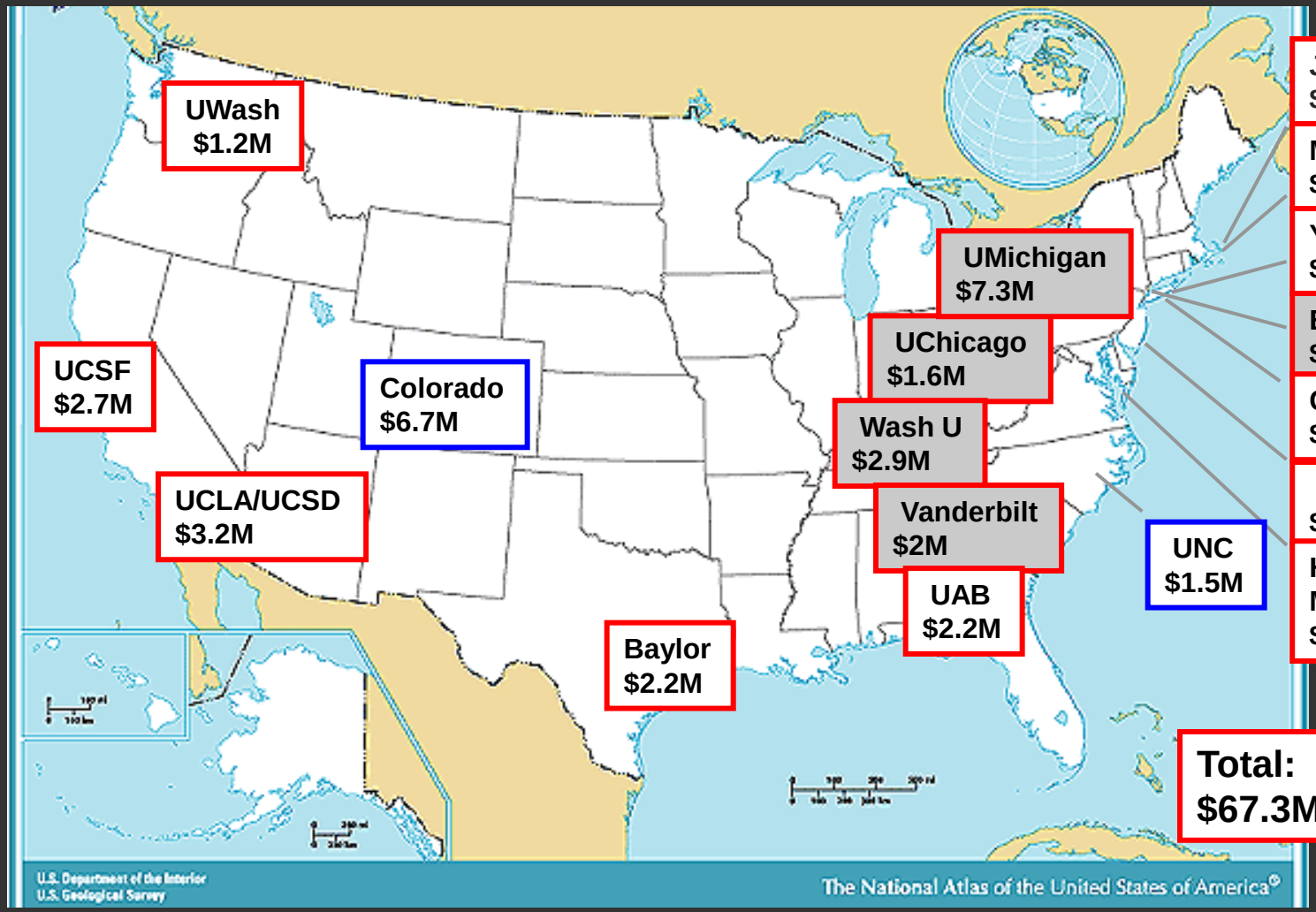
In 2012 the Association:

- Made $34.6 million available for research,
- Supporting more than 450 active research projects, with
- 400 investigators at 130 leading academic research institutions across the U.S.

American Diabetes Association®

NIH-Funded Diabetes Centers

2008-2012 American Diabetes Association Grant Support



Diabetes Research Center (DRC)

DRC and CDTR Center for Diabetes Translation Research (CDTR)

American Diabetes Association®

Volunteer Leadership

Research Grant Review Committee

- **Approximately 250 volunteer members, three study sections**
- **Review and evaluation of grant applications**
- **Committee Chairs**
  - **Clayton Mathews, MD, University of Florida**
    Basic Science: Immunology, Beta Cell Biology, Complications
  - **Jeffrey Pessin, MD, Albert Einstein Medical College**
    Basic Science: Insulin Signaling, Integrated Physiology and Complications
  - **Steven Smith, MD, Burnham Research Institute**
    Clinical Science: Clinical Research, Translational Science, and Epidemiology

American Diabetes Association.

Volunteer Leadership

Research Policy Committee

Alvin Powers, MD, Chairperson Vanderbilt University	**Martin G. Myers, Jr., MD, PhD** University of Michigan
Debra Haire-Joshu, PhD, MSE Washington University	**Vincent Poitout, DVM, PhD** University of Montreal
Mary Elizabeth Hartnett, MD University of Utah	**Jane E.B. Reusch, MD** University of Colorado and Denver VAMC
James F. Hyde, PhD National Institutes of Health	**Alan R. Saltiel, PhD** University of Michigan
C. Ronald Kahn, MD Joslin and Harvard Medical School	**Kumar Sharma, MD** University of California, San Diego
Steven Kahn, MD University of Washington	**Katalin Susztak , MD, PhD** University of Pennsylvania
Orville G. Kolterman, MD Bristol-Myers Squibb	**Steven A. Smith, MD** Mayo Clinic Rochester
Michelle Magee, MD, MBBCh, BAO, LRCPSI Medstar Research Institute	**Matthias von Herrath, MD** La Jolla Institute for Allergy and Immunology
Elizabeth Mayer-Davis, PhD, MPH University of North Carolina, Chapel Hill	**Gretchen Youssef, RD, CDE** Medstar Research Institute

American Diabetes Association.

Research Program Objectives

Support high-quality academic science across the broad spectrum of diabetes research

- Investigator-initiated submissions
- Independent peer-review process

Encourage new investigators to dedicate their careers to diabetes research

- Successful career development and training programs that support early investigators in diabetes research

Support innovative research with high potential to have a significant impact

- Specific grant opportunities for high-risk/high-impact and translational diabetes research
- Targeted research in high priority areas

American Diabetes Association®

Funding Opportunities

Core Research Program

- Two application cycles/year, deadlines in January and July
- Majority of program funding (~90%) dedicated to core program activities

Targeted awards (Periodic Requests for Applications)

- Targeted funding opportunities in specific research areas
- Means to increase research activity in areas of strategic focus

Federal/Collaborative Co-support

- Co-support of larger collaborative efforts, often with NIH or other federal funders

Pathway to Stop Diabetes

- New program, launched in 2013, to attract a new generation of researchers to diabetes

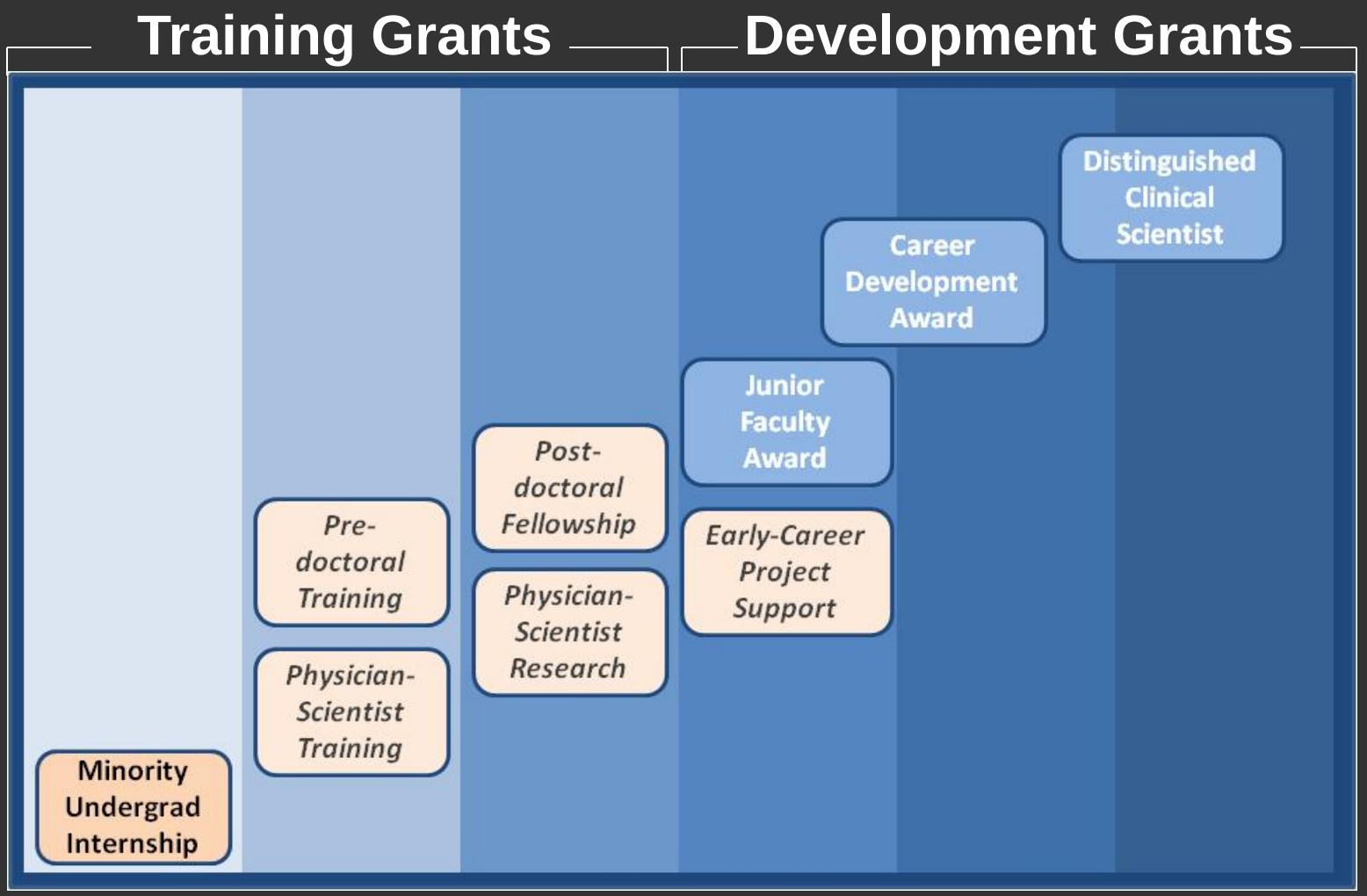
American Diabetes Association®

Core Program Grant Mechanisms

Research Awards	Development Awards	Training Awards
Basic Science $115,000/yr, 3 yrs	*Early Career Project Support*	**Undergraduate Internship** $3000
Clinical Science & Epidemiology $200,000/yr, 3 yrs	**Junior Faculty** $138,000/yr, 3 yrs	*Medical Student*
Translational Science $200,000/yr, 3 yrs	**Career Development** $172,500/yr, 5 yrs	*Pre-doctoral*
Innovation $50,000/yr, 2 yrs	**Distinguished Clinical Scientist** $200,000/yr, 4 yrs	*Physician Scientist* *Post-doctoral*

American Diabetes Association®

Career Development and Training



American Diabetes Association.

Targeted/Collaborative Research

Collaborations with research partners allow program expansion in areas of shared strategic interest, and provide funding for science in critical and emerging areas of diabetes research

Recent Targeted RFAs

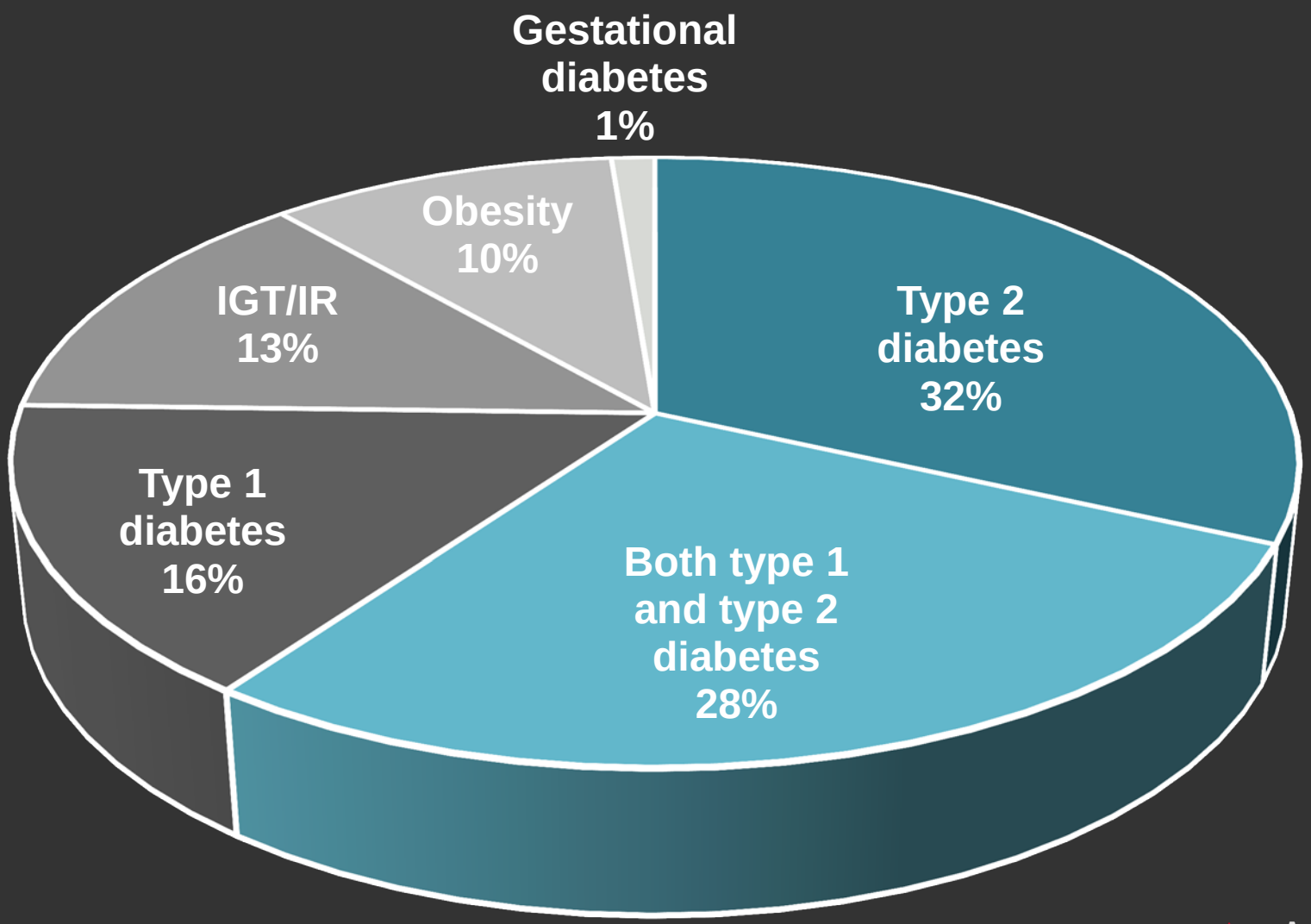
- Bariatric Surgery in Diabetes
  (Covidien & Ethicon Endosurgery)
- Hypoglycemia and Diabetes, Neurohormonal Control of Metabolism
  (Novo Nordisk)
- Microbiome and Metabolic Changes in Diabetes and Obesity
  (GlaxoSmithKline)

Federal/Collaborative Co-Support

- DCCT, HAPO, VADT, ACCORD, TrialNet

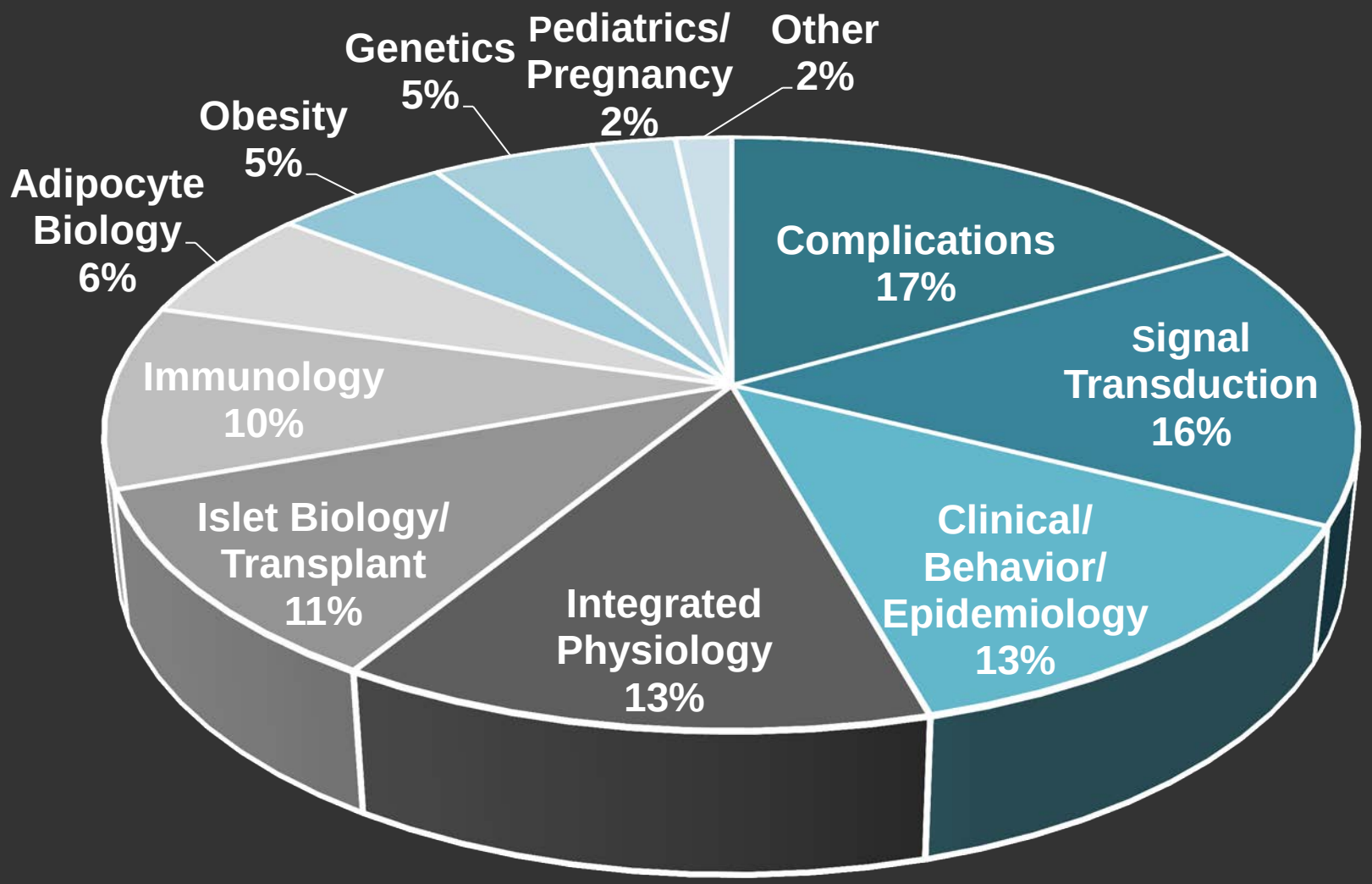
American Diabetes Association®

Research Portfolio by Diabetes Type



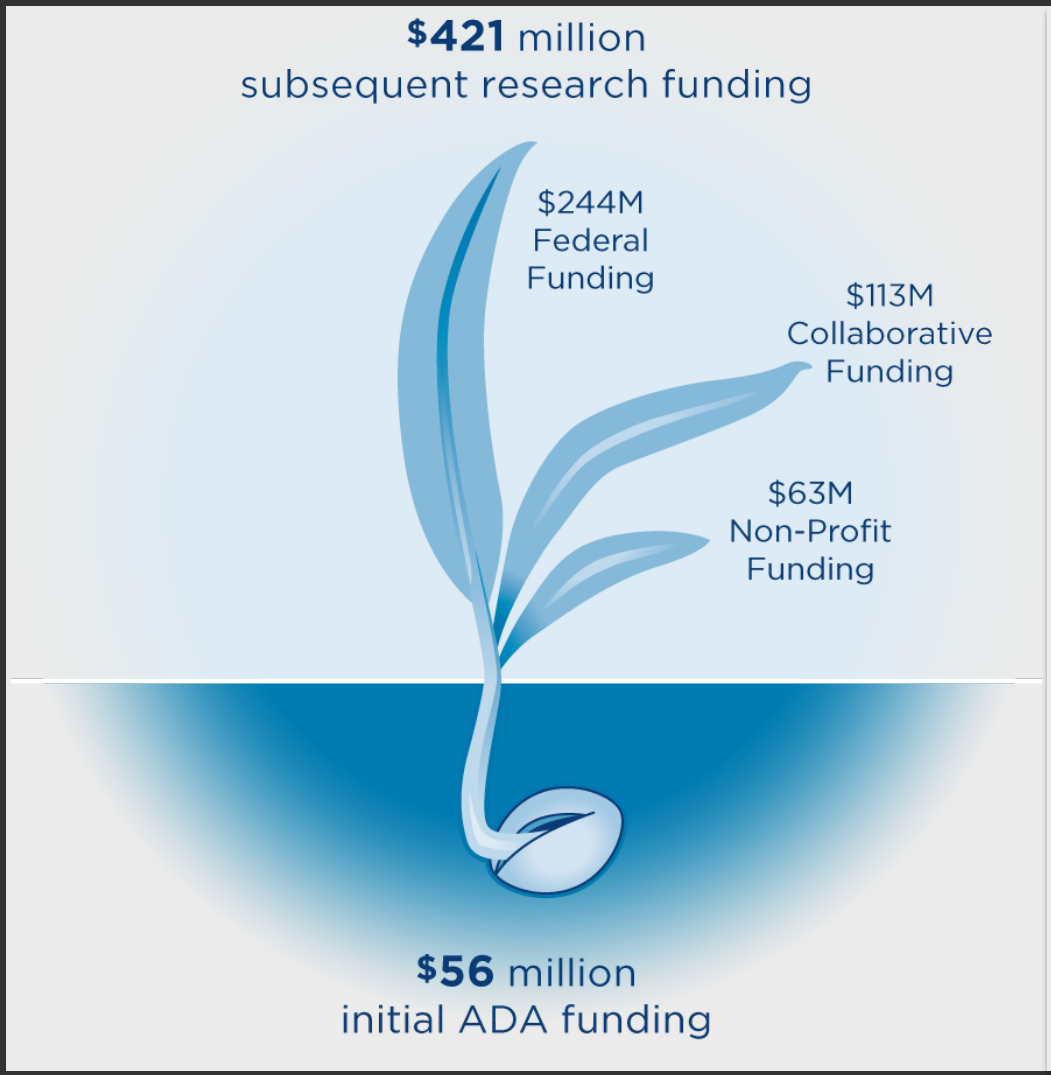
American Diabetes Association.

Portfolio by Research Focus



American Diabetes Association.

Expanding the Field of Diabetes Research

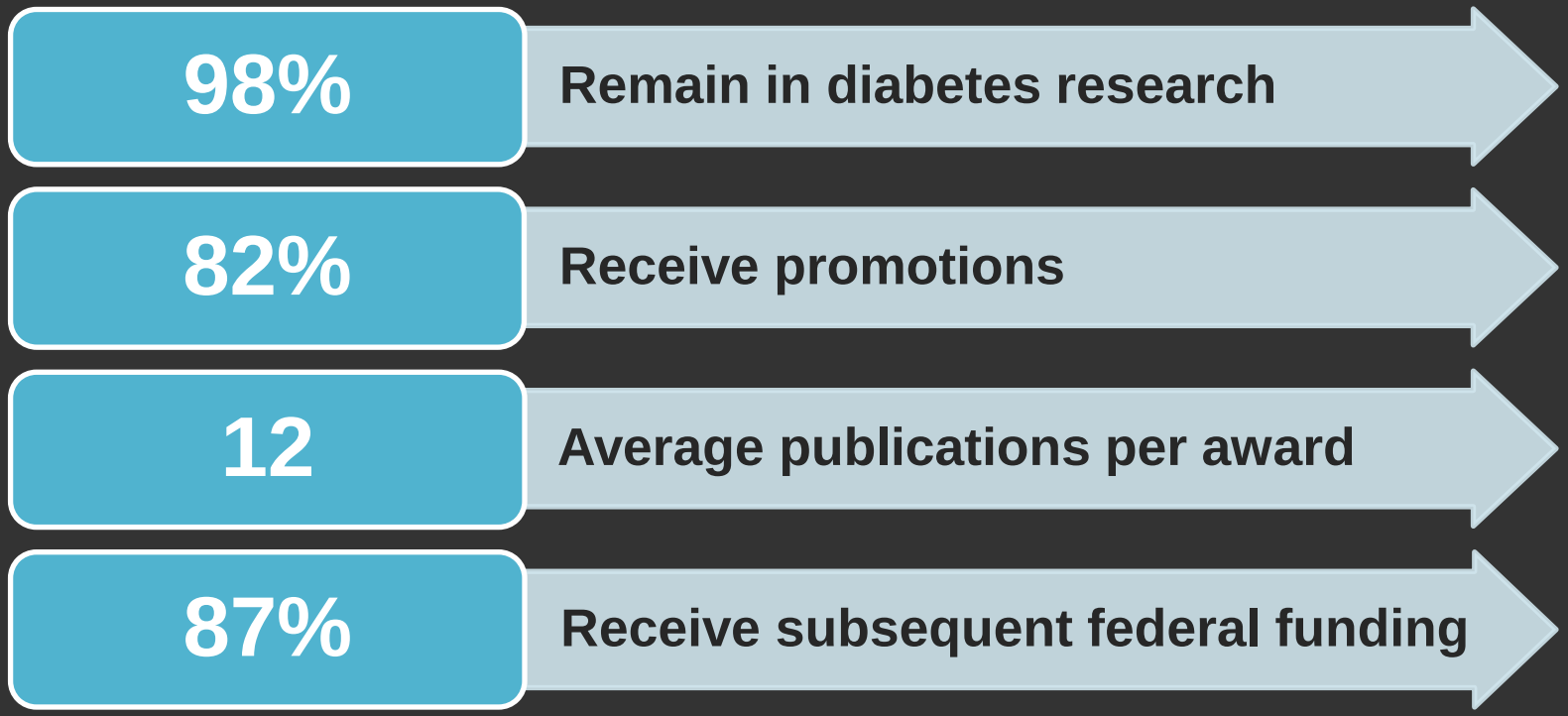


85% **received subsequent federal funding to support their work**

Association support leveraged into *7.5X* additional funding

American Diabetes Association.

Promoting Career Development



American Diabetes Association®


PATHWAY
TO STOP DIABETES
A RADICAL NEW ROAD FOR RESEARCH
American Diabetes Association.
ResearchFoundation℠
Science. Progress. Hope.

A NEW GENERATION OF BRILLIANT SCIENTISTS

Funding …	**Awards of up to $1.625 Million**
Flexibility …	**The freedom to innovate, to explore, to blaze new trails**
Security …	**Five to seven years of support**
Mentoring …	**Guidance from distinguished scientists, business leaders and other major donors**
Collaboration …	**Opportunities to advance research and careers through mentoring, symposia, speaking opportunities and technology**

Nomination and application materials available March 2013
http://professional.diabetes.org/grants-pathway

Mentor Advisory Group

C. Ronald Kahn, MD *Chair, Mentor Advisory Group* *Joslin and Harvard Medical School*	**Barbara Howard, PhD** Medstar Research Institute
Mark Atkinson, PhD University of Florida	**Steven Kahn, MB, ChB** University of Washington
Richard Bergman, PhD Cedar Sinai Medical Center	**Barbara Kahn, MD** Beth Isreal Deaconess and Harvard
Jeffrey Bluestone, PhD University of California, San Francisco	**Chris Newgard, PhD** Duke University
Charles Burant, MD, PhD University of Michigan	**Jeffrey Pessin, PhD** Albert Einstein College of Medicine
John Buse, MD University of North Carolina	**Alvin Powers, MD** Vanderbilt University
Lawrence Chan, MD Baylor University	**Chris Rhodes, PhD** University of Chicago
Sylvia Corvera, MD University of Massachusetts	**Philipp Scherer, PhD** University of Texas Southwestern
Robert Eckel, MD University of Colorado	**Elizabeth Seaquist, MD** University of Minnesota
Michael German, MD University of California, San Francisco	**Randy Seeley, PhD** University of Cincinnati
Margaret Grey, DrPH, RN, FAAN Yale University	**Gerald Shulman, MD, PhD** Yale University

NOMINATIONS

Pathway **awards are by nomination only**

- **Nominations are invited from U.S. accredited academic and non-profit research institutions**
- **Each institution is allowed a maximum of one nomination per grant cycle**
- **Individual components of multi-component institutions may nominate one candidate each**
- **Nominations of candidates from diverse disciplines are encouraged**

Nomination and application materials available March 2013
http://professional.diabetes.org/grants-pathway

Diabetes Research *INITIATOR*

AWARD TYPE	SUPPORT		TERM
Researchers in training transitioning to independence	**Up to $1.625M total over two phases**		
	Phase 1 *(mentored)*	$100,000/year maximum	2 years
	Phase 2 *(independent)*	$325,000/year maximum	5 years

Eligibility:

- Currently in **mentored training position** (post-doctoral fellow, research fellowship)
- **<7 years** since terminal doctoral degree

Diabetes Research *ACCELERATOR*

AWARD TYPES	SUPPORT	TERM
Early-career diabetes investigators	**$1.625M total** $325,000/year	5 years
Established investigators new to diabetes	**$1.625M total** $325,000/year	5 years

Eligibility:

- **Early-career diabetes investigators** may have independent funding through initial NIH-R01
- **Established investigators new to diabetes research** in any discipline who *have not had* previous national diabetes research funding

THE PROBLEM IS UNPRECEDENTED

THE SOLUTION MUST MATCH IT

Involvement in Local ADA Activities

Why

- **Brings the research mission to the community**
- **Puts a face to research**
- **Interact with people who are impacted by diabetes and who may benefit from research advances**
- **Establish local connections to organization**

How

- **Step Out Walk and Tour de Cure events**
- **Community Volunteer Leadership activities**
- **Research education and awareness**
  - Research presentations at local events
  - Community lab tours
  - Spokespersons for research

American Diabetes Association®

Resources

Submission deadlines, detailed application instructions, and open Requests for Applications (RFAs)

- **Online at http://professional.diabetes.org/grants**

Research Program Contacts:

Priya Selvakumar

Associate Director, Research Programs (Core Programs)

- **pselvakumar@diabetes.org**
- **grantquestions@diabetes.org**

Magda Galindo

Associate Director, Research Programs (Pathway)

- **mgalindo@diabetes.org**
- **pathway@diabetes.org**

American Diabetes Association.

American Diabetes Association®

OUR VISION:

Life free of diabetes
and all its burdens

American Diabetes Association®

Thank you!

Questions?

American Diabetes Association®

DIABETES RESEARCH CENTERS

The Diabetes Research Center Directors welcome applications for the Summer **Medical Student Research Training Program in Diabetes**.

This Program is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and allows medical students during the summer between the first and second year or second and third year to conduct independent research under the direction of an established scientist at one of the 17 Diabetes Research Centers.

Prior research experience is not required.

The objectives of this Program are to provide the opportunity for the student to conduct diabetes-related research and to gain an improved understanding of career opportunities in biomedical research. Participants will also develop a comprehensive understanding of diabetes, its clinical manifestations and its unsolved problems.

The diabetes-related research opportunities are quite broad and range from basic laboratory studies to clinical studies in humans. The preceptor and the medical student jointly design a research project that is then conducted over the course of the summer. In addition to working on his/her own research project, each student attends a series of web-cast seminars addressing various clinical and research aspects of diabetes mellitus and its complications. At the conclusion of the summer, each student presents a brief summary of his/her work at a scientific symposium for all Program participants.

Each student receives a stipend (currently calculated at a rate of approximately $399 per week) from which expenses for food and housing may be paid. Students are expected to spend eight-twelve (8-12) weeks in the Program, but commencement and conclusion dates are reasonably flexible.

You must be a U.S. Citizen and/or permanent resident to participate in this Program.

Questions regarding the Program should be directed to:

Medical Student Research Program in Diabetes

E-mail: niddk.diabetes.student.research@vanderbilt.edu

Website: http://medicalstudentdiabetesreseach.org/

Application and Program Statistics
NIDDK Medical Student Symmer Research Program
Summer 2012 and 2013

Applications:

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

2012 Program				2013 Program		
Center	# participants	# participants from medical schools associated with a DRC	# applicants listing Center as #1, 2, 3 choice	# participants	# participants from medical schools associated with a DRC	# applicants listing Center as #1, 2, 3 choice
AECOM	4	4	78	2	2	115
Baylor	3	0	39	3	1	73
Boston Area	5	1	42	5	0	69
Columbia	4	1	124	6	0	185
JHopkins/Univ MD	6	1	82	8**	0	124
Joslin	6	0	60	6	1	94
UAB	6	3	31	6	5	43
UCLA/UCSD	4	2	168	8**	0	158
UCSF	3*	2	98	4	2	135
Univ Chicago	4	1	101	4	0	130
Univ Colorado	5	2	53			
Univ Michigan	5*	0	42	10**	2	74
Univ Pennsylvania	4	1	69	6	1	111
Univ Washington	5	0	41	6	0	68
Vanderbilt - NIDDK	6	0	66	6	0	100
Washington Univ	4	1	35	4	1	54
Yale	4	0	51	4	0	66
Sub-total	78			88		
Vanderbilt - T35 grant	24*			26		
TOTAL	102			114		

*indicates student withdrawing too late to be replaced

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

Student Participant Demographics 2012		**US Medical School Enrollment 2012****		**US Census 2010**	
Race		Race		Race	
African American	8 (8%)	African American	5,630 (7%)	African American	38.9 million (13%)
American Indian	0	American Indian	630 (>1%)	American Indian	2.9 million (0.9%)
Asian	41 (40%)	Asian	18,449 (22%)	Asian	14.7 million (5%)
Caucasian	35 (34%)	Caucasian	48,625 (59%)	Caucasian	173.1 million (56%)
Hispanic	8 (8%)	Hispanic	7,225 (9%)	Hispanic	50.5 million (16%)
Native Hawaiian or Pacific Islander	0	Native Hawaiian or Pacific Islander	223 (>1%)	Native Hawaiian or Pacific Islander	0.5 million (0.2%)
Other/No answer	10 (10%)	Other/No answer	4,086 (5%)	Other/two races	28.1 million (9%)
Gender		Gender		Gender	
Female	62 (61%)	Female	38,434 (47%)	Female	156,964,000 (50.8%)
Male	40 (39%)	Male	43,633 (53%)	Male	151,781,000 (49.2%)

Citizenship	
US Citizens	99
Permanent Res.	3

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

NIDDK Medical Student Research Program - Summer 2012

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 select 3 Diabetes Research Centers
- Each Center reviews and lists students
- "Matching" of students/centers

DIABETES RESEARCH CENTERS

Some Stats – See Handout

	2009	2010	2011	2012	2013
# applicants	197	431	486	395	568
# medical schools	82	104	111	114	138
# participants	83	92	101*	102*	114^
# medical schools	40	49	49*	58*	65^

*** Includes students in Vanderbilt T-35 program**
^ Includes students in Vanderbilt T-35 program plus students supported nu Diabetes Complications Consortium

Additional Students Supported by the Diabetic Complications Consortium (DCC)

Institution	# Additional Students
J Hopkins/U Maryland	2
U Michigan	4
UCSD	4

Other Medical Student Research Program Supported by NIDDK

- **Started by NIDDK-supported O'Brien Kidney Center Program for summer of 2012**
- **36 students from 24 medical schools applied; 13 students from 12 schools selected**
- **Diabetes student program provided template, administrative advice, and support**
- **Combined research symposium with national NIDDK Diabetes Program**

NIDDK Medical Student Research Symposium

- Visiting Professors
  - Arshiya Baig, M.D. (U. Chicago)
  - Art Castle, Ph.D. (NIDDK)
  - Steven Kahn, M.D. (U. Wash.)
  - Mike Rickels, M.D. (Penn)
  - John Sedor, M.D. (Case Western Reserve)
  - Scott Soleimanpour, M.D. (Penn)
- Career advice

New in 2012: Panel of Previous Participants

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)
  - Fred Wondisford / Sally Radovick (Johns Hopkins)

Items for Discussion

- Number of students applying to some Diabetes Centers
- Need suggestions for visiting professors for this summer's research symposium (July 31, August 1)
- Follow up in place
  - Applied/accepted into year-off programs (NIH, NIDDK-supported)

National Institute of Diabetes and Digestive and Kidney Diseases

NIDDK Diabetes Center Medical Student Summer Research Program

Jim Hyde, Ph.D.
Program Director, NIDDK

National Institute of Diabetes and Digestive and Kidney Diseases

Competing Renewal T32 Applications

- Jim/Art will send out an e-mail reminder to each participating T32 PI (cc: Diabetes Center Director) to include a request to add or continue the summer medical student slots to the T32 renewal application.
- T32 PI needs to describe the summer program in the renewal application: (How are student applications solicited? Reviewed? How are mentors for students identified? How will students interact with other T32 students, fellows, etc.? What activities besides research will enrich the summer students' experiences? Seminars? Workshops? Opportunities to have lunch with endocrine fellows/faculty?)

Competing Renewal T32 Applications

- Tables need to be provided for the summer program: appointed medical student trainees (Table 12A; Predoctoral Trainees Supported by This Training Grant); applicant pool (Table 9A; Qualifications of the Current Predoctoral Trainees Clearly Associated with the Training Program)—use the students who applied to your program, but were not selected as the appointed short-term trainees
- Ask Al Powers to provide a letter in the T32 application? Could be a uniform letter describing the program, number of applications received nationally, selection of Centers, summer research symposium, webinars, etc.

National Institute of Diabetes and Digestive and Kidney Diseases

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

Overview of the NIH Common Fund Metabolomics Program

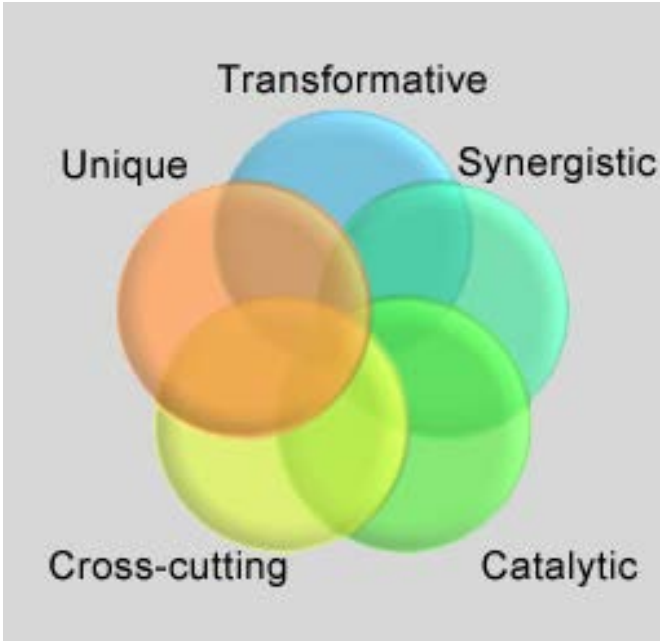
Arthur L. Castle, PhD

Program Director
Metabolomics and Informatics Program
National Institute of Diabetes and Digestive and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

NIH Common Fund

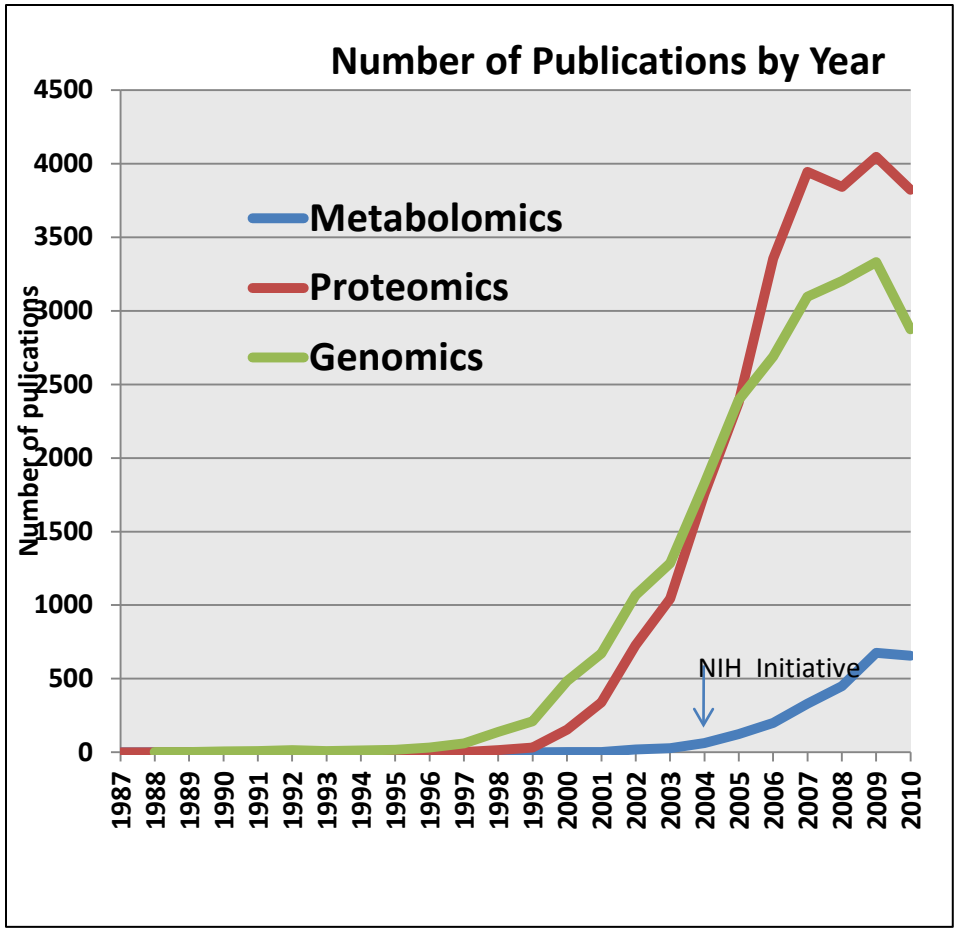
- ~$500M annual budget
- 3-4 new programs introduced each year
- Limited duration (5 to no more than 10 years)
- Managed by the Office of Strategic Coordination in the Office of the Director, NIH



National Institute of Diabetes and Digestive and Kidney Diseases

Comparing Literature Trends in Other "Omics" Fields

Number of Journal Articles in Metabolomics (Scopus Database: searched in Abstract, Title and Keywords)			
Year	Metabolomics	Proteomics	Genomics
1987	0	0	
1988	0	0	1
1989	0	0	1
1990	0	0	7
1991	0	0	9
1992	0	0	15
1993	0	0	9
1994	0	0	13
1995	0	0	18
1996	0	0	32
1997	0	2	61
1998	0	15	139
1999	0	33	210
2000	3	155	484
2001	2	339	671
2002	19	728	1,068
2003	29	1,044	1,284
2004	63	1,756	1,827
2005	123	2,376	2,394
2006	199	3,354	2,690
2007	330	3,943	3,095
2008	449	3,841	3,203
2009	675	4,046	3,331
2010	656	3,822	2,874
Total	2,548	25,454	23,436



National Institute of Diabetes and Digestive and Kidney Diseases

Metabolomics Needs

- Increase national metabolomics capacity in specialized facilities that provide high quality data, analyses, and interpretation.
- Train a new generation of scientists in metabolomics with the skills in technology, biochemistry and physiology needed for metabolomics studies.
- Need for more standard compound preparations and mechanism to make new ones as needed.
- Development of new technologies and adoption of existing technologies and methods.

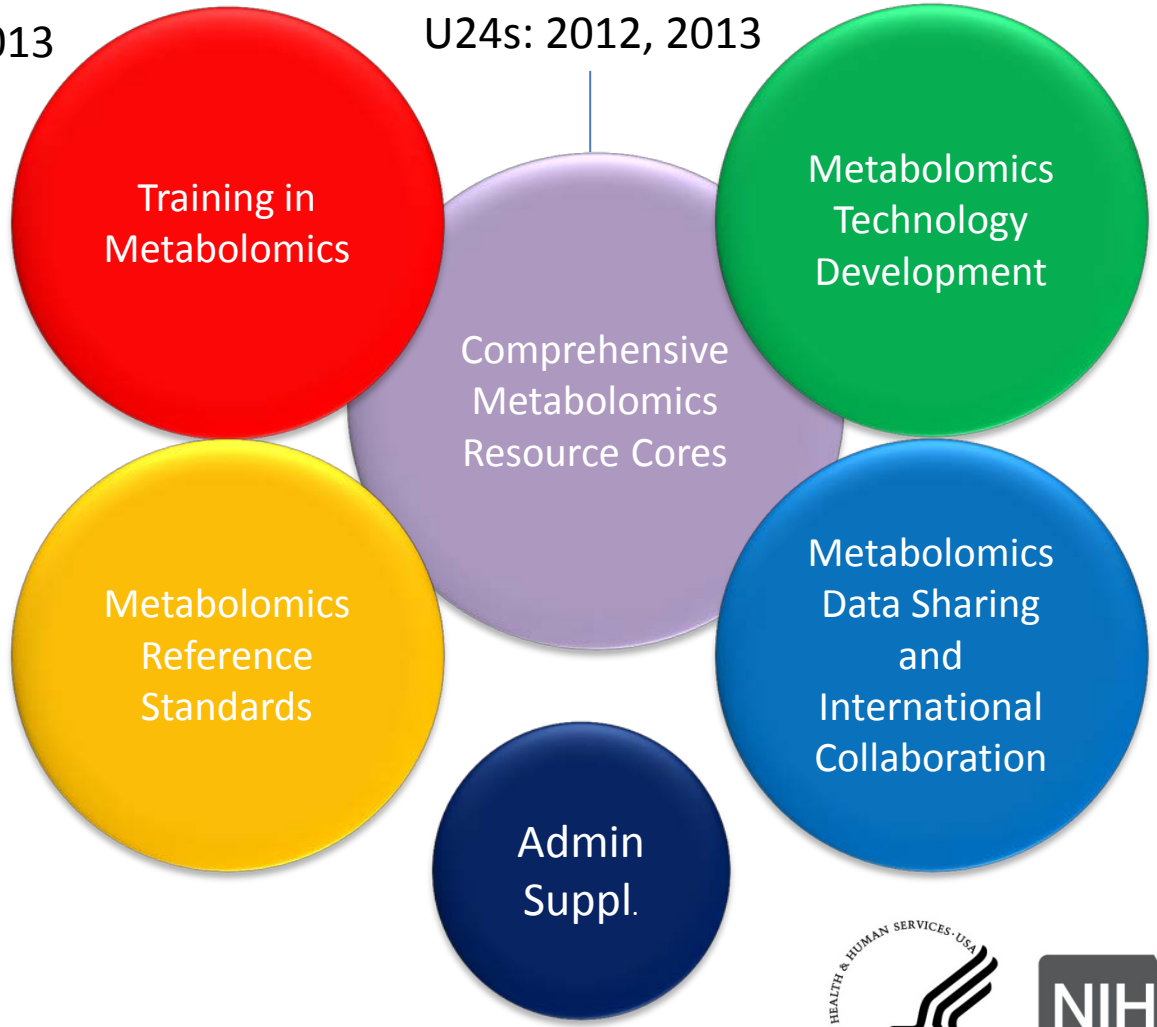
National Institute of Diabetes and Digestive and Kidney Diseases

Common Fund Program to build metabolomics capacity and bridge discovery to translation

K01s: 2012, 2013
R25s: 2102

U24s: 2012, 2013

R01s : 2012



Synth Contract 2013

U01 DRCC: 2012

2012-2017

National Institute of Diabetes and Digestive and Kidney Diseases

Currently Supported Projects
https://commonfund.nih.gov/Metabolomics/

DRCC

U01 DRCC SUBRAMANIAM, SHANKAR, UNIVERSITY OF CALIFORNIA SAN DIEGO The Metabolomics Data Center and Workbench (MDCW)

CMRC

U24 BURANT, CHARLES F, UNIVERSITY OF MICHIGAN AT ANN ARBOR Michigan Regional Comprehensive Metabolomics Resource Core (MRC2)

CMRC

U24 FIEHN, OLIVER, UNIVERSITY OF CALIFORNIA DAVIS West Coast Central Comprehensive Metabolomics Resource Core (WC3MRC)

CMRC

U24 SUMNER, SUSAN J, RESEARCH TRIANGLE INSTITUTE RTI's Regional Comprehensive Metabolomics Resource Center

TrEd

R25 BARNES, STEPHEN, UNIVERSITY OF ALABAMA AT BIRMINGHAM
UAB Metabolomics Workshop: from design to decision

TrEd

R25 KOHLMEIER, MARTIN, UNIVERSITY OF NORTH CAROLINA CHAPEL HILL
Online learning platform: introducing clinicians and researchers to metabolomics

Currently Supported Projects
https://commonfund.nih.gov/Metabolomics/

R01 BAKER, ERIN S, BATTELLE PACIFIC NORTHWEST LABORATORIES
Platform Providing Increased Throughput, Sensitivity and Specificity for Metabolo

R01 FAN, TERESA W-M (contact) HIGASHI, RICHARD M MOSELEY, HUNTER NATHANIEL NANTZ, MICHAEL H ,UNIVERSITY OF LOUISVILLE
Integrated Chemoselective and Informatic Platform for Large-Scale Metabolomics

R01 HU, JIAN ZHI , BATTELLE PACIFIC NORTHWEST LABORATORIES
Slow-MAS NMR Metabolomics

R01 MURPHY, ROBERT CARL , UNIVERSITY OF COLORADO DENVER
High Throughput Lipidomics Analysis by MALDI/Ion Mobility Mass Spectrometry

R01 PATTERSON, ANDREW , PENNSYLVANIA STATE UNIVERSITY-UNIV PARK
Optimized Metabolite Extraction, Separation, and Identification for Metabolomics

R01 PATTI, GARY JOSEPH , WASHINGTON UNIVERSITY
Developing the Untargeted Metabolomic Workflow for High-Throughput Analyses

K01 AMMONS, MARY CLOUD BOSWORTH, MONTANA STATE UNIVERSITY - BOZEMAN
Metabolomic Analysis as a Tool to Understanding the Use of Novel Therapeutics in

K01 NGO, DEBBY, MASSACHUSETTS GENERAL HOSPITAL
The Kynurenine Pathway in Pulmonary Arterial Hypertension

K01 PHELAN, VANESSA V, UNIVERSITY OF CALIFORNIA SAN DIEGO
The Interactive Metabolome of Polymicrobial Biofilms

K01 ROWLEY, JESSE, UNIVERSITY OF UTAH
The Platelet Metabolome in Obesity

Support for Metabolomics Analysis https://commonfund.nih.gov/Metabolomics/

Direct fee for service from resource cores

Low cost, year round, Design to Analysis

Pilot and Feasibility funding from resource cores

50 K, apply directly through CMRCs, 1 time/yr

Next application time TBA, winter/spring

Administrative supplements to existing NIH grants

100K, Apply to FOA issued yearly

New collaboration, any metabolomics facility and currently funded investigator

P01, P50, DP2, DP3, R00, R01, R37, U01

Due date TBA probably around March.

Data Repository and Coordinating Center

- Cloud Based Data Repository at UCSD
  - Public access database for all data from the Regional Cores and Tech Development projects
- Develop analytical tools and data standards.
- International Coordinating Committee will provide interface between metabolomics efforts in North America, Europe and Asia
- Future Plans as General Metabolomics Repository

More Information

Common Fund Metabolomics Web Site : links to all FOAs, resources
https://commonfund.nih.gov/metabolomics/

DRCC: Database and links to funded metabolomics resources
http://www.metabolomicsworkbench.org/

UC Davis
http://metabolomics.ucdavis.edu/

U. Michigan
http://mrc2.umich.edu/index.php

RTI
http://www.rti.org/page.cfm?objectid=3BC41B11-068E-1405-9A6F79D91D8D69EC

National Institute of
Diabetes and Digestive
and Kidney Diseases

FAQs for Costing of NIH-Funded Core Facilities

Notice Number: NOT-OD-13-053

Key Dates
Release Date: April 8, 2013

Related Announcements
NOT-OD-10-138

Issued by
National Institutes of Health (NIH)

Purpose

The National Center for Research Resources and the NIH Office of Extramural Research co-sponsored a meeting in July 2009 on the Efficient Management and Utilization of Core Facilities. One of the items discussed during that meeting was costing issues for these facilities. Representatives of universities, academic health centers and non-profit institutions cited difficulty in interpreting the requirements in Federal cost principles and implementing these requirements in a manner consistent with the objectives of NIH projects which support these resources.

A draft set of Frequently Asked Questions was developed to provide NIH staff and awardees with a set of common questions and answers to aid in assuring core facilities operate in compliance with the terms of award including applicable Federal cost principles. This was published for comment in NOT-OD-10-138. Based on this input and further consideration by NIH staff revised FAQs are provided in this Notice.

The purpose of these FAQs is to provide answers to common questions raised regarding NIH-funded core facilities and other applicable research related facilities that support NIH grants. These FAQs are not intended to establish new policies or interpretations of applicable Federal cost principles, nor are they meant to represent broad guidance on the costing treatment of all institutional service and recharge centers.

The range and approaches employed by institutions in the implementation and management of cores is very broad and often complex. Accordingly, while these FAQs are provided to serve as a helpful resource they are not intended to provide definitive or final interpretations for all circumstances that may be applicable to core facilities beyond that provided in applicable Federal cost principles and the terms of a NIH grant award. Specific questions regarding appropriate costing of such facilities and the application of these FAQs to individual awards should be addressed to the NIH Grants Management contacts on grant awards.

When Federal cost principles are revised or as other matters may require, these FAQs may be revised and updated in the future.

Administrative Requirements and Federal Cost Principles Applicable to NIH Grants

An overview of the terms and conditions of NIH Grant Awards is provided in the NIH Grants Policy Statement (10/12) http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch3.htm#terms_conditions_overview

The administrative and costing requirements for all NIH grants, including projects supporting core facilities, are referenced in the terms of award. These terms incorporate Title 45 CFR Part 74 or 45 CFR 92 as applicable, which apply the applicable Federal cost principles by reference.

For NIH grants which support core facilities one of the following cost principles are most commonly applicable:

- Cost Principles for Educational Institutions OMB Circular A-21 (2 CFR Part 220), http://www.whitehouse.gov/omb/circulars_a021_2004/
- Cost Principles for Non-Profit Institutions OMB Circular A-122 (2 CFR Part 230), http://www.whitehouse.gov/omb/circulars_a122_2004
- Cost Principles for Hospitals Title 45 CFR Part 74, Appendix E (http://www.access.gpo.gov/nara/cfr/waisidx_09/45cfr74_09.html).

Frequently Asked Questions

1) General Information about Core Facilities

a. What is a core facility?

For the purpose of these FAQs core facilities are centralized shared research resources that provide access to instruments, technologies, services, as well as expert consultation and other services to scientific and clinical investigators. The typical core facility is a discrete unit within an institution and may have dedicated personnel, equipment, and space for operations. In general, core facilities recover their cost, or a portion of their cost, of providing service in the form of user fees that are charged to an investigator's funds, often to NIH or other federal grants.

A core facility can share similar operating principles with other service (or recharge) centers, which also may provide resources necessary to support the research objectives of an institution. Institutions establish core facilities, including the corresponding costing structure of the facility, to provide required services to users generally with all or a portion of the cost of these services charged to users' accounts.

It should also be noted that institutions may determine, consistent with applicable Federal cost principles that it is appropriate to include the cost (or a portion of the cost) of a centralized resource in the facilities and administrative (F&A) costs. Consequently, the cost would be charged to NIH grants as an F&A cost by application of the appropriate F&A rate to the allowable direct costs of a NIH grant. When this is the case, an institution should take steps to ensure that there is no duplication of cost recovery as a direct and indirect (F&A) cost (see FAQ 2g).

Institutions may determine that establishing a core facility is appropriate to address required services based on a variety of expected advantages which may include, but are not limited to assuring access to resources/services required to address institutional needs; providing for required compliance with applicable regulations and institutional policy and achieving economies of scale. Accordingly, these facilities can take many forms to address institution needs and objectives.

Core facilities may be fiscally supported by institutional funds, Federal funds, external revenue, other funding, or any combination of these. No matter the source of funding for a core facility, the costing issues discussed in this document should be observed if NIH grants are charged for the use of a core facility or if an NIH grant provides general support to establish or operate a core facility.

b. How are NIH funds used to support core research facilities?

NIH project support for core facilities takes many forms. Some examples are provided below. However, these examples are not all-inclusive and specific circumstances of the core facility should be considered when applying these FAQs. Institutional policies and other Federal requirements and guidance also should be considered.

- Example 1: NIH grant provides direct support for the operation of a core facility

In this example the NIH grant (generally a Center, Program Project Grant or Resource award) provides direct support for the operation of a core. This support may provide full support of the core or partial support per the example below:

Total Allowable Direct Cost of Core	$110,000
NIH Direct Cost Support	$60,000
Net Direct Cost	$50,000
Number of Units of Service 1,000 Net cost per Unit = $50 (user fee)	

If the institution allocates $10,000 of its funds to support the service the net direct cost would be reduced to $40,000 and the user fee to $40

- Example 2: NIH grant (generally a Center, Program Project Grant or Resource award) provides funds to reduce user charges on other NIH supported projects or a subset of NIH supported investigators (potentially: targeted support for a specific Institute or Center or to support Early Stage Investigators or for Pilot and Exploratory Projects) Note: This example assumes that usage supported by NIH is relatively stable or reliably predicted. If this is not the case,

institutions may determine that this methodology is not practical or appropriate.

Total Allowable Direct Cost of Core $110,000

Number of Units of Service 1,000
Net Direct Cost per Unit = $110

NIH projects are estimated to use 800 units in the budget period.

NIH grant provides $40,000 in direct costs to reduce net charges to NIH supported projects.

NIH project support is estimated to offset $50 of the cost per unit ($40,000/800).

The net direct cost charged to the NIH project using the service would be $60 ($110-$50)

Or the NIH support could be directed to fully fund the service until funds are exhausted. In this case NIH support could cover 363 units ($40,000/$110) (F&A cost to NIH grants would be charged at the negotiated F&A rate)

- Example 3: Any type of NIH grant uses a core facility and is charged all or a portion of the allowable direct cost of the service.

Note: In each of the above examples, it is expected that the NIH grants would reimburse the F&A costs associated with the direct costs charged to the projects for these core facilities in accord with the provisions of the applicable indirect cost rate agreement that is negotiated by the Federal cognizant agency.

c. Are cores limited in the types of costs they may recover on an NIH grant?

A core facility's cost may be limited by the terms of award, and in general, may only recover the allocable, allowable and reasonable direct cost (consistent with applicable Federal cost principles) of providing a service to an NIH grant. This is the case when the core's infrastructure costs, commonly referred to as Facilities and Administration (F&A) or also known as indirect costs, will be recovered by the application of the institution's negotiated F&A rate to the allowable direct charges to the grant. However, there may be instances when a portion of the core's costs include items often associated with facilities and administrative costs, but are not included in the costs recovered by application of the negotiated F&A rate. In these situations, it may be appropriate to include the core's costs associated with those F&A costs in the unit cost charged to NIH grants. One example of such treatment is when a core facilities costs include allowable depreciation costs for non-federal equipment utilized by the core and additional operations and maintenance expenses associated with this equipment such as service and repair contracts and others expenses for such equipment. (If these costs were not included in the depreciation or O&M costs recovered by the application of the F&A rate, then including them in the core facility would not represent an unallowable duplication in cost recovery.)

d. How should specialized service facilities (SSF) be considered in the context of these FAQs?

Some core facilities may have unique requirements which could generate a disproportionate share of F&A burden or present other extraordinary circumstances where it may be in the best interest of the Federal government and the institution to establish an alternate costing arrangement) (see A-21 J.47.d and A-122 (Attachment B 46.)), which may be termed a specialized service facility and recover both direct and allocable indirect costs in the fees charged to users. The applicable cost circulars do not specify an annual amount or other threshold for determining this or another alternative treatment.

Accordingly, these FAQs are not generally applicable to SSF and institutions should consult with their cognizant Federal agency when considering such matters. Animal research facilities should utilize the Cost Analysis and Rate Setting Manual for Animal Research Facilities (5/2000) (http://grants.nih.gov/grants/policy/air/rate_setting_manual_2000.pdf) when determining rates for their services.

e. How should costs be allocated when a core facility offers multiple services?

Within the provisions of applicable cost principles institutions have flexibility in defining core facilities. It is possible for an institution to group multiple types of instruments/services under a centralized core infrastructure. Such an organizational structure may spread costs among subcomponents of the central core (such as a mass spectrometry component and a microscopy component). For example, if a core facility provides a range of integrated services for a generally overlapping

set of investigators (e.g., RNA-sequence sample preparation and next generation sequencing data generation), then one service in a core can support the services of another (i.e., the cost recovery from one service can recover the cost of another service integral to the service of the core; provided the institution has in place controls necessary to assure expenses are allocated on a reasonable and consistent basis in accord with Federal cost principles, are not double billed, and rates are reviewed and adjusted to address over/under recoveries).

f. Can one core support another?

Generally, no. The charges for one core can't directly subsidize the implementation or operations of another core. However, if a core benefits or utilizes the services of another core; then appropriately determined and documented charges can be incorporated into the charges of the core utilizing the services. The rates for a service should include only the costs of that service, and investigators should be charged only the cost of the service that they are using.

g. How should institutional fiscal contributions to the operation of the core be managed and described in an application to NIH?

As noted in these FAQs, amounts charged to NIH grants for core facilities need to be managed to ensure that over a reasonable period of time the amounts charged do not exceed the actual costs of the core facility.

In general, most institutions will manage these charges to ensure that the institution is not required to cover under recoveries of a core facility. However, the cost circulars do not require that the core as a whole break even, only that the cost allocations are consistent with the Federal cost principles and the charges for services are reviewed and adjusted as needed to assure that there is no over-recovery/overcharge to federal projects.

Accordingly, the institution may decide to utilize institutional funds to support the operation of a core facility.

If the institution decides to formally commit its funds to the operation of the core facility, this support should be included in the requested budget for support of the core by describing the support (for example, salaries for staff who will work on the core but will not be charged against NIH funding) with either no costs requested, or less commonly, as the "non-federal share". These commitments are considered "voluntary committed cost sharing" when the project is awarded.

If the institution decides not to formally commit its funds to an NIH project it should not include these items in the requested budget submitted for NIH grant support, and further, should not identify them as a non-federal share. Absent such formal commitments, these costs would not be considered "voluntary committed cost sharing" when the project is awarded.

2) Charges to NIH Supported Users

a. How should core facilities set charges for users – particularly for use on federally funded projects?

Core facilities should set user fees based on actual costs and actual usage.

b. What are the considerations for determining appropriate charges?

Summarizing the requirements of applicable cost principles (see A-21 J.47. and A-122 (Attachment B 46.))**:**

The costs of core facility services must be charged directly to the applicable awards in the form of user fees, charges or rates.

- Rates must be based on actual usage of the services.
- The schedule of rates should be established using a documented method.
- Rates may not discriminate against federally supported activities of the institution, including usage by the institution for internal purposes.
- Rates must be designed to recover only the aggregate costs of the services. The costs charged for providing each service from a core facility generally consist of its direct costs only (with exceptions, for example, as those noted in FAQ 1.c).

- Rates shall be reviewed and if required adjusted at least every other year, and shall take into consideration any operating deficit or surplus of the previous period(s).
- Records must be retained and made available to federal officials as required by 45 CFR 74 .53.

c. What are typical allowable costs for determining rates?

Typical allowable costs may include:

Labor (salary and fringe for staff)

- Operating supplies and materials
- Service contracts for core equipment
- Depreciation on non-federally purchased equipment (not included in the F&A cost pool)

This list is not comprehensive. Other costs that support the day-to-day operations of the core facility, excluding unallowable costs (see d. below), are allowable for determining rates. Refer to OMB Circular A-21 Section J or the other cost principles as applicable for more details.

d. What are types of costs that are unallowable or excluded for determining rates?

Some examples include:

- Advertising (exclusive of exemptions deemed allowable in applicable cost principles, e.g., personnel recruiting)
- Alcoholic beverages
- Bad debts
- Contributions and donations
- Entertainment expenses
- Fund raising
- Public relations

This list is not comprehensive. Refer to OMB Circular A-21 Section J or the other cost principles as applicable for more details.

e. What is an aggregated cost?

Aggregated cost is a term used in OMB Circulars A-21 (J.47.b. (2)) and A-122 (Attachment B 46.) for specialized services facilities with a requirement that charges only recover the aggregated cost of each service normally of both its direct costs and its allocable share of all F&A costs.

f. Is it acceptable to estimate costs in determining initial rates that will be charged for new services?

Initial rates may be developed based upon estimates of how actual costing will accrue and apply. These rates subsequently should be adjusted to reflect actual costing as required by the applicable cost principles.

Some key principles include:

- Institutions may group homogeneous costs in order to cover those cases where it is not practical to itemize costs. For example: instead of itemizing each pipette tip and test tube used in providing each service every time it is performed, it is acceptable to determine an average or estimated consumable supply cost that will apply across all users of a given service.

- The method for estimating costs should be applied consistently within the core and without discriminating against any one user.

- The method and details involved in any cost estimate must be documented and available for review by the institution or the funding agency as appropriate.

g. How should core facilities recover F&A costs from users?

F&A costs associated with a core facility should be recovered in a manner that is determined by the policies of the institution, consistent with applicable federal requirements and guidelines including the applicable indirect cost negotiation agreement. This includes necessary steps to ensure that there is no duplication of F&A cost recovery – i.e., F&A costs included in a service rate cannot be included in the F&A rate, and conversely, those F&A costs not included in a service rate may be included in the F&A rate.

3) General core operating principles

Effective practices for a core facility ensure that:

- The costs of providing service are allowable, allocable, consistently applied and reasonable.

- The rates established to recover these costs are documented and systematically evaluated against actual costs and revised on a regular basis to reflect actual costs (see A-21 J. 47 and A-122 (Attachment B 46.)).

- Rates are charged to internal institutional users on a consistent basis, regardless of funding source(s) consistent with the concept of "one service, one rate"

a. Can a core facility purposefully accumulate profit from its charges to internal users?

Generally, no. Any operating surplus for charges to internal institutional users must be applied to reduce the rates charged. It is not appropriate to purposefully "bank" funds for expenses that may be incurred in future years. In general, core facilities are expected to operate in a manner that allows them to recover only the cost of providing service.

See FAQ 5.a. for a discussion of program income for charges to external users.

b. Can a core use an operating surplus to purchase equipment?

The acquisition cost of new equipment cannot be funded with an internal operating surplus of a core or other recharge center.

Acceptable methods of acquiring equipment for a core facility include:

- Recovery of existing equipment depreciation expense in user fees. The accumulated credits from this portion of the service rates can be used to fund the purchase of new equipment.

- Institutional or other non-federal funding

- Shared instrumentation grants or other grant programs designed to establish or support shared resources. Note: the cost of equipment supported from these programs, or other federally funded programs, cannot be recovered as depreciation in the rates applied by the core facility.

- Program income which may accrue on NIH grants.

- Lease or rental of equipment.

See the Depreciation (FAQ 6) for more information.

c. My core provides a project level service that may take months to complete, and may be subject to change in scope or cost as the project moves forward. How can I develop an acceptable system of custom charges?

It is understandable that at a project level, there may be services for which it is difficult to determine a standard rate to charge per project or if the time required to complete a service would unreasonably delay cost recovery if charges were delayed until final completion. In these circumstances institutions may establish (1) definable billable units of service, or (2) set up accounts to accumulate costs that can be charged as a specific project cost as they are incurred. These charges whether a billable unit or from an institutionally defined subaccount should be established according to institutional policy and are subject to applicable federal cost principles. These approaches provide two options to ensure timely recovery of costs incurred. Other methods also may be acceptable provided they are in accord with the terms of award, including compliance with applicable cost principles.

d. How often should my core bill for usage?

Billing cycles are established by institutions and in general can be expected to occur at regular intervals to provide for timely and accurate accounting and cost recovery. While institutions may opt to bill on a monthly basis, other cycles and approaches can be adopted as appropriate. Accordingly, billing cycles of the core facilities at an institution need not be synchronized or occur with the same frequency.

e. Can my core bill now for work that will be performed later?

All billing and cost recovery must be based on services provided (i.e., after service has been provided and expense has been incurred). Billing in advance of the work or receipt of pre-payment is not allowable. However, institutions may choose to divide the work into smaller billable units in order to appropriately recover incremental costs for long-term project work on a timely basis. For example, if a core needs to purchase specialized reagents or supplies to perform work for a specific project, these expenses may be charged to the user once the purchase has been made, with separate charges for completion of later work.

f. If a core director or other staff scientist is awarded a shared instrumentation grant, may the PI of that grant and other co-PIs be charged discounted user fees as a benefit of the award?

User fees that have been appropriately developed will not include any costs associated with federal support provided for acquisition of the new shared instrument supported by a NIH grant or other Federal project. So, there is no basis for a discount or credit to be applied.

g. Can fee schedules cap the amount charged to a user in a particular time period?

Generally, no. Fee schedules that cap charges at a certain dollar amount per month if more than a certain number of hours or units are used are not consistent with applicable cost principles unless the institution or some other non-Federal funding supports the difference between the allocable cost and the amount charged to a heavy user. If appropriate for a particular facility, it may be possible to create fee schedules that have different charges depending on timing and level of usage as long as the charges are determined and consistently applied in accord with applicable Federal cost principles.

4) Accommodating Different Types of Rate Payers

a. Where do funds to pay core facility rates come from?

Funds to pay user charges can come from a variety of sources: internal users (e.g., federal and non-federal grants and contracts, institutional funds, other sources); and external users (e.g., third parties, etc. – see FAQ 5). . In general, the fees collected on behalf of each internal user of a specified service should be the same. However, the amount charged to the user may vary according to the type of user, as long as the difference is made up from some other source or the institution will be required to absorb the difference. See FAQs 3) and 5) for additional references.

For example, a senior PI in a tenure track position with external support may be required to pay the full charge for a service provided by the facility. However, net charges to junior faculty might be lower or waived if some other source of funding (e.g., an institutional or other fund) will make up the difference. Net charges to members who participate in a particular sponsored project may also be lower than the established price schedule, as long as the difference is charged to the sponsored project (if allowable) or other appropriate source of funds. In all examples, the same cost for each unit of usage must be allocated for that usage. It is not allowable to offset lower rates to some users by applying higher rates to other users.

Proper documentation and accounting for the rates paid by all users will mitigate concerns related to apparent differential

treatment. Any appropriate accounting methodology that ensures that federally funded users are not charged more than cost or discriminated against is acceptable if consistent with applicable Federal cost principles.

b. How can my core acceptably bill usage to users with multiple federal awards and funding agencies?

Institutions may have investigators funded by multiple awards and federal agencies. Individual awards and federal funding agencies, while all applying Federal cost principles, may result in different service needs or incorporate slightly different administrative and fiscal allowances as terms of a grant award. While rates for federal users should be consistent when the same service is applicable to multiple federal awards and funding agencies, in order to accommodate situations where there are different service needs, a core may develop separate documented service lines to meet specific program requirements or opt to account for these activities in separate institutional defined subaccounts as costs are incurred for each project. Each of these service lines or subaccounts must be managed in accord with the applicable cost principles and be adjusted to reflect actual cost as described above. Other approaches which are in accord with applicable federal cost principles and the terms of award will also be acceptable.

c. Is it allowable for core facilities to be subsidized for particular sets of users or grants?

The costs associated with use of the core facility must be allocated on a consistent basis for all users. However, some grant programs may provide resources to stimulate research for a particular disease or for the purpose of assuring the stability of critical shared resources for particular constituent groups of investigators at an institution (e.g., members of a Center for AIDS Research, a Cancer Center Support Grant, or a Clinical Translational Science Award etc.). In these cases, individual usage may be subsidized by the project likely following one of the models described in FAQ 1.b. For example, NCI funds provided under a Cancer Center Support Grant specifically support cancer research by Cancer Center members. Thus, resource usage by Cancer Center members may be supported in whole or in part through appropriate charges to the Cancer Center Support Grant award. While the rate charged for the cost of a service cannot be different for different users, Cancer Center members will be charged only the remaining (non-subsidized) portion of the standard rate.

d. Are there any circumstances when one federally-supported project may be charged a reduced or discounted rate compared to another?

Except for the example provided above (FAQ 4.c.), rates used to charge the cost of the service must be charged to internal institutional users, on a consistent basis, regardless of funding source(s). Billing rates should not discriminate between federal and non-federal users, including internal institutional activities. The schedule of rates will apply to all users of the core facility on the basis of actual utilization and cannot discriminate against any one segment of the population. However, institutions may consider differential pricing for large quantity purchases, high-volume usage, off-peak usage or other factors. A core may have multiple rates for the same service when those rates are properly established, consistent with applicable Federal cost principles, and the favorable rates are available to all users that meet established criteria.

e. If an investigator acquires an instrument on a project and this instrument is assigned to a core facility, can the investigator's usage be given preferential treatment?

Yes, provided that it can be clearly shown: (1) the preferential treatment does not constitute the equivalent of a reduced rate; (2) preferential treatment for any investigator does not increase the rates for any other investigators, particularly instrument users with federally supported activities; (3) the preferential treatment is clearly defined in operating policies and procedures of the core; and (4) similar terms are available to any investigator willing to donate equipment. Some examples include: **providing priority to samples from the donating investigator in the instrument's sample processing queue; allowing only the donating investigator or members of the donating investigator's group hands-on access to the instrument; and/or allocating a defined percentage of capacity of the instrument exclusively for the benefit of the donating investigator.**

Note: When equipment purchased in whole or part with Federal research grant funds, the title to the equipment generally passes to the grantee (generally with limited authority retained by the Federal funding entity). The grantee has the authority to direct the usage of the equipment. It is expected that the equipment would be available for the research that was supported in the grant that supported the cost of acquiring the equipment.

5) Non-institutional usage

a. Can a third-party, for example another biomedical research institution, educational institution, or commercial (for-profit) organization be charged at a different rate from the institutional rate?

Yes. NIH encourages the sharing of available research resources including core facilities at reasonable rates. So, charges to external users will need to be appropriately established including application of any applicable Facilities and Administrative (F&A) costs and may include an additional fee in excess of the cost of the service. However, net fees should remain reasonable for the service provided to facilitate the provision of access to the core facility to external users.

b. How should an additional fee in excess of the cost of service generated by a third-party be treated?

Such recoveries in excess of full costs may need to be treated as program income if the costs of the core facility charged to external users are supported on any NIH grant and if other conditions pertinent to recognizing program income are applicable. If NIH funding support does not cover costs of the core service facility, institutional and federal policy will determine handling of any identified income.

Program income is addressed in the NIH Grants Policy Statement (10/12) http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch8.htm#_Toc271264957 "Program income is gross income—earned by a grantee, a consortium participant, or a contractor under a grant—that was directly generated by the grant-supported activity or earned as a result of the award. Program income includes, but is not limited to, income from fees for services performed; charges for the use or rental of real property, equipment or supplies acquired under the grant; the sale of commodities or items fabricated under an award; charges for research resources; registration fees for grant-supported conferences, and license fees and royalties on patents and copyrights. (Note: Program income from license fees and royalties from copyrighted material, patents, and inventions is exempt from reporting requirements unless otherwise specified in the terms and conditions of award.) Specific questions about program income should be directed to the assigned grants management specialist for an NIH award."

6) Depreciation

a. What is depreciation?

Depreciation is an accounting tool that is used to recognize and apply the cost of acquiring an instrument or piece of equipment across its useful life. Purchases below an institutionally defined threshold are generally treated as supplies, which are expensed when purchased, and are not depreciated.

b. How is depreciation used by core facilities in setting rates?

Appropriately applied depreciation would be considered an allowable cost of providing service, with some limitations discussed below.

c. Can depreciation charges be applied to the cost of instruments and equipment purchased with Federal funds, for example the ORIP S10 Shared Instrumentation Grant program?

No. If the acquisition cost of the equipment was fully supported by federal funds, the core cannot recover depreciation expenses in its user fees, because inclusion would result in duplicate charges when used on a Federal project (first in the support for acquisition and again if depreciation costs are included in usage charges). However, if some of the funds for an equipment purchase were from non-federal sources, depreciation charges for that portion of the equipment purchase can be incorporated in the determination of charges for the core facility.

d. Must depreciation charges be applied to the cost of instruments and equipment purchased with non-Federal funds?

For instruments and equipment purchased with non-Federal funds, institutions can allocate or not allocate depreciation charges in its usage fees on a discretionary basis, based on sources of funding and institutional policy. Another option might be to include the depreciation in the F&A cost pool of the institution. In all cases, if the equipment qualified for capitalization, and therefore depreciation was recognized, it would not be appropriate for the institution to treat the equipment purchase expense as a 'current expense' of the core facility when determining charges.

Inquiries

Please direct inquiries to:

Inquiries regarding individual grant awards may be directed to the Grants Management Specialist identified on the Notice of

Award.

General inquires in regard to this Notice:

David Curren
Office of Policy for Extramural Research Administration
National Institutes of Health
6705 Rockledge Drive, Suite 350
Bethesda, MD 20892
Email: CurrenD@mail.nih.gov

Weekly TOC for this Announcement
NIH Funding Opportunities and Notices

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

Shared *vs*. Diabetes-specific Cores

What's in the most recent RFA

- Diabetes Research Centers are designed around research cores that provide shared, specialized technical resources and/or expertise that enhance the efficiency, productivity, and multidisciplinary nature of research performed by Center-affiliated investigators.

What's in the most recent RFA (2)

- Particular emphasis should be placed on services that support and foster interdisciplinary, integrated and translational approaches (). Preference will be given to core support services that are not readily available or cost-effective when supplied from commercial sources, and techniques or technologies that may be technically challenging or require specialized expertise, equipment or infrastructure. Proposed Diabetes Center research cores may be an institutional shared research core. In such cases, the research core support provided by the Diabetes Center should be proportional to the use of the institutional research core by Diabetes Research Center members.

How Are Cores Shared With Other DK Centers Supposed to Interact?

- Participants in the Diabetes Center program are encouraged to become fully integrated into, and synergistic with, other NIDDK- and NIH-funded Core Centers within their institutional setting.
- This includes () any related NIDDK-funded Center programs such as the Nutrition Obesity Research Center () and the Centers for Diabetes Translation Research

Core Breakdown 2013

- Diabetes-specific = 25
  - 15 Animal Phenotyping Cores
  - 5 Human Studies Cores
  - 5 Islet Cores
- Shared = 56
  - 10 Assay cores
  - 5 Flow Cores
  - 14 Histopath Cores
  - 14 Genetics/genomics Cores
  - 13 Transgenic Cores

What happens in practice

- Cores are reviewed by experts on technical matter
- Not all reviewers are familiar with the scope of DRC-related work
- Different reviewers weigh shared vs. diabetes-specific cores differently
- Sharing with other DK Centers is mostly frowned upon by reviewers, even as it's specifically encouraged by program
- Evaluation of costs borne by DRC also a gray area
- Specific evaluation of how individual Cores fit the overall mission of individual DRCs is not commonly done

Considerations for upcoming review

- Even diabetes-specific cores cannot be sustained by DRC alone
- Sharing with other DK Centers should be rewarded, not penalized
- DRC is the only vehicle available to diabetes researchers to access institutional cores at an affordable price
- Today's "diabetes-specific" cores will be tomorrow's institutional cores
- Should "diabetes-specific" vs. "institutional" even be a review criterion?
- Growing emphasis on business plans and usage metrics overshadows the key mission of Cores: what key advances have they supported?
- How is the specific mission of the individual DRC furthered by a Core?

What is to be done?

- Become more “prescriptive” in the RFA?
- Encourage reviewers to weigh core results over process/business plan?
- Do away with a formal “Core” structure, and let Centers provide two services: diabetes-specific *vs*. institutional/shared?
- ??


JETS
SHARKS
DRCs
NORCs

Institutions with DRCs & NORCs

Shared Cores

- Boston Area
  - Transgenic
- Columbia
  - Hormone & Metabolite
- Johns Hopkins/Univ Maryland
  - Molecular & Translational Genomics
- Univ of Alabama
  - Aminal Physiology/Small Animal Phenotyping
  - Human Physiology/Metabolism
- Univ of Michigan
  - Animal Phenotyping
- Washington Univ
  - Mass Spectrometry/Biomolecular Analysis
  - Diabetes Models Phenotyping/Animal Model

No Shared Cores

- Univ of Washington

Rationale for Shared Cores

- Extends impact of expertise, provides consistency
- Adds economy of scale and avoids duplication of services--both lower costs
- Expansion of user volume can lower costs
- Leverages limited support from each program

Potential Pitfalls of Shared Cores

- Diabetes (T2DM) and obesity lie on a continuum of metabolic disease that may be difficult to parse
- Some in research base may be appropriate members of both types of Centers—how do we attribute use?
- Reviewers must be familiar with program designs that address RFA

Important Principles

- All research base members benefit from same subsidy whether member of one or both centers
- For each project, science should dictate the assignment of support
- Design/management of coordinated cores should be transparent to program, reviewers, research base

DISCUSSION

Penn Medicine

Penn Diabetes Research Center
NIH DK19525:
Regional Metabolomics Core at Princeton

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

2013 Annual DRC Directors Meeting

Bethesda, MD

May 29, 2013

The Penn Diabetes Research Center

- P30 DK19525: Year 38 began April 1, 2013
- There are ~136 current members of the Penn DRC
- Cores:
  - Administrative (Lazar)
    - Pilot and Feasibility Grant Program (Doris Stoffers)
    - Academic Enrichment (Kendra Bence)
    - Translation (Mike Rickels)
  - Biomarkers/RIA (Stephen Master)
  - Functional Genomics (Klaus Kaestner)
  - Islet Biology (Franz Matschinsky and Doris Stoffers)
  - Mouse Phenotyping, Physiology, and Metabolism (Rex Ahima)
  - Transgenic (Nancy Cooke)
  - Viral Vectors (Julie Johnston)

2011 RFA

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	**Diabetes Research Centers (P30)**
Activity Code	P30 Center Core Grants
Announcement Type	Reissue of RFA-DK-08-008

1) To broaden the scope and reach of current research core services, a Center may propose to serve a wider scientific community on a geographic or national level through the establishment of a Regional/National Shared Resource Core that is located at a different institution. Such a Regional/National Core may not be established with an affiliated hospital of the applicant organization; such an arrangement would be considered an institutional, rather than a regional/national, core for the purposes of this FOA. If the Center is primarily located at an affiliated hospital, core(s) based at another affiliated hospital of the same academic institution will not be considered Regional/National Shared Resource Cores. With a regional or national core located at a different institution, the Center will service a specific research base that is expanded beyond investigators at the academic institution and/or affiliated hospitals where the Center is primarily located. Support for the expansion of the Center P&F program to investigators at the institution where the Regional/National Shared Resource Core is located may also be requested (see below).

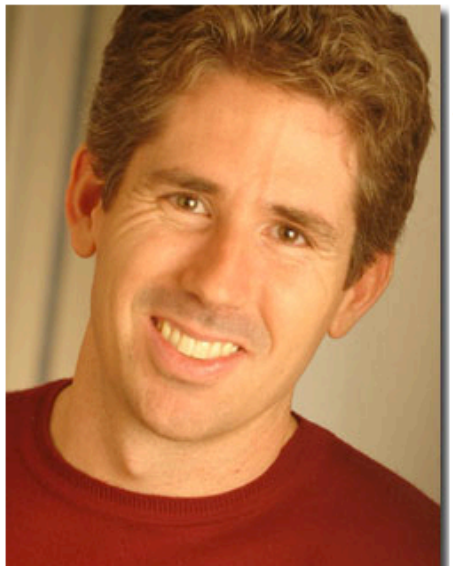
Josh Rabinowitz at Princeton

"Metabolomics Core" presented to Penn DRC EAB: March 16, 2011

DEPARTMENT OF MOLECULAR BIOLOGY
PrincetonUniversity

in Molecular Biology search

About | Faculty & Research | Postdocs | Graduate | Undergraduate | Resources | Associated Programs | Calendar



Joshua Rabinowitz

Rabinowitz Lab Webpage
joshr@genomics.princeton.edu
Carl Icahn Lab-241
Phone: 609-258-8985

Faculty Assistant:
Marybeth Fedele
Phone: 609-258-7058

Towards a Holistic Understanding of Cellular Metabolism

- Actively collaborating with Morrie Birnbaum
- Several interested DRC investigators

1) Mass measurements of key metabolites

2) Flux studies using heavy isotope

- Incorporation into lipophilic metabolites to measure fat metabolism
- Enrichments in soluble metabolites to measure carbohydrate metabolism

Propose: $250K for core including pilot grants to incent core use and collaboration

2012 DRC Renewal

- Submitted June, 2012
- Regional Metabolomics Core directed by Josh Rabinowitz at Princeton

Penn Medicine

March 6, 2013: Presentation to the Penn DRC EAB

Inter-institutional U Penn-Princeton Metabolomics Core

Joshua Rabinowitz, M.D., Ph.D., Director

Wenyun Lu, Ph.D., Technical Director

Objectives

To provide DRC members with state-of-the-art metabolomic services, including

1) Targeted metabolome quantitation
2) Flux measurement
3) Novel metabolite discovery

Services

1) Water-soluble metabolites from adherent cell culture

2) Water-soluble metabolites from suspension cell culture

3) Fatty acid extraction from plasma/serum

4) Water-soluble metabolites from plasma/serum

5) Tissue extraction for water soluble metabolites and lipids

How do we facilitate proper usage?

Example SOP text:

Consideration: Some metabolites can degrade rapidly. In addition, the metabolic pathways we're looking at (glycolysis, TCA cycle, etc) are easily perturbed during the sample prep. Therefore, be sure to follow the protocol precisely.

Please discuss with Wenyun Lu (wlu@princeton.edu) to schedule the exact date of the experiment 10-days IN ADVANCE so appropriate machine time can be reserved.

Step 1: ~48 hours prior to metabolite collection: switch to medium with dialyzed serum (SH3007903 from Fisher) and keep using it for the duration of the experiment. In addition, the medium should contain no sodium pyruvate (we use mediatech cellgro DMEM, MT-10-017-CV, Fisher Scientific).

Step 2: 1-2 hour(s) prior to metabolite collection: aspirate of all media, and replace with 5 ml of new, respective media. PARTICULARLY IMPORTANT FOR FLUX STUDIES.

How do we encourage usage?

1. Rabinowitz talk at Penn Diabetes Spring Symposium (March 28, 2012)

2. Present poster at Penn Diabetes Spring symposium (March 6, 2013)

3. Updated website: (including SOPs, links to capabilities and papers, etc)

http://www.med.upenn.edu/idom/derc/cores/metacore/index.html

4. Pilot awards

First Metabolomics Core Pilot

High throughput profiling of pancreatic islet metabolites

Nicolai Doliba, Scott Soleimanpour,
Franz Matschinsky, Doris Stoffers
(UPenn)
Wenyun Lu, Josh Rabinowitz
(Princeton University)

Collaboration between DRC Islet Cell Biology Core and the Regional Metabolomics Core at Princeton to optimize methods for metabolomic profiling of primary islets.

Aims:

1. Optimize metabolite extraction in Min6 insulinoma cells (Stoffers lab)
2. Continue optimization in wild type mouse islets (Doliba)
3. Apply the methodology to an experimental model of altered mitochondrial function in bets cells (Soleimanpour)

What is current usage?

SERVICES RENDERED (as of March 6, 2013 EAB meeting):

A. Water-soluble metabolite quantitation by positive mode LC-MS/MS
B. Water-soluble metabolite quantitation by negative mode LC-high resolution MS
C. Water-soluble metabolite quantitation by negative mode LC-MS/MS
D. Fatty acid quantitation by negative mode LC-high resolution MS
E. Metabolite quantitation by GC-MS/MS
F. Flux analysis with isotope tracers
G. Unknown/unexpected metabolite discovery
H. Consultation

User	PI	Period of Performance	A	B	C	D	E	F	G	H	Actual use and comments
Howerton	Bale	1/2013	X	X		X					50 samples from mice
Chen	Birnbaum	4/2012-5/2012		X	X	X		X			150 samples from mice
Lu	Birnbaum	4/2012	X	X							20 samples from mice
Miller	Birnbaum	6/2012-7/2012	X	X	X	X		X			80 samples from cell culture
Spiegelstein	Birnbaum	8/2012-1/2013	X	X	X	X	X	X			120 samples from mice (tissue+serum)
Lee	Choi	10/2012								X	
Gerhart-Hines	Lazar	9/2012-11/2012	X	X	X				X		120 samples from mice (tissue+serum)
Sun	Lazar	7/2012	X	X							20 samples from mice
Tuteja-Stevens	Rader	11/2012								X	
Ferguson	Reilly	11/2012								X	
Soleimanpour	Stoffers	8/2012-9/2012	X	X	X						50 samples from cell culture
Stein	Van Dang	9/2012								X	

Penn Medicine

Penn Medicine | Institute for Diabetes, Obesity & Metabolism

60 years 1950 2010 NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

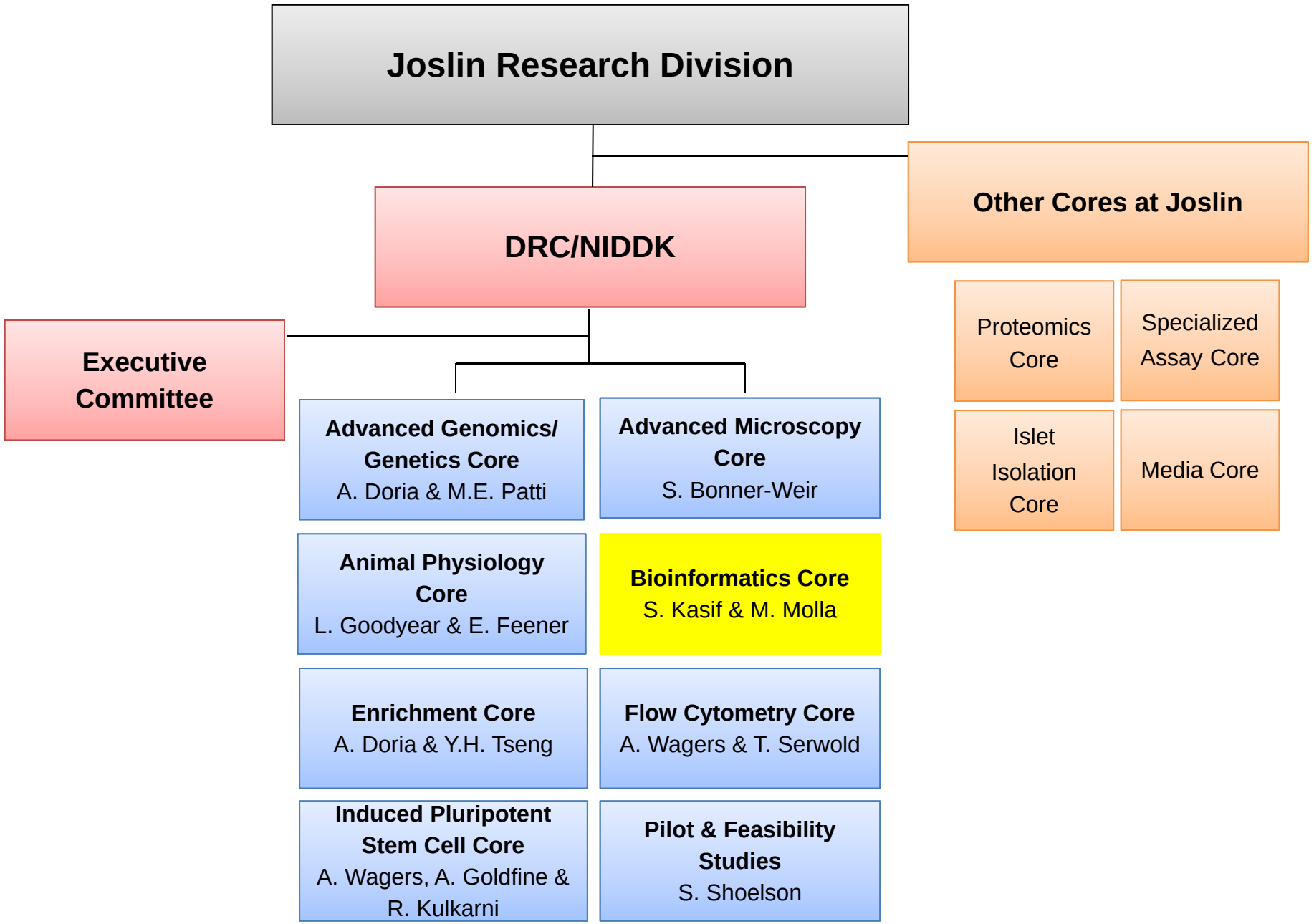
60 Years of Advancing Research to Improve Health

Research supported by NIDDK

DK19525

Diabetes Research Center funded by NIDDK

George L. King, M.D.


Joslin Research Division
DRC/NIDDK
Other Cores at Joslin
Executive Committee
Proteomics Core
Specialized Assay Core
Islet Isolation Core
Media Core
Advanced Genomics/ Genetics Core
A. Doria & M.E. Patti
Advanced Microscopy Core
S. Bonner-Weir
Animal Physiology Core
L. Goodyear & E. Feener
Bioinformatics Core
S. Kasif & M. Molla
Enrichment Core
A. Doria & Y.H. Tseng
Flow Cytometry Core
A. Wagers & T. Serwold
Induced Pluripotent Stem Cell Core
A. Wagers, A. Goldfine & R. Kulkarni
Pilot & Feasibility Studies
S. Shoelson

Boston University / Joslin Diabetes Center Regional Computational (BUJRC) Core

Started: September 1, 2012

Core PI :Simon Kasif, Boston University

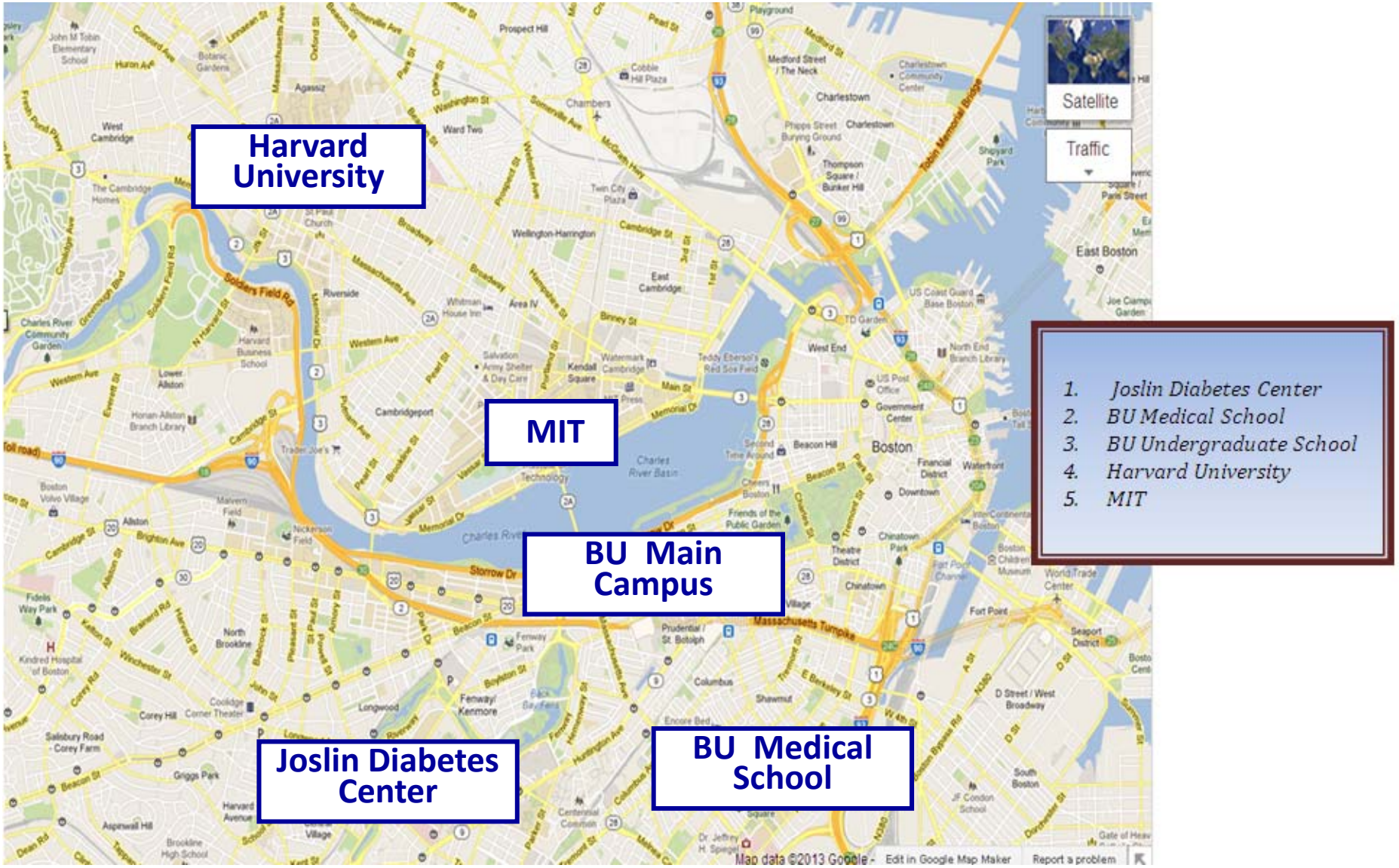
Joslin Director of Bioinformatics : Michael Molla, Boston University and Joslin
Joslin Senior Data Analyst : Dr. Gung-wei Chirn, Boston University and Joslin

Bioinformatics / Computational Biology Service branch provides state-of the-art analysis of high-throughput biomedical data generated from "omic" data and its integration with physiological and clinical data.

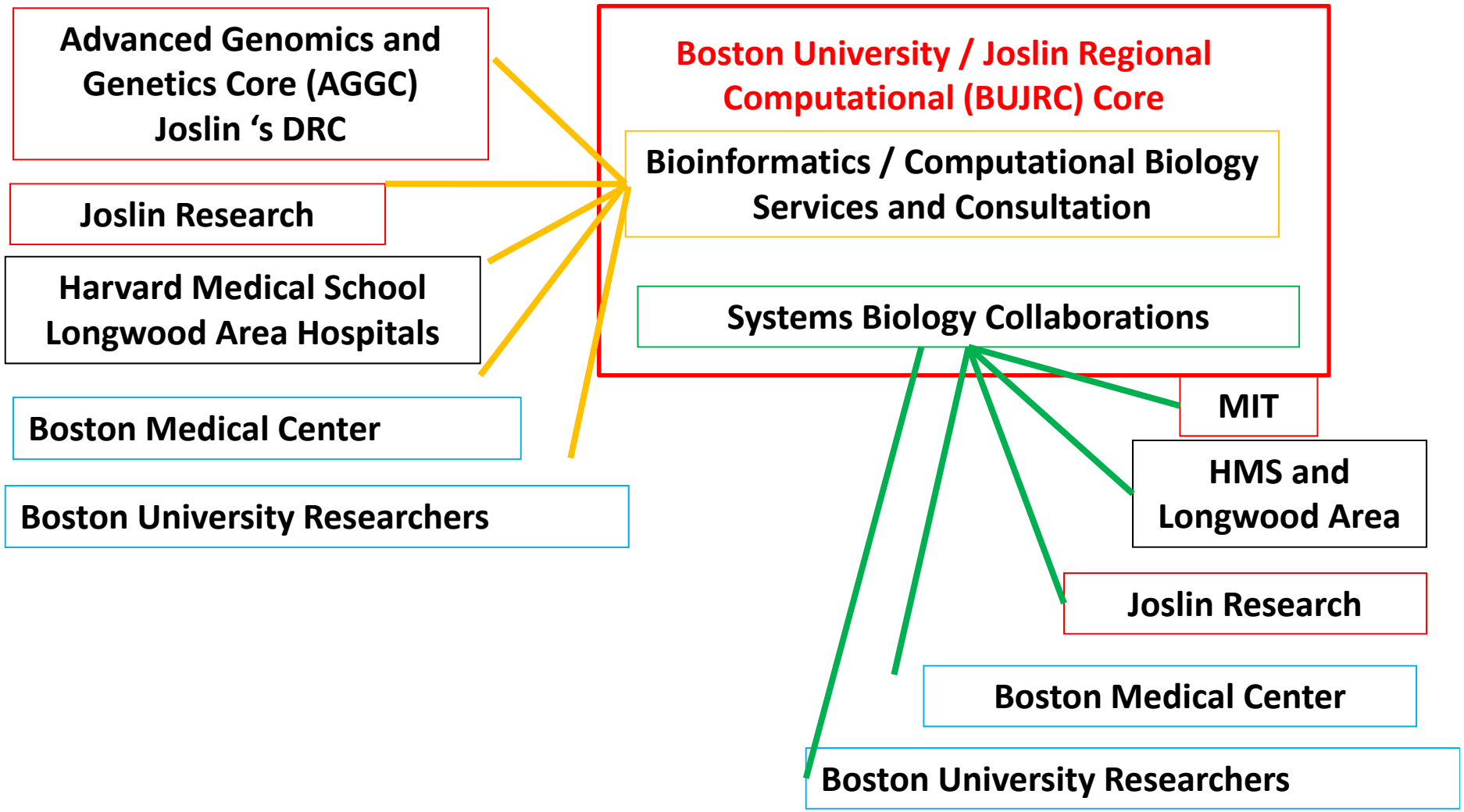
Collaborative Systems Biology/Medicine branch will help to create an environment that incorporates computational systems biology/medicine methodologies into clinically relevant translational and basic diabetes research. This branch includes local faculty with a proven track record of having worked together to produce innovative systems biology and translational research.

Collaborating Institutions

Boston and Cambridge



BUJRC Core Interactions With Area Researchers



Bioinformatics Directors –Joslin: Simon Kasif & Michael Molla

Joslin liaison: C. Ronald Kahn

Senior Data Analyst: Gung-wei Chirn

Collaborators

Jim Collins, PhD (HHMI, McArthur) (BU/BME/WYSS)	Regulatory Network Inference
Charles DeLisi, (BU/BME)	Visant NETWORK Visualization Tool
Bonnie Berger, PhD -- Head Computational Biology,(MIT)	Human Mouse Orthology Tool for mapping mouse studies into human
Zak Kohane, MD/PhD (Harvard/I2B2)	I2B2 software and architecture, Functional Genomics
Daniel Segre, PhD (BU/BME)	Metabolic Network Modeling, Synthetic Lethality, and Epistasis
Alan Herbert, MD/PhD (BU)	- Computational Genetics, Genomics Data Analysis GWAS - Herbert A et al Science. 2006 Apr 14;312(5771):279-83. A common genetic variant is associated with adult and childhood obesity
Tomer Shlomi, PhD (Technion)	Human Tissue Metabolic Network, Metabolic Modeling and Disease
Eric Kolatzyk, PhD (BU)	Network Statistics

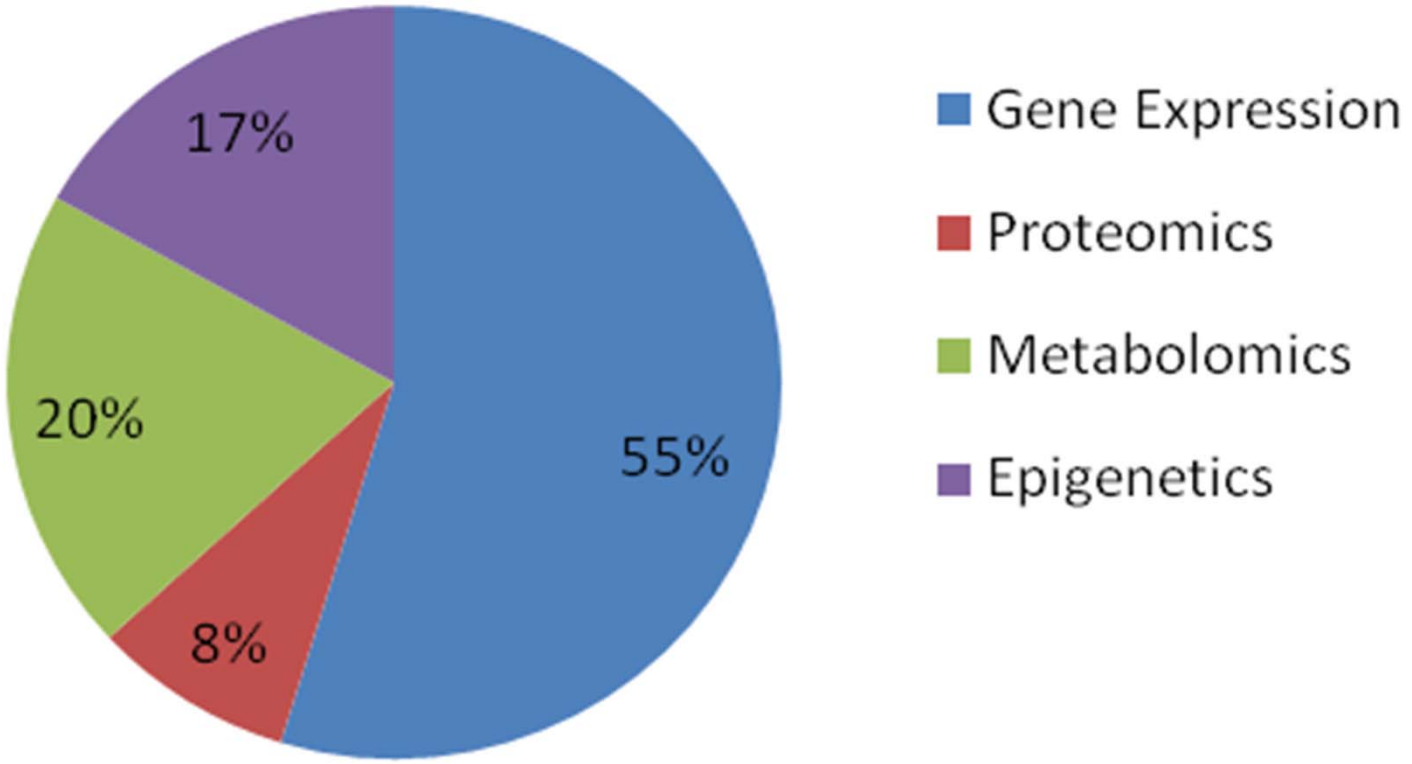
Additional Collaborators: Steven Salzberg (UMD), Jill Mesirov (Broad), Brad Bernstein (Broad), Tarjei Mikkelsen (Broad/Stem Cell Institute)

BUJRC Core – Functions and Expertise

- Next Generation Sequencing
  - RNA Sequencing
  - DNA Methylation
  - Copy Number Variation
  - Sequence Variation
- Microarrays
- Proteomics & Phosphoproteomics
- Metabolomics
  - Mapping Data onto Metabolic Networks
  - Metabolic Network Modeling and Inference
  - Causal Inference of Metabolic Phenotypes
- Network Biology
  - Identifying Genes Associated with Insulin Resistance or other Phenotypes Using Network Methods
  - Building Tissue Specific Metabolic Networks
  - Drug Response / Phenotypic Signatures
  - Identification of Drug Targets and Novel Biomarkers

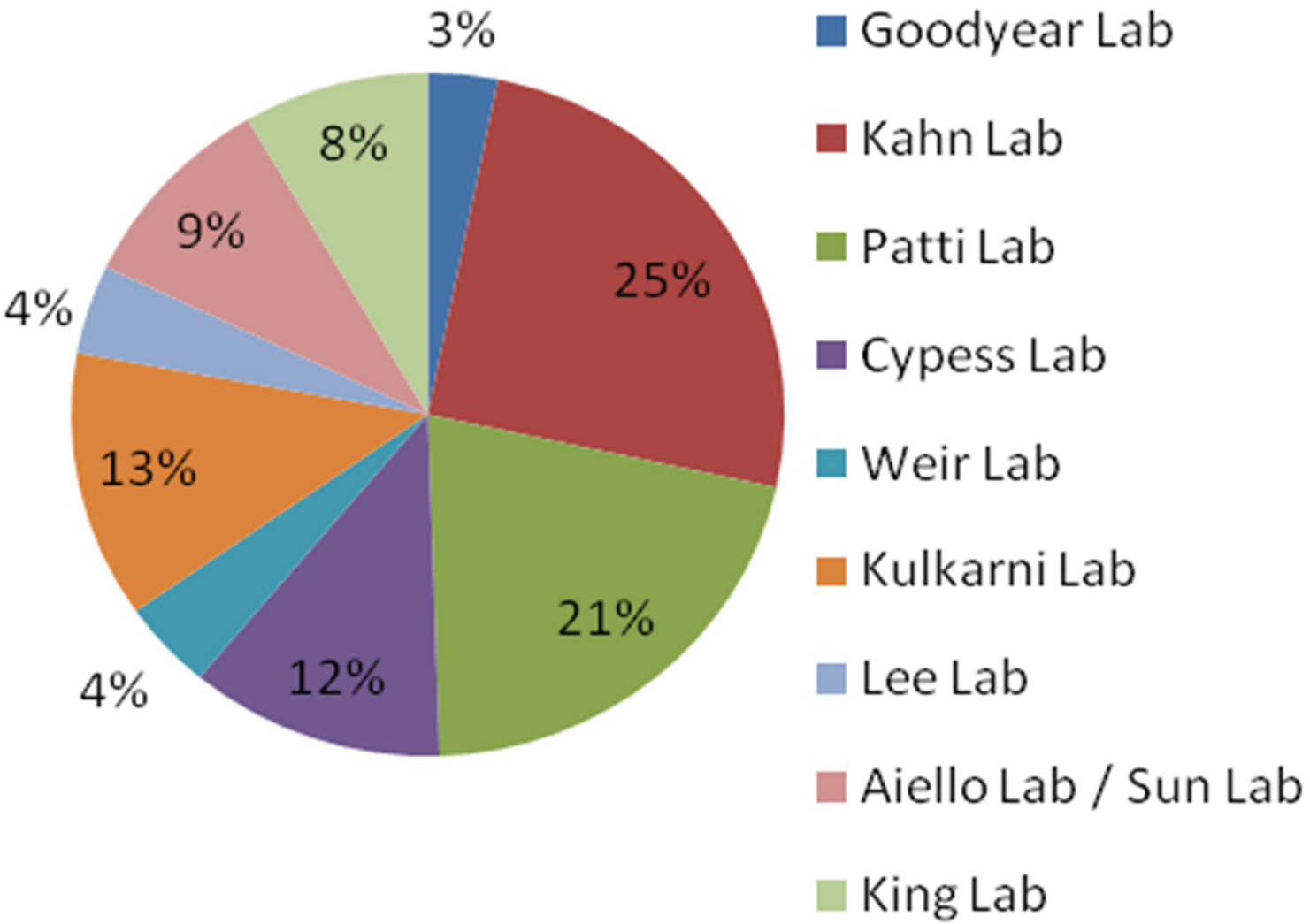
BUJRC Core Activities

October 1, 2012 – April 1, 2013



BUJRC Analyses at Joslin

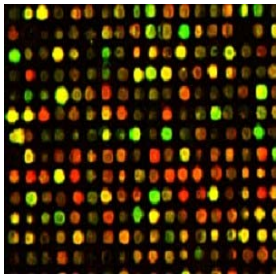
October 1, 2012 – April 1, 2013



Examples of how the BUJRC Core is being used

Epigenomic Profiling of Prenatal Underutrition
Michael Molla/ME Patti

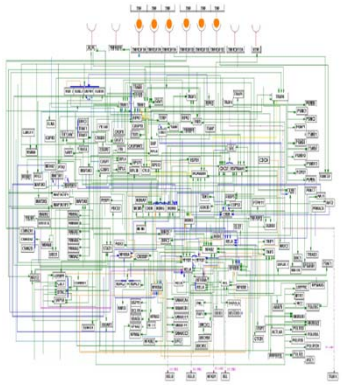
Towards Molecular Network Anomaly Detection:
Detect Networks Commonly Disregulated in Different Mouse models of Insulin Resistance and Human Tissues
Goldfine/Kahn/Kohane/Patti/Kasif



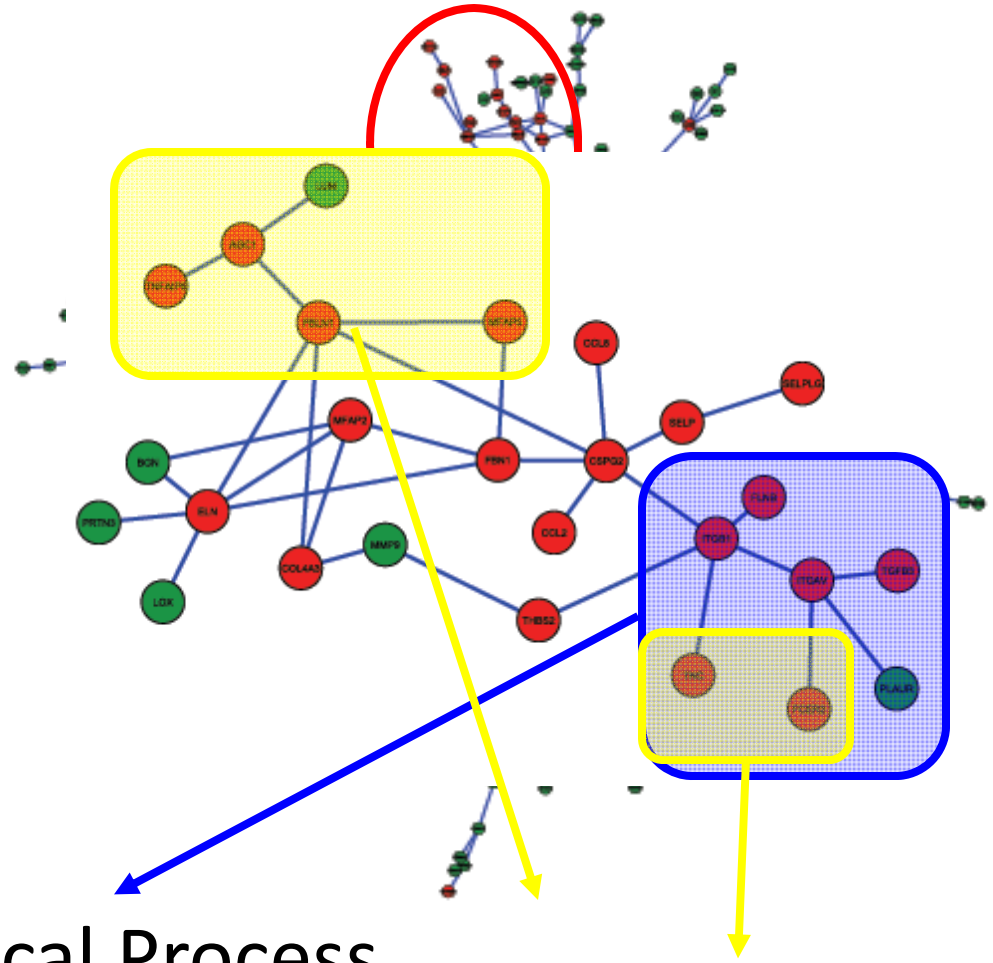
Microarray data

+

=



Protein protein interaction network



Biological Process Network

Molecular Function

The New DRC-Funded BU/JDC P&F Program

- **The initial plan is to fund 3 grants/year, each for $33,000 and of 1 year duration.**

- **Focus of these P&Fs is on systems biology/bioinformatics related diabetes research in the Boston area**

- **Year 1 (2013) of the new BU/JDC P&F program has been initiated:**

  ♦ 15 Letters of intent received from researchers at local institutions excluding Joslin.

  ♦ 11 Full applications received
  - **6 Boston University Medical Center**
  - **4 Boston University**
  - **1 Massachusetts Institute of Technology**

  ♦ 3 Applications funded

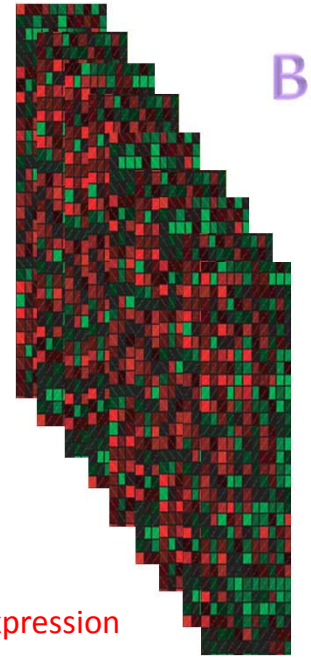
Three Newly Funded BU/JDC P&F Applications

- **Bonnie Berger:** MIT Professor of Applied Math and Computer Science at; Head of the Computation and Biology group at MIT's Computer Science and Artifical Intelligence Lab

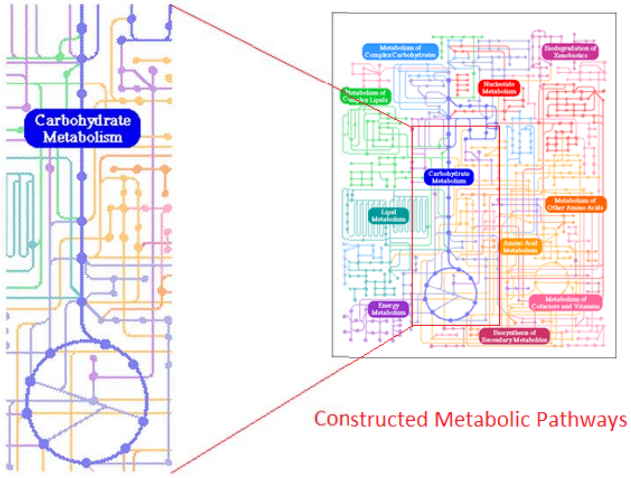
- **Mi-Jeong Lee:** Instructor, Boston University School of Medicine

- **Valentina Perissi:** Assistant Professor, Boston University School of Medicine, Biochemistry

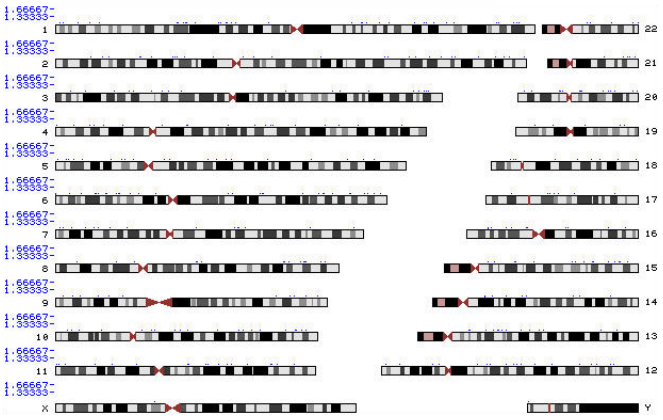
Pilot 1
Data Integration
Bonnie Berger, Senior Investigator
MIT



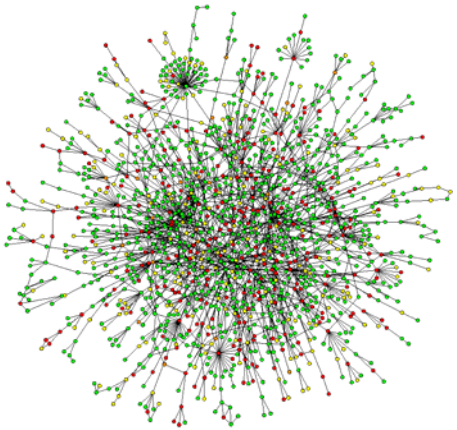
Expression



Constructed Metabolic Pathways



GWAS



PPI network

Integrative Analysis to Discover Genetic Factors behind Diabetes-Bonnie Berger

Abstract

We propose an integrative analysis, covering public datasets as well as potentially novel datasets from Joslin researchers, to identify the pathways and genes involved in diabetes. Sequencing data from diabetes patients has been increasingly accumulating due to the recent advances in Next Generation Sequencing (NGS). We propose to perform an integrative analysis of this data together with metabolomics data to demystify the hidden genetic factors behind diabetes. Our first aim is to construct multiple molecular subnetworks by mapping each diabetes-related dataset (expression, exomic variation, copy number variations (CNVs)) to protein-protein interaction (PPI) networks that involve related genes identified by genome-wide association and family-based analysis. The Berger lab has extensive experience in the statistical and graph-theoretic techniques required for constructing robust networks from such data. Our second aim is to construct comprehensive metabolic network models from patient data. We have recently developed several effective tools to build and analyze putative metabolic network models. Our final aim is to compare the constructed PPI networks and the metabolic network models to identify possible genes of key importance to diabetes. By bringing our expertise in network analysis to the problem, we aim to discover the consensus network of genes and pathways involved in metabolism in diabetes that best agrees with the combined data.

Pilot 2

M.J. Lee (Junior Investigator)
Boston University Medical Center

- **Abnormalities in adipocyte metabolic and endocrine dysfunctions contribute to risk of metabolic diseases, especially type 2 diabetes.**

- **Converting white to ‘brown-like’ or ‘brite’ adipocytes:**

  - has the potential to relieve oxidative stress and normalize the production and secretion of ‘good’ adipokines.

  - could contribute to energy dissipation and hence weight control.

Pilot 2

M.J. Lee (Junior Investigator)
Boston University Medical Center

Goal of the project:
Use a systems biology approach to integrate transcriptome and secretome data to elucidate pathways/networks that distinguish metabolic and endocrine differences between white and brite adipocytes

Pilot 3

Valentina Perissi: Assistant Professor

Boston University Medical Center

G-Protein Suppressor 2 (GPS2) is as a novel coactivator for PPARγ in adipocytes

Define the molecular mechanism of GPS2 regulation of resistin gene expression as a transcriptional cofactor of PPARg.

Determine the subset of PPARg-dependent transcriptional program that is regulated by GPS2 in 3T3-L1 and mouse adipose tissues.

Next Steps For the Core

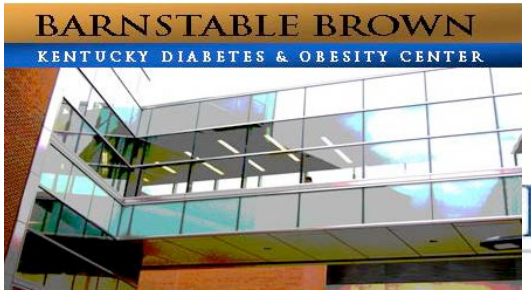
- Current research highlights:
  - Multiple publications in preparation with King, Patti,
  - Publications under review with Cypess Lab, Kulkarni Lab, and Sun Lab.
- Planning “Seminar Series in Bioinformatics at The Joslin” for Spring
- Development of an Integrated Central Database.
- Evaluation of Transformative Platforms.

Washington University in St. Louis

Shared National Resource Component (SNRC)

- Leverage Washington University DRC's experience with P&F program that has funded 166 investigators over 35 years

- Expand program to University of Kentucky and University of Utah
  - Existing scientific collaborations & professional interactions
  - Strong diabetes research efforts, but no DRC
  - Shared focus on diabetic cardiovascular complications



UK UNIVERSITY OF KENTUCKY College of Medicine

UNIVERSITY OF UTAH SCHOOL OF MEDICINE

Program Design

- Design and management mirrors WU program
- Fully integrated into WU P&F solicitation, review and award
- Leadership at WU: Schaffer and Semenkovich
- Leadership from Univ of Kentucky and Univ of Utah Pis

Philip Kern, MD
Prof of Medicine
Dir,CTSA
Dir, Barnstable Brown
Diabetes & Obesity Center
University of Kentucky

E. Dale Abel, D Phil
Prof of Medicine
Chief of Endocrinology
Dir, Metabolism Interest Group
University of Utah

- Requested funds dedicated solely to supporting projects at Kentucky and Utah through subcontract mechanisms

WU-DRC SNRC Progress to Date

University of Kentucky

- 22 apps received/reviewed
- 8 discussed/2 selected
  - Michael Murphy, PhD, Assoc Prof: Diabetes, Alzheimer's Disease and Exercise Intervention
  - Venkateswaran Subramanian, PhD, Asst Prof: Role of macrophage-specific calpain in diabetic atherosclerosis

University of Utah

- 10 apps received/reviewed
- 5 discussed/2 selected
  - Amnon Schlegel, MD, PhD, Asst Prof: Interrogating Liver X Receptor Function with Zebrafish
  - Balamar Ambati, MD, PhD, Prof: COMP-Ang1 for Vascular Normalization and Neuroprotection in Diabetic Retinopathy

DIABETES RESEARCH CENTERS

2013 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

Baylor College of Medicine: http://www.bcm.edu/diabetescenter/

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

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

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

University of Alabama at Birmingham: http://alpha.webcenter.uab.edu/sites/drtc/

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://www.med.umich.edu/mdrtc/

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

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

Vanderbilt University: http://www.mc.vanderbilt.edu/diabetes/drtc/

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

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

NIDDK DIABETES CENTERS
NON-COMPETING RENEWALS
(TYPE 5 PROGRESS REPORT)
INSTRUCTIONS
2012-2013

I. FORM PAGES

- Face page
- Cumulative Budget for Center (PHS 2590 Form Page 2)
- Budget and Justification for each Core (PHS 2590 Form Page 2 for each Core)
- List of **NEW key personnel** followed by their biographical sketches
- **Other support for all key personnel ONLY** (i.e. only for personnel listed as "KEY" in the Notice of Grant Award, or their recent replacements); please verify that "other support" information is current and that effort does not exceed 12 calendar months for any individual.
- **Budget note (2013):** increases due to inflation are NOT allowed [see: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-12-036.html]; for example, budget requests in progress reports that include salary increases (i.e. above FY2012 level of support) will be administratively reduced, and therefore the total amount of funds awarded will be decreased in the non-competing Notice of Award.

CENTER PROCESS MEASURES *(#sII-V)*

II. RESEARCH BASE (1-2 pages MAX for narrative text)

(Table) A. List Current Center Investigators – **list only changes** in membership since last year's report

- New members (name, dept, area of interest)
- Members lost (due to transfer, inactivity or other)

(Table) B. Enumeration & list of

- Publications directly traceable to center activities during the past year (include PMCID#; **see attached format Table from most recent RFA as an example**); Publications during the past year not citing center support should be noted with an asterisk.
- Major changes in research grant support (new and/or ending from prior year)

(Text) C. New collaborative activities

- List & briefly describe any new Center programs, projects, or collaborations that would not have been possible without Center resources (including new collaborations with other DK Center Programs, e.g. NORCs, CDTRs)
- List collaborative publications, if applicable (include PMCID#)

III. ADMINISTRATION + ENRICHMENT CORES (1-2 pages MAX for narrative text)

(Text or Table) A. Activities raising awareness and interest in diabetes research and clinical care at center institutions, locally, regionally, and nationally:

- Center website developments

- Diabetes Research Center-sponsored seminars & symposia (only those sponsored by or supported with Diabetes Center funds)
- Regional and national presentations (list all that were sponsored by or supported with Diabetes Center funds; i.e. presentations of research that was supported by Diabetes Center funds)
- Collaborations with other Diabetes Research Centers, institutions and centers

(Text or Table) B. Activities enhancing diabetes education and training opportunities for patients, students, scientists and clinicians:

- Enumerated changes in related Ts, F & K awards
- Joint activities (training, symposia, etc.; may be incorporated in IIIA, if desired)

IV. BIOMEDICAL & TRANSLATIONAL RESEARCH CORE REPORTS (1-2 pages MAX for narrative text per core)

For each core:

(Table or Text) A. Significant changes from previous year

- New personnel
- New services or changes in existing services

(Table or Text) B. Usage metrics (tabulated)

- Number of users - broken down by members vs nonmembers, including the distribution of core activity for each
- Number of assays/services over the past year (**see attached format Table from most recent RFA as an example for each core)**
- Number of publications citing center support that used the core

(Text) C. Significant accomplishments

- R&D to improve core services; briefly describe new, innovative services that are being developed
- Surveys to evaluate core services
- 2-3 papers highlighting scientific advances supported by the core (PMCID# plus brief description)

V. PILOT & FEASIBILITY PROGRAM (1-3 pages MAX for any narrative text)

(Table) A. Solicitation

- Number of new (or continuing) P&F applications reviewed (may also include number of letters of intent received, if applicable)
- Types of applications reviewed
  - new invest, established investigator new to field, innovative partnership
  - basic, clinical, phase I translation, prevention & control
  - diabetes, endo, obesity, autoimmunity, transplantation
  - inter or trans-disciplinary
- Review process (only if altered from previous years)

(Table or Text) B. New Awards

- Number of new (or continuing) P&F awards
- Types of awards
    - new investigator, established investigator new to field, innovative partnership
    - basic, clinical, phase I translation, prevention & control
    - diabetes, endo, obesity, autoimmunity, transplantation
    - inter or trans-disciplinary
    - joint funding (with other centers or programs)
- P&F Award titles, PI names, brief descriptions (the supported P&F project descriptions should be 2-3 sentences at a minimum; the best P&F project descriptions are usually written by the P&F awardees)
- **see attached format Table from most recent RFA as an example**

(Table or Text) C. Awards funded in previous year(s)

- Titles, PIs, brief description (repeated from prior year report)
- Progress – brief description (short paragraph)
- Presentations, manuscripts, publications (include PMCID#)
- New funding

CENTER IMPACT MEASURES *(#VI)*

VI. MAJOR RESEARCH ACCOMPLISHMENTS (1-2 pages MAX for narrative text)

A. Select **up to three significant findings** and provide PMCID# for supporting center citations that typify activity at your center and that highlight recent research accomplishments.

B. Describe progress along a translational continuum in your center for a selected topic area/project. This can be a retrospective analysis, or an example of a current project or area that is actively progressing along the translational continuum.

VII. PROGRESS MADE WITH ANY SUPPLEMENTAL FUNDS

If your Diabetes Center received supplemental funds in the past 1-3 years, please be sure to include an update on progress made with these funds. Examples include:

- ARRA or NIDDK funds for equipment (list equipment purchased, if not reported previously)
- ARRA or NIDDK funds for P&F projects (designate the P&F projects supported with ARRA vs. NIDDK funds, and research progress on each)
- NIDDK funds for a diversity supplement (report research progress and include budget request if supplement was approved for second year of support)
- NIDDK funds for “R24 seeding projects” (report research progress)

VIII. CHECKLIST, HUMAN SUBJECTS, VERTEBRATE ANIMALS, & OTHER REQUIRED FORM PAGES

Specific Examples:

- Inclusion Enrollment Report Format Page (submit this form page for each P&F awardee using human research subjects during the past 1-2 years)
- Targeted/Planned Enrollment Format Page (submit this form page for each new P&F awardee who plans to study human research subjects, but whose study is just beginning and enrollment hasn't started yet)
- IRB and/or IACUC approval information for all P&F studies involving human research subjects and/or vertebrate animals (a listing, with approval dates, PI names, project title, etc., is acceptable)
- For Center cores using human research subjects and/or vertebrate animals, provide a list of approvals that are specific to the core (i.e. NOT the approvals of all investigators using a core during the past year), if any
- Note: If your Center grant is currently approved for research involving human research subjects and/or vertebrate animals, we will need at least one current copy of the appropriate approval information in order to keep this designation active.
- All Personnel Report
- **External Advisory Report: If your Center has a report from an External Advisory meeting during the past year, please include a copy in your annual progress report.**
- **The Difference Between PMID and PMCID when reporting publications to NIH**

CONSOLIDATED PUBLICATION LIST
during last 12-month budget period

Publications	Core B - Imaging	Core C – Animal Models	Core D – Morphology	Molecular Core
Jones, J; Smith, A.. Title, YR, Vol., page#, PMCID	P			
Brown, A.; **Chu, D.;** Anderson, J.C. Title, YR, Vol., page #, PMCID	P			
Cheng, C.; **Olson, F.** Title, YR, Vol., page#, PMCID	S	P	S	
***Sands, W**.; Cheng, C. Title, YR, Vol., page#, PMCID		P		
Smith, L.; Davis, S.; **Taylor, E**. Title, YR, Vol., page#, PMCID			P	
Hathaway, J.; Schultz, A. Title, YR, Vol., page#, PMCID				P

Note: An asterisk (*) indicates a publication that fails to cite DRC support.

Sort this table by Primary Core used, recognizing that the services of several cores might have contributed to the publication. Center members' names should be in **bold**; P/F recipients and users from outside the Center in *italics*. The number of publications (not the list) that resulted from any use of the core, i.e. including secondary core use, should also be indicated in the core write-up.

List each publication only once, regardless of how many cores were utilized or how many Center members are authors.

USE OF CORE FACILITIES
during last 12-month budget period

For each Core provide information on the use of the Core's services for the last 12-month period of support.

To avoid unwieldy tables, group services whenever possible, i.e all 'assays', all 'animals', all 'consultations' and provide more details in the core description.

CORE: Biochemistry

DETERMINATIONS/SERVICES RENDERED

A. Insulin, Ghrelin, CCK, leptin measurements
B. RNA, DNA isolations
C. Serum, cell, tissue storage
D. Consultation

User	Funded Project	Period of Performance	A	B	C	D	Actual use and comments
Adams	R01DK 099999	03/1/2009 – 07/30/2012		X		X	B. 5 per month for months D. 20 hours over the course of 12 months
Knight	P/F project	07/01/2010-06/30/2011	X			X	A. 100 samples per month for 3 months D. 10 hours

List Center Members first, alphabetically, followed by users who are not Center Members, also alphabetically.

PILOT PROJECT OUTCOME TABLE

Provide information on the most recent 5 or, if possible, 10 yr period. By adding to this Table each year in the progress report, you should have less work for the renewal application.

P/F #	PI (Dept)	Dates/Amount of P/F project	Title of Project	A	P	Applications Funded/Pending	Project Period	Still in Diabetes Research?
01	John Doe (Physiology)	07/01/10 - 06/30/11 $10,000	Role of NPY in the Regulation of Energy Balance	1		NIH R01 - pending	01/01/12 – 12/31/16	Yes
02	Mary Hathaway (Medicine/Endo)	07/01/10 – 06/30/12	Role of GI Hormones in Insulin Resistance	2	1	R21DK088888	09/01/12 – 8/31/14	Yes
03								
etc								

A = Abstracts

P = Publications

* Under "Applications Funded/Pending", list the grant received most proximate in time to the P/F award, i.e. for investigators who received funding 5-10 years ago, this may not be current funding.

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 2015

- **RFA**: Published in NIH Guide in summer/early fall 2013 (electronic applications begin!)
- Application deadline: Early Summer 2014
- Initial Review: Fall 2014
- Earliest Funding: April 2015 (FY2015)
- Renewal Applications:
  - Albert Einstein College of Medicine
  - Boston Area DRC
  - UCSF

National Institute of Diabetes and Digestive and Kidney Diseases

Fiscal Year 2017

- **RFA**: Published in NIH Guide in summer/early fall 2015
- Application deadline: Early Summer 2016
- Initial Review: Fall 2016
- Earliest Funding: April 2017 (FY2017)
- Renewal Applications:
  - Joslin Diabetes Center
  - University of Pennsylvania
  - Vanderbilt University

NIH National Institute of Diabetes and Digestive and Kidney Diseases

National Institute of
Diabetes and Digestive
and Kidney Diseases

National Institute of Diabetes and Digestive and Kidney Diseases

Submitting Complex Electronic NIH Grant Applications

National Institute of Diabetes and Digestive and Kidney Diseases

Application Submission System & Interface for Submission Tracking (ASSIST)

What is ASSIST?

ASSIST is a web-based system used to prepare multi-project (also known as multi-component or complex) applications using the SF424 Research & Related form set and to submit electronically through Grants.gov to NIH.

ASSIST allows participants to do the following:

- Delegate application preparation responsibilities to multiple users within and outside the applicant organization
- Populate data from established eRA Commons profiles
- Run validations on federal-wide and agency business rules prior to submission
- Generate Table of Contents, headers, footers, page numbers, etc. automatically
- Print/Preview applications prior to submission in the format used by the agency
- Present to reviewers clear, color PDF images rather than scanned versions of the application
- Avoid the hassle of preparing and shipping multiple paper copies

Application Format Using ASSIST

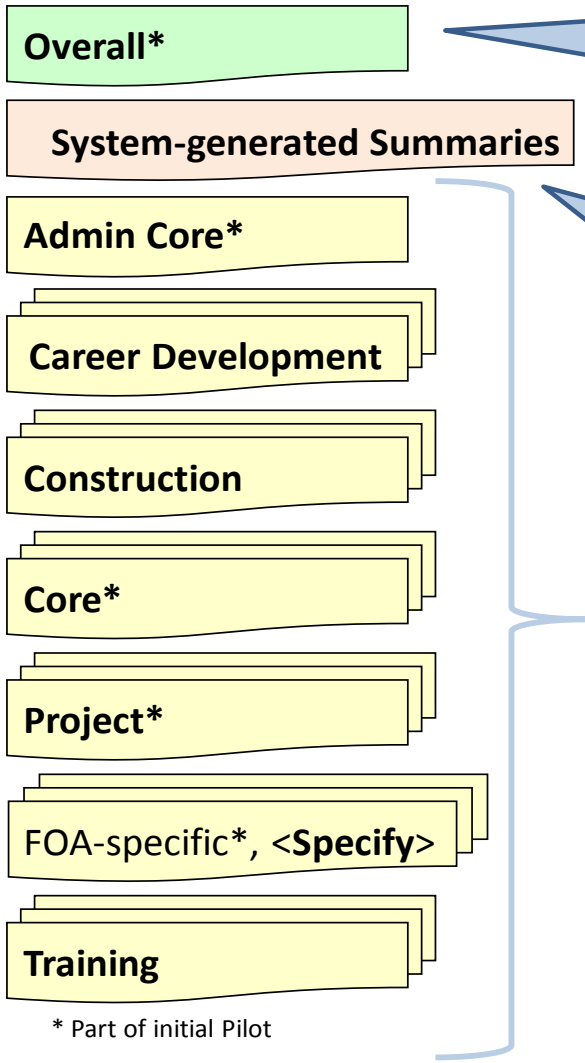
All electronic multi-component applications prepared in ASSIST will include:

- A single Overall Component: The Overall component describes the entire application and how each of the additional components fit together.

- Other Components: Some number of other component types (e.g., Admin Core, Core, P&F Program, Enrichment Program) with predefined data collection requirements set by the agency when posting the opportunity in Grants.gov

- Summaries: Information compiled from the data provided in the individual components (e.g., component and categorical roll-ups of budget data).

ASSIST Application Format



The Overall Component is present in every complex application and describes the entire application. Overall will always be displayed first in the application image.

Summaries of component data will be included in the application image with the Overall component.

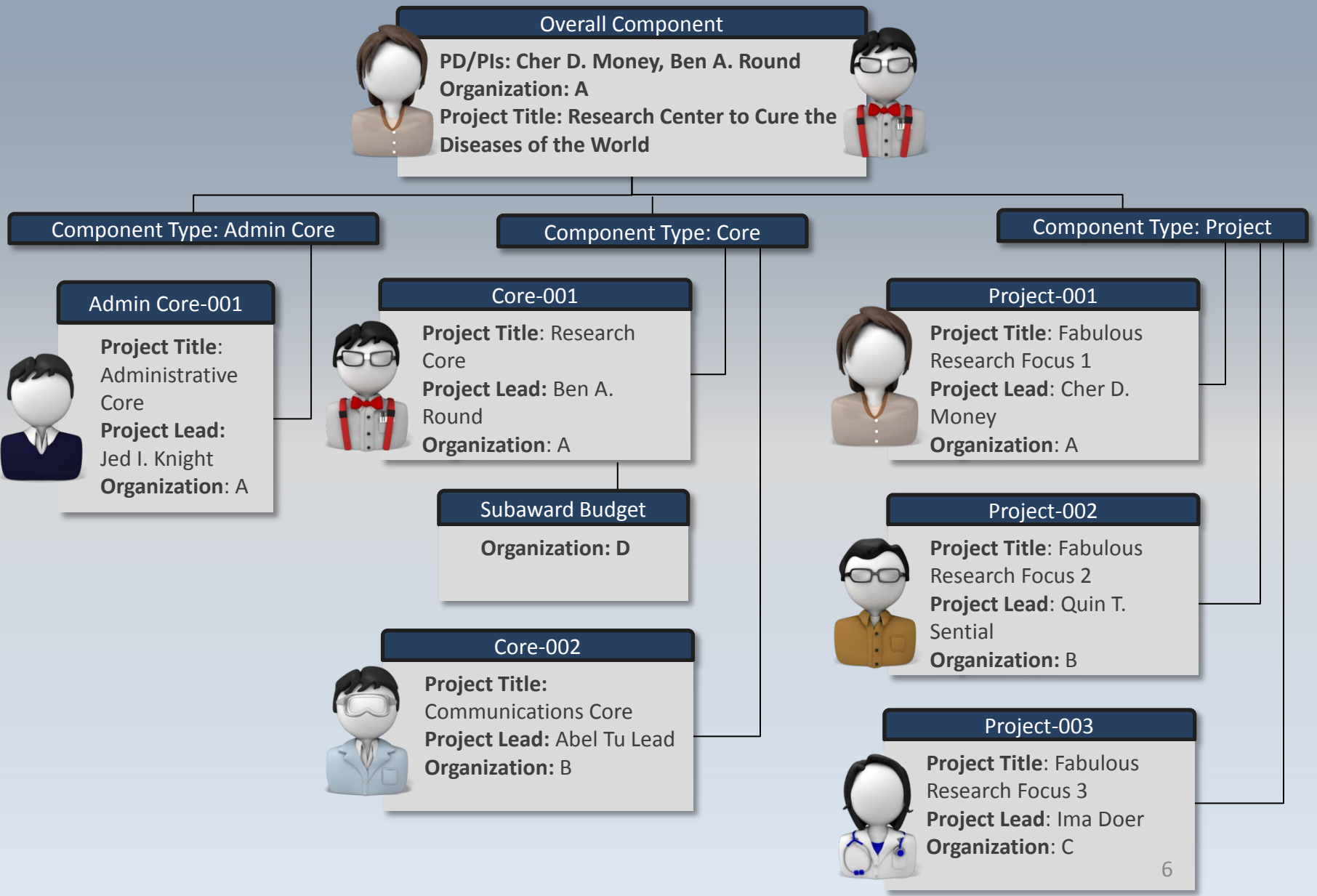
The additional component types allowed for an application will vary by FOA. Each component type will have a pre-defined set of forms for data collection.

Admin Core, Core, Project & FOA-specific components will have data collection similar to a single-project research application.

Training will be similar to an NRSA Training application.

Construction and Career Development will be similar to their single-project counterparts.

Sample Application Layout



Recent 'Pilot' Electronic RFA: Environmental Health Sciences Core Centers (P30)

Component Types Available in ASSIST	Research Strategy/Program Plan Page Limits
Overall	12 pages
Admin Core •Use for: Administrative Core and Leadership component	12 pages
Institutional Org •Use for: Institutional Commitment and Organization component	6 pages
Pilot Program •Use for: Pilot Projects Program component	6 pages
Careers •Use for: Career Development for Environmental Health Investigators component	6 pages
Core •Use for: • Integrative Health Sciences Facility Core • Facility Cores • Community Outreach and Engagement Core (COEC)	12 pages

Application Format Using ASSIST

Each ‘Component’ within a multi-component application prepared in ASSIST may include the following, as appropriate:

- Project Summary/Abstract
- Biographical Sketches for key/senior personnel
- Facilities & Other Resources
- Budget Information
- Other Attachments: suggested Tables of information
- Specific Aims
- Research Strategy/Program Plan

Application Format Using ASSIST

- ASSIST will use many of the SF424 forms that you already use with other NIH electronic applications (e.g. R01)

- ASSIST uses .pdf format for uploaded narrative sections (e.g. Specific Aims, Research Strategy/Program Plan)

- Biographical Sketches for key/senior personnel are collated automatically into a single section

- Budget Information is collated automatically across the application

- Table of Contents is generated automatically

- Applications will be bookmarked automatically to assist review

Resources

- ASSIST User Guide: http://era.nih.gov/files/ASSIST_user_guide.pdfASSIST

- ASSIST Webinar: "Initial Look at the Electronic Submission Process for Multi-Project Applications":

  http://grants.nih.gov/grants/webinar_docs/webinar_20121213.htm

- ASSIST Frequently Asked Questions: http://era.nih.gov/era_training/assist.cfm

- eRA Help Desk: http://era.nih.gov/help/index.cfm#era

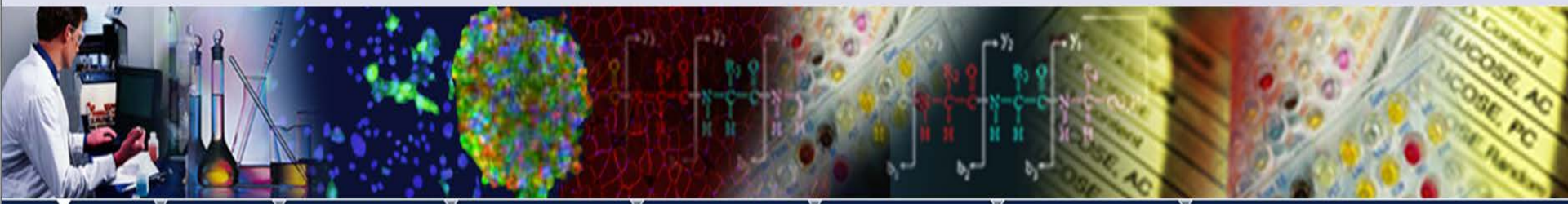
National Institute of
Diabetes and Digestive
and Kidney Diseases

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



What's New

- NIDDK Summer Medical Student Research Program in Diabetes
- NIH Intramural Research Program Newsletter: The NIH Catalyst
- Type 1 Diabetes Research: Initiative Concepts to Be Pursued in FY 2012-2013
- Diabetes Research Strategic Plan
- Upcoming Symposia & Meetings
- Drug Discovery News Interviews the FNIH on the

Diabetes Research Centers

IN THE SPOTLIGHT

Dr. Russell Rothman

Dr. Russell Rothman is an Associate Professor of Medicine and Pediatrics. He currently serves as the Director of the Vanderbilt Program o.. MORE

DRC - Vanderbilt University

The Vanderbilt DRTC, an interdisciplinary program involving 98 participating faculty distributed among 18 departments in two schools and four colleges at Vanderbilt and at neighboring Meharry Medical College, consists of:Administrative Component... MORE

Centers In The News

- YALE: Dr. Robert Sherwin Wins ADA's 2011 Albert Renold Award
- UWASH: Dr. Jerry Palmer - ADA's 2011 Outstanding Physician Clinician in Diabetes Award
- WASHU: Surprising culprits behind cell death from fat and sugar overload
- JHU/UMD: Dr. Fred Brancati Wins ADA's 2011 Kelly West Award
- JHU/UMD: DNA 'End-Caps' Length Linked to Diabetes

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy
- For National Diabetes Month, NIH urges actions to reach health goals
- "Biggest Loser" study finds modest diet and exercise can sustain weight loss

Diabetes Research Centers

IN THE SPOTLIGHT

Robert S. Sherwin, M.D.

Robert S. Sherwin. MD is the C.N.H. Professor of Medicine in the Section of Endocrinology at Yale University School of Medicine in New Haven, CT. He graduated from the Albert Einstein College of Medicine in 1967. After completing his residency in internal m.. MORE

Yale University

The Yale Diabetes Endocrinology Research Center (DERC) was established in the spring of 1993 with the goal of promoting research in diabetes and related metabolic and endocrine disorders at the university. The DERC brings together a multidisciplinary group ... MORE

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge
- COLUMBIA: New Brain

DIABETES RESEARCH CENTERS

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy
- For National Diabetes Month, NIH urges actions to reach health goals
- "Biggest Loser" study finds modest diet and exercise can sustain weight loss

Diabetes Research Centers

IN THE SPOTLIGHT

Robert S. Sherwin, M.D.

Robert S. Sherwin. MD is the C.N.H. Professor of Medicine in the Section of Endocrinology at Yale University School of Medicine in New Haven, CT. He graduated from the Albert Einstein College of Medicine in 1967. After completing his residency in internal m.. MORE

Yale University

The Yale Diabetes Endocrinology Research Center (DERC) was established in the spring of 1993 with the goal of promoting research in diabetes and related metabolic and endocrine disorders at the university. The DERC brings together a multidisciplinary group ... MORE

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge
- COLUMBIA: New Brain

DIABETES RESEARCH CENTERS

Niddk Admin

- Manage Site Content
- P&F SlickGrid Test

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy

Diabetes Research Centers

View | Revisions

IN THE SPOTLIGHT

John Darga

My Master's thesis research involved the identification and characterization of sulfidogenic bacteria from Bahamian blue holes. As a grad student I had the pleasure of spending a summer working in Mitch Sogin's lab at the MBL at Woods Hole on the Giardi.. MORE

Boston Area

The Boston Area Diabetes Endocrinology Research Center (BADERC) is a consortium of laboratory-based and clinical investigators whose efforts are directed toward addressing many of the major research questions bearing on the etiology, pathogenesis, treatment... MORE

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge

DIABETES RESEARCH CENTERS

Contact Us | Login

Search

Home | Centers | Publications | Funding Opportunities | Research Cores | Diabetes Resources | Pilot & Feasibility | Other NIDDK Centers

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy
- For National Diabetes Month, NIH urges actions to reach health goals
- "Biggest Loser" study finds modest diet and exercise can sustain weight loss

Diabetes Research Centers

IN THE SPOTLIGHT

Robert S. Sherwin, M.D.

Robert S. Sherwin. MD is the C.N.H. Professor of Medicine in the Section of Endocrinology at Yale University School of Medicine in New Haven, CT. He graduated from the Albert Einstein College of Medicine in 1967. After completing his residency in internal m.. MORE

Yale University

The Yale Diabetes Endocrinology Research Center (DERC) was established in the spring of 1993 with the goal of promoting research in diabetes and related metabolic and endocrine disorders at the university. The DERC brings together a multidisciplinary group ... MORE

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge
- COLUMBIA: New Brain

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy
- For National Diabetes Month, NIH urges actions to reach health goals
- "Biggest Loser" study finds modest diet and exercise can sustain weight loss
- Family problem-solving sessions help teens better manage diabetes

more

Funding Opportunities

- Phenotyping Embryonic Lethal Knockout Mice (R01)
- Biomarkers for Diabetes, Digestive, Kidney and Urologic Diseases Using Biosamples from the NIDDK Repository (R01)
- Improvement of Animal Models for Stem Cell-Based Regenerative Medicine (R01)
- High-End Instrumentation Grant Program (S10)

Pilot And Feasibility

P&F program

The Diabetes Center Pilot and Feasibility (P&F) program provides seed support for new and innovative research projects directed at basic biomedical, clinical and translational research questions relevant to diabetes and its complications.

The P&F program is designed to support:

- 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.

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
Baylor College of Medicine	University of Chicago
Boston Area DRC	University of Michigan
Columbia University	University of Pennsylvania
Johns Hopkins/University of Maryland	University of Washington
Joslin Diabetes Center	Vanderbilt University
UCSD/UCLA	Washington University
UCSF	Yale University

P&F database

The P&F database provides access to researchers' institutions, departments, and interests. It is a secure web page requiring users to **login** before viewing or searching the database.

Once you are logged in, please visit the **P&F database**.

Diabetes Center P&F Program - commonalities and diversities of practices

- Diabetes Centers P&F Grant Programs

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge
- COLUMBIA: New Brain Target for Appetite Control Identified

more

Featured Publications

- Weight Change, Psychological Well-Being, and Vitality in Adults Participating in a Cognitive-Behavioral Weight Loss Program.
- The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention: an intent-to-treat analysis of the DPP/DPPOS.
- Cross-sectional associations between measures of sleep and markers of glucose

Pilot and Feasibility Reviewers Database

[Edit] [Export] [Clone]

The following fields allow you to select a subset of all the pandf reviewers to display, click on Apply to submit. **Note:** All fields are ANDed together.

Reviewer | **Center** <Any> | **Institution** <Any> | **Interests**

State <Any> | **Search all fields** | Apply

Reviewer	Degrees	Center	Institution	Department	Position	Interests	Email	Telephone	State
Abbas, Abul	M.D.	UCSF	University of California San Francisco	Pathology	Chair Professor	Immune tolerance and regulatory T-cells	abul.abbas@ucsf.edu	415-514-0681	California
Abcouwer, Steven F.	Ph.D.	University of Michigan	University of Michigan	Ophthalmology & Visual Science	Associate Professor	Diabetic retinopathy, microglia, neurodegeneration	sabcouwe@umich.edu	734-232-8227	Michigan
Abel, E. Dale	M.D.	Johns Hopkins University/ University of Maryland	University of Utah		Professor of Biochemistry, Human Genetics and of Medicine	Cardiac dysfunction in diabetes and the regulation of myocardial growth and metabolism by insulin signaling.	dale.abel@hmbg.utah.edu	801-581-7761	Utah
Abendschein, Dana	Ph.D.	Washington University	Washington University in St. Louis	Cardiology Medicine	Associate Professor	Complications	dabendsc@wustl.edu	314-362-8925	Missouri
Ablamunits, Vitaly	Ph.D.	Yale University	Yale	Immunobiology	Associate Research Scientist	Immune monitoring of human T cells	Vitally.ablamunits@yale.edu	203-785-6515	Connecticut


Sort by Center
Click here to download as Excel spreadsheet
Pilot and Feasibility Reviewers Database
Reviewer
Center
Institution
Interests
State
Search all fields
Apply
Degrees
Department
Position
Email
Telephone
NIDDK
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Niddk Admin

- Manage Site Content
- P&F SlickGrid Test

What's New

- Upcoming Symposia & Meetings
- NIH Director's Blog: "Brown Fat, White Fat, Good Fat, Bad Fat"
- NIH launches study of long-term effects of blood glucose during pregnancy
- For National Diabetes Month, NIH urges actions to reach health goals
- "Biggest Loser" study finds modest diet and exercise can sustain weight loss
- Family problem-solving sessions help teens better manage diabetes

more

Funding Opportunities

- Phenotyping Embryonic Lethal Knockout Mice (R01)

Publications

View | Edit | Revisions

Featured Publications | Center Publications | Publication Search

[Edit] [Export] [Clone]

- **Effects of proximal gut bypass on glucose tolerance and insulin sensitivity in humans.**
  Breitman I., Isbell JM, Saliba J., Jabbour K., Flynn CR, Marks-Shulman PA, Laferrère B., Abumrad NN, and Tamboli RA
  Journal Title: Diabetes care, **Volume:** 36(2013), **Pages:** e57
  Abstract **Journal Site** **Pubmed** **PMCID:** PMC3609508 **Collaboration Columbia University, Vanderbilt University**

- **A behavioral weight-loss intervention in persons with serious mental illness.**
  Daumit, GL, Dickerson FB, Wang NY, Dalcin A., Jerome GJ, Anderson CA, Young DR, Frick KD, Yu A., Gennusa JV 3rd, et al.
  Journal Title: The New England journal of medicine, **Volume:** 368(2013), **Pages:** 1594-602
  Abstract **Journal Site** **Pubmed** **Johns Hopkins University/ University of Maryland**

- **Patient-reported outcomes in the practice-based opportunities for weight reduction (POWER) trial.**
  Rubin, RR, Peyrot M., Wang NY, Coughlin JW, Jerome GJ, Fitzpatrick SL, Bennett WL, Dalcin A., Daumit G., Durkin N., et al.
  Journal Title: Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation, **Volume:** (2013), **Pages:**
  Abstract **Journal Site** **Pubmed** **Johns Hopkins University/ University of Maryland**

- **Primary aldosteronism and impaired natriuresis in mice underexpressing TGFβ1.**
  Kakoki, M., Pochynyuk OM, Hathaway CM, Tomita H., Hagaman JR, Kim HS, Zaika OL, Mamenko M., Kayashima Y., Matsuki K., et al.
  Journal Title: Proceedings of the National Academy of Sciences of the United States of America, **Volume:** 110(2013),

Centers In The News

- Frederick L. Brancati, M.D., M.H.S., 1959-2013
- UCHICAGO: Dr. Graeme Bell awarded 2012 Manpei Suzuki International Prize
- John C. Hutton, PhD: 1948-2012
- George S. Eisenbarth, MD, PhD: 1947-2012
- EINSTEIN: Preventing Diabetes and Its Complications Offers Public Health Challenge
- COLUMBIA: New Brain Target for Appetite Control Identified

more

Featured Publications

- Generation of functional insulin-producing cells in the gut by Foxo1 ablation.
- Diabetes and co-morbid depression among racially diverse, low-income adults.
- Adipocyte NCoR Knockout Decreases PPARγ

Website Updates

- Website is now located in a 'cloud' environment
- 2012 Website Project: P&F awardees' database
  - Web-based data input
  - May be downloaded by each Center
  - Beta-test site: http://diabetescenters.org/niddktest/pandf_outcomes

Pilot Project Outcomes

[Edit] [Export] [Clone]

We are providing the following pre-formatted table to assist in preparing your application.

1. Provide information on the most recent 5 or, if possible, 10-year period. Complete and organize by Year Funded.
2. P&F Type Key:
   N= New Investigator; NTD= Established Investigator, New to Diabetes research; E = Established Investigator; with new, innovative research idea
3. A = # of Abstracts; P = # of Publications; include only those supported by the P&F award.
4. If grant applications or funding resulted from the pilot and feasibility project, please complete Columns G (P) and H (Applications/Grants). List the grant received, or application submitted, most proximate in time to the P&F award, i.e. for investigators who received funding 5-10 years ago, this may not be current funding.

Center	P&F #	PI	Department	Award Period	Amount of P&F Award (direct costs)	Pilot Project Title	P&F Type	A	P	Application
Albert Einstein College of Medicine	1	Smith, Elizabeth	Biochemistry	07/01/2012 - 06/30/2013	$50,000	Role of serotonin receptors in T1D autoimmunity	E	1	0	Applicat Applicat
Albert Einstein College of Medicine	2	Doe, Jane	Surgery	05/01/2013 - 04/30/2014	$75,000	AECOM Pilot Project 2	N	1	1	Grant 1 Grant 2
University of Michigan	1	Doe, John	Physiology	06/30/2009 - 06/29/2010	$45,000	Role of leptin receptors in the arcuate nucleus	N	1	1	NIH/NI ADA 12 NIH/NH
University of Michigan	2	Wilson, Mary	Endocrinology	07/01/2010 - 06/30/2012	$100,000	Regulation of Beta Cell Mass in T2D	NTD	2	2	R21DK0

Pilot Project Outcomes

[Edit] [Export] [Clone]

We are providing the following pre-formatted table to assist in preparing your application.

1. Provide information on the most recent 5 or, if possible, 10-year period. Complete and organize by Year Funded.
2. P&F Type Key:
   N= New Investigator; NTD= Established Investigator, New to Diabetes research; E = Established Investigator; with new, innovative research idea
3. A = # of Abstracts; P = # of Publications; include only those supported by the P&F award.
4. If grant applications or funding resulted from the pilot and feasibility project, please complete Columns G (P) and H (Applications/Grants). List the grant received, or application submitted, most proximate in time to the P&F award, i.e. for investigators who received funding 5-10 years ago, this may not be current funding.

Center	P&F #	PI	Department	Award Period	Amount of P&F Award (direct costs)	Pilot Project Title	P&F Type	A	P	Application
Albert Einstein College of Medicine	1	Smith, Elizabeth	Biochemistry	07/01/2012 - 06/30/2013	$50,000	Role of serotonin receptors in T1D autoimmunity	E	1	0	Applicat Applicat
Albert Einstein College of Medicine	2	Doe, Jane	Surgery	05/01/2013 - 04/30/2014	$75,000	AECOM Pilot Project 2	N	1	1	Grant 1 Grant 2
University of Michigan	1	Doe, John	Physiology	06/30/2009 - 06/29/2010	$45,000	Role of leptin receptors in the arcuate nucleus	N	1	1	NIH/NI ADA 12 NIH/NH
University of Michigan	2	Wilson, Mary	Endocrinology	07/01/2010 - 06/30/2012	$100,000	Regulation of Beta Cell Mass in T2D	NTD	2	2	R21DK(

Example: Click here to edit

Pilot Project Outcomes

We are providing the following pre-formatted table to assist in preparing your application.

1. Provide information on the most recent 5 or, if possible, 10-year period. Complete and organize by Year Funded.
2. P&F Type Key:
   N= New Investigator; NTD= Established Investigator, New to Diabetes research; E = Established Investigator; with new, innovative research idea
3. A = # of Abstracts; P = # of Publications; include only those supported by the P&F award.
4. If grant applications or funding resulted from the pilot and feasibility project, please complete Columns G (P) and H (Applications/Grants). List the grant received, or application submitted, most proximate in time to the P&F award, i.e. for investigators who received funding 5-10 years ago, this may not be current funding.

Center	P&F #	PI	Department	Award Period	Amount of P&F Award (direct costs)	Pilot Project Title	P&F Type	A	P	Application
Albert Einstein College of Medicine	1			13	$50,000	Role of serotonin receptors in T1D autoimmunity	E	1	0	Applicat Applicat
Albert Einstein College of Medicine	2			14	$75,000	AECOM Pilot Project 2	N	1	1	Grant 1 Grant 2
University of Michigan	1	Doe, John	Physiology	06/30/2009 - 06/29/2010	$45,000	Role of leptin receptors in the arcuate nucleus	N	1	1	NIH/NI ADA 12 NIH/NH
University of Michigan	2	Wilson, Mary	Endocrinology	07/01/2010 - 06/30/2012	$100,000	Regulation of Beta Cell Mass in T2D	NTD	2	2	R21DK(

PI Name:

Smith, Elizabeth

Save Cancel

Pilot Project Outcomes

Pilot Project Outcomes | Pilot Project Outcomes Test | PandF Reviewers

[Edit] [Export] [Clone]

We are providing the following pre-formatted table to assist in preparing your application.

Form is downloadable, in format for RFA

1. Provide information on the most recent 5 or, if possible, 10-year period. Complete and organize by Year Funded.
2. P&F Type Key:
   N= New Investigator; NTD= Established Investigator, New to Diabetes research; E = Established Investigator; with new, innovative research idea
3. A = # of Abstracts; P = # of Publications; include only those supported by the P&F award.
4. If grant applications or funding resulted from the pilot and feasibility project, please complete Columns G (P) and H (Applications/Grants). List the grant received, or application submitted, most proximate in time to the P&F award, i.e. for investigators who received funding 5-10 years ago, this may not be current funding.

Center	P&F #	PI	Department	Award Period	Amount of P&F Award (direct costs)	Pilot Project Title	P&F Type	A	P	Applications/ Grants	Pending/ Funded	Project Period	Still in Diabetes
Albert Einstein College of Medicine	1	Smith, Elizabeth	Biochemistry	07/01/2012 - 06/30/2013	$50,000	Role of serotonin receptors in T1D autoimmunity	E	1	0	Application 1 Application 2	Pending Pending	04/01/2013 - 03/31/2014 05/01/2013 - 06/30/2014	Yes
Albert Einstein College of Medicine	2	Doe, Jane	Surgery	05/01/2013 - 04/30/2014	$75,000	AECOM Pilot Project 2	N	1	1	Grant 1 Grant 2	Pending Pending	05/01/2013 - 04/30/2014 05/01/2013 - 04/30/2014	Yes
University of Michigan	1	Doe, John	Physiology	06/30/2009 - 06/29/2010	$45,000	Role of leptin receptors in the arcuate nucleus	N	1	1	NIH/NIDDK R01 ADA 12345 NIH/NHLBI R21	Pending Pending Pending	12/01/2013 - 11/30/2018 09/01/2013 - 08/31/2015 09/01/2013 - 08/31/2015	Yes
University of Michigan	2	Wilson, Mary	Endocrinology	07/01/2010 - 06/30/2012	$100,000	Regulation of Beta Cell Mass in T2D	NTD	2	2	R21DK012345	Funded	07/01/2012 - 06/30/2013	Yes
University of Michigan	3	Smith, John	Medicine	07/01/2012 - 06/30/2014	$30,000	Michigan Pilot Project 3	NTD	0	0	NIH 1	Pending	10/01/1010 - 09/30/0015	Yes

XLS

Diabetes Research Centers Website

- P&F reviewers in database (2013): 1,421
- In the Spotlight: 161 (2011); 210 (2013)
- Note: We presently have 320 center personnel listed on website, however we only have images and brief descriptions for 210.
- Banner Images: Send images to Jim or Jodee Allen
- Centers in the News: Send web links to Jim

Diabetes Research Centers Website

September 2010

- Total Visits: 248
- Unique Visits: 208
- New Visitors: 80.2%
- Page Views: 859

May 2013

- Total Visits: 1,986
- Unique Visits: 1,031
- New Visitors: 47.3%
- Page Views: 8,916

DIABETES TRANSLATION RESEARCH CENTERS

New CDTR Website!

HOME ABOUT US CENTERS CORES RESOURCES

Diabetes Translation Research Centers Overview

The NIDDK-supported Centers for Diabetes Translation Research (CDTR) are part of an integrated program whose cores support and enhance diabetes type II translation research (e.g. bedside to practice and the community). The purpose of the CDTRs is to enhance the efficiency, productivity, effectiveness and multidisciplinary nature of diabetes translation research.

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

www.diabetes-translation.org

National Institute of
Diabetes and Digestive
and Kidney Diseases

Health Psychology
2013, Vol. 32, No. 4, 439–446

0278-6133/13/$12.00 DOI: 10.1037/a0029186

Weight Change, Psychological Well-Being, and Vitality in Adults Participating in a Cognitive–Behavioral Weight Loss Program

Charles Swencionis and Judith Wylie-Rosett
Albert Einstein College of Medicine, Yeshiva University

Michelle R. Lent
Yeshiva University

Mindy Ginsberg, Christopher Cimino, Sylvia Wassertheil-Smoller, Arlene Caban, and Carol-Jane Segal-Isaacson
Albert Einstein College of Medicine, Yeshiva University

Objective: Excess weight has been associated with numerous psychological problems, including depression and anxiety. This study examined the impact of intentional weight loss on the psychological well-being of adults participating in three clinical weight loss interventions. ***Methods:*** This population consisted of 588 overweight or obese individuals randomized into one of three weight loss interventions of incremental intensity for 12 months. Psychological well-being was measured at baseline and 6, and 12 months using the Psychological Well-Being Index. ***Results:*** Mean weight loss was 5.0 pounds at 12 months. Weight change at 12 months was associated with higher overall psychological well-being ($r = -.20$, $p < .001$), lower levels of anxiety ($r = -.16$, $p = .001$) and depression ($r = -.13$, $p = .004$), and higher positive well-being ($r = -.19$, $p < .001$), self-control ($r = -.13$, $p = .004$), and vitality ($r = -.22$, $p < .001$). Vitality was found to be the best predictor of weight change at 12 months ($p < .001$). ***Conclusions:*** Weight loss was associated with positive changes in psychological well-being. Increased vitality contributed the largest percentage of variance to this change.

Keywords: psychological well-being, weight loss, vitality, obesity

Obesity poses one of the greatest threats to the future of our public health, with more than 400 million people worldwide estimated to be obese and 1.6 billion estimated to be overweight (World Health Organization, 2006). Weight loss, while strongly encouraged by health care professionals, remains challenging to achieve and maintain for many living with excess weight. Though the multiple potential benefits of weight loss for obese individuals span medical, social, and psychological domains, barriers to losing weight and maintaining reductions in weight remain for much of this population. Furthermore, many individuals living with significant excess weight encounter more health and psychosocial problems, as well as prejudice and discrimination, all of which can lower quality of life (QoL) (Gregg & Williamson, 2002; Rand & Macgregor, 1990; Wadden, Womble, Stunkard, & Anderson, 2002).

The relationship between excess weight and dimensions of both QoL and health-related quality of life (HRQL) has been extensively examined (de Zwaan et al., 2009; Han, Tijuis, Lean, & Seidell, 1998). QoL and HRQL assessments typically measure multiple aspects of well-being and functioning, including vitality, perceptions of general health, pain, social and physical functioning, impairment or disability, and mental health. Individuals with larger waist circumferences and higher body mass indices (BMI) report lower QoL and more impairment in completing tasks of everyday living (Han et al., 1998). Middle-aged overweight and obese women (BMI > 25 kg/m^2) describe lower levels of vitality, as well as worse overall mental health, compared to women in the normal weight range (Brown, Dobson, & Mishra, 1998). Additionally, women who perceive themselves as overweight report lower levels of general health, vitality, and physical functioning, and women with a history of frequent weight loss displayed notable reductions in scores on the mental health and emotional functioning areas of QoL measures (Burns, Tijhuis, & Seidell, 2001). Obese individuals may also experience significant levels of chronic pain, particularly back and knee pain, which may reduce perceptions of QoL (Barofsky et al., 1997). As with other serious medical conditions, obesity has been associated with the presence of mental health problems, including depression and anxiety, in

This article was published Online First August 13, 2012.

Charles Swencionis, Ferkauf Graduate School of Psychology, Yeshiva University and Departments of Epidemiology and Population Health and Psychiatry, Albert Einstein College of Medicine, Yeshiva University; Judith Wylie-Rosett, Mindy Ginsberg, Sylvia Wassertheil-Smoller, Arlene Caban, and Carol-Jane Segal-Isaacson, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Yeshiva University; Michelle R. Lent, Ferkauf Graduate School of Psychology, Yeshiva University; Christopher Cimino, Albert Einstein College of Medicine, Yeshiva University.

Christopher Cimino is now at the New York Medical College.

This research was supported in part by the National Institutes of Health, National Heart, Lung, and Blood Institute Grant R01 HL50372-01; the Diabetes Research and Training Center Grant P60 DK020541; and Clinical and Translational Science Award Grant UL1 RR025750 (ClinicalTrials.gov Identifier: NCT00674180).

Correspondence concerning this article should be addressed to Charles Swencionis, Ferkauf Graduate School of Psychology, Yeshiva University, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: Charles.Swencionis@einstein.yu.edu

Intervention Costs and Cost-Effectiveness of a Successful Telephonic Intervention to Promote Diabetes Control

Clyde B. Schechter, MD, MA[1]
Hillel W. Cohen, DrPH, MPH[2]
Celia Shmukler, MD[3]
Elizabeth A. Walker, PhD, RN[4]

OBJECTIVE—To characterize the costs and cost-effectiveness of a telephonic behavioral intervention to promote glycemic control in the Improving Diabetes Outcomes study.

RESEARCH DESIGN AND METHODS—Using the provider perspective and a time horizon to the end of the 1-year intervention, we calculate the costs of a telephonic intervention by health educators compared with an active control (print) intervention to improve glycemic control in adults with type 2 diabetes. We calculate the cost-effectiveness ratios for a reduction of one percentage point in hemoglobin A_{1c} (A1C), as well as for one participant to achieve an A1C <7%. Base-case and sensitivity analysis results are presented.

RESULTS—The intervention cost $176.61 per person randomized to the telephone group to achieve a mean 0.36 percentage point of A1C improvement. The incremental cost-effectiveness ratio was $490.58 per incremental percentage point of A1C improvement and $2,617.35 per person over a 1-year intervention in achieving the A1C goal. In probabilistic sensitivity analysis, the median (interquartile range) of per capita cost, cost per percentage point reduction in A1C, and cost per person achieving the A1C goal of <7% are $175.82 (147.32–203.56), $487.75 (356.50–718.32), and $2,312.88 (1,785.58–3,220.78), respectively.

CONCLUSIONS—The costs of a telephonic intervention for diabetes self-management support are moderate and commensurate to the modest associated improvement in glycemic control.

Diabetes Care 35:2156–2160, 2012

The goal for glycemic control for most individuals with type 2 diabetes is a hemoglobin A_{1c} (A1C) of <7% (1); however, more than 40% of individuals with diabetes in the U.S. do not reach this goal (2). Implementation of a diabetes management plan should include clinical care, diabetes self-management education, and ongoing support (1). Most individuals with diabetes report lack of or access challenges for receiving diabetes self-management education or support (3,4). Several studies have reported self-management education and support delivered in the community by community peer educators (5,6), by Web-based applications (7), or by telephone (8,9). A telephonic intervention delivered by health educators was successful for a poor, urban, bilingual diabetes population for a single behavior, such as going for a dilated eye examination for retinopathy screening (10), and at a moderate cost (11). This telephonic behavioral counseling intervention was broadened in the Improving Diabetes Outcomes (I DO) study to promote change in multiple behaviors, including medication adherence, healthy eating, and increased physical activity (12,13). Information about the costs of such an intervention is necessary for knowledgeable translation of the intervention to the broad community.

From the [1]Department of Family and Social Medicine, Albert Einstein College of Medicine, Bronx, New York; the [2]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York; [3]1199SEIU Benefit and Pension Funds, New York, New York; and the [4]Division of Endocrinology and Diabetes, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York.
Corresponding author: Elizabeth A. Walker, elizabeth.walker@einstein.yu.edu.
Received 9 January 2012 and accepted 18 May 2012.
DOI: 10.2337/dc12-0048

RESEARCH DESIGN AND METHODS

—The I DO study was a randomized controlled trial of a behavioral telephonic intervention delivered by health educators with improvement in glycemic control as the primary outcome in adults with type 2 diabetes. All randomized participants received selected diabetes self-management print materials in English or Spanish, as requested (12). The telephonic intervention successfully led to a significantly greater decrease in A1C compared with the active control (print) group (13). I DO study baseline data and methods (12) and main results (13) have been published previously. In summary, these 526 lower-income, urban adults with type 2 diabetes and a baseline A1C ≥7.5%, were members/spouses in a health care workers' union; as such, they received full health care coverage, including medications. They were 62% non-Hispanic black and 23% Hispanic, and 77% were foreign-born. Those randomized to the telephone group (n = 262) were assigned to a health educator for up to 10 self-management support phone calls to discuss self-management as found in the print materials mailed to them. Those randomized to the print group (n = 264) received only the print materials. At the end of the 1-year intervention, the primary outcome, change in A1C, showed a mean (95% CI) decrease of 0.36% (0.02–0.69) more in the telephone group than in the print group (P = 0.04). Adjusted for baseline A1C, the mean reduction in A1C for the telephone group compared with the print group was 0.42% (0.11–0.73; P = 0.008).

Here we present a cost accounting and cost-effectiveness analysis of that intervention. Our perspective is that of a provider of health services, and our time horizon is the duration of the intervention of up to 1 year. Because all costs and effects occurred within 1 year, no discounting was applied in these analyses. The telephonic intervention group received up to 10 telephone calls from a health educator, one call every 4–6 weeks,

The Validity of Medication Adherence Self-Reports in Adults With Type 2 Diabetes

Jeffrey S. Gonzalez, phd[1,2]
Havah E. Schneider, ma[1]
Deborah J. Wexler, md[3]
Christina Psaros, phd[4]
Linda M. Delahanty, ms[3]
Enrico Cagliero, md[3]
Steven A. Safren, phd[4]

OBJECTIVE—To assess the validity of self-report measures of diabetes medication adherence and evaluate the effect of depression on the validity of these reports.

RESEARCH DESIGN AND METHODS—Adults with type 2 diabetes, treated with oral medications, completed a set of medication adherence self-reports that varied response scales and time frames, were administered structured clinical interviews for depression, and provided blood samples for HbA_{1c} as part of a screening for an intervention study. A subsample of participants with $HbA_{1c} \geq 7.0\%$ and clinically significant depression received Medication Event Monitoring System (MEMS) bottle caps to record adherence. Analyses examined relationships between adherence measures and HbA_{1c} and, in the subsample, MEMS. Moderated linear regression evaluated whether depression severity modified relationships with HbA_{1c}.

RESULTS—Participants' (n = 170, 57% men, 81% white, mean HbA_{1c} = 8.3% [SD, 1.7]) adherence self-reports were significantly ($r = -0.18$ to -0.28; $P < 0.03$) associated with lower HbA_{1c}. In the subsample (n = 88), all self-reports were significantly (r = 0.35 to 0.55; $P \leq 0.001$) associated with MEMS-measured adherence. Depression significantly moderated the relationship between three of six self-reports and HbA_{1c}; at high levels of depression, associations with HbA_{1c} became nonsignificant.

CONCLUSIONS—Results support the validity of easily administered self-reports for diabetes medication adherence. One-month, percentage-based ratings of adherence had the strongest associations with MEMS and HbA_{1c}; those requiring the report of missed doses had weaker associations. One-week self-ratings and measures that require respondents to record the number of missed doses appear to be vulnerable to bias from depression severity.

Treatment nonadherence is a common and important problem in diabetes care that negatively impacts treatment outcomes (1). Many studies have focused on the identification of factors that contribute to adherence and on the evaluation of interventions to improve adherence. However, the value of these studies is often limited by measurement challenges in the assessment of medication adherence. Although objective measures, such as electronic monitoring caps that record the timing of pill bottle openings, are often thought to be preferable to subjective self-reports, they are costly, often not feasible for certain study designs, and are impractical for clinical practice. Given the continued need for valid adherence self-report measures, understanding the factors that influence the validity of self-reports remains important (2). However, information on the validity of self-reported medication adherence often goes unreported in studies investigating the psychometric properties of widely used diabetes treatment adherence scales (3), and few studies focus directly on the issue of validity in the evaluation of self-reported medication adherence measures.

For self-report measures of diabetes medication adherence to be valid, there should be evidence of a robust correlation with other adherence measures, preferably those not subject to the biases associated with self-report (concurrent validity). In addition, because diabetes medication adherence has a causal impact on glycemic control, participants' adherence self-reports should correlate with HbA_{1c} levels (criterion validity). Relationships with HbA_{1c} levels are expected to be relatively weaker than with other measures of adherence because medication adherence is not the only factor that impacts glycemic control (2).

Nevertheless, studies often rely exclusively on HbA_{1c} to demonstrate the validity of adherence measures in diabetes. For example, studies have documented significant relationships between self-reported adherence and HbA_{1c} in adults with type 2 diabetes, using versions of the Morisky Medication Adherence Scale (4–8) and other self-reports of medication adherence (9,10). Other large studies, however, have failed to find relationships between similarly measured self-reported adherence and HbA_{1c} (11). Relatively few studies have examined the relationship between self-reported adherence and objectively monitored medication adherence; fewer still have examined how the characteristics of self-report questions affect this relationship.

Significantly closer concordance has been observed between single-item global self-ratings of adherence and concurrently assessed adherence measured by electronic monitoring cap, compared with the concordance for self-report measures that focused on frequency of missed doses in HIV/AIDS (12). The recalled time frame of these measures also related to concordance: 1-month recall periods were more accurate than 3- and 7-day recall periods (12). Another study

From the [1]Ferkauf Graduate School of Psychology, Yeshiva University, Bronx, New York; the [2]Diabetes Research Center, Albert Einstein College of Medicine, Bronx, New York; the [3]Diabetes Center, Department of Medicine, Massachusetts General Hospital/Harvard Medical School, Boston, MA, and [4]Behavioral Medicine, Department of Psychiatry, Massachusetts General Hospital/Harvard Medical School, Boston, Boston, Massachusetts.
Corresponding author: Jeffrey S. Gonzalez, jeffrey.gonzalez@einstein.yu.edu.
Received 1 March 2012 and accepted 14 September 2013.
DOI: 10.2337/dc12-0410

Diabetes Care Publish Ahead of Print, published online November 30, 2012

EMBO reports

scientific report

Loss of autophagy in hypothalamic POMC neurons impairs lipolysis

Susmita Kaushik[1,2], Esperanza Arias[1,2*], Hyokjoon Kwon[1,3,4], Nuria Martinez Lopez[1,3,4], Diana Athonvarangkul[1,3,4], Srabani Sahu[1,3,4], Gary J. Schwartz[1,2,4,5], Jeffrey E. Pessin[1,2,3,4] & Rajat Singh[1,2,3,4+]*

[1]Department of Medicine, [2]Institute for Aging Research, [3]Department of Molecular Pharmacology, [4]Diabetes Research Center, and [5]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA

Autophagy degrades cytoplasmic contents to achieve cellular homeostasis. We show that selective loss of autophagy in hypothalamic proopiomelanocortin (POMC) neurons decreases α-melanocyte-stimulating hormone (MSH) levels, promoting adiposity, impairing lipolysis and altering glucose homeostasis. Ageing reduces hypothalamic autophagy and α-MSH levels, and aged-mice phenocopy, the adiposity and lipolytic defect observed in POMC neuron autophagy-null mice. Intraperitoneal isoproterenol restores lipolysis in both models, demonstrating normal adipocyte catecholamine responsiveness. We propose that an unconventional, autophagosome-mediated form of secretion in POMC neurons controls energy balance by regulating α-MSH production. Modulating hypothalamic autophagy might have implications for preventing obesity and metabolic syndrome of ageing.

Keywords: autophagy; ageing; hypothalamus; obesity; POMC

EMBO *reports* (2012) **13**, 258–265. doi:10.1038/embor.2011.260

INTRODUCTION

Nutritional surplus and reduced energy expenditure promote adiposity and glucose intolerance, the hallmarks of the metabolic syndrome. The hypothalamic agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons integrate nutritional and hormonal cues to control energy balance [1]. The AgRP neurons release AgRP that promotes food intake, whereas POMC neurons express POMC preproprotein that is processed to generate adrenocorticotrophic hormone (ACTH) and α-melanocyte-stimulating hormone (MSH) [2]. α-MSH activates central melanocortin receptors to curtail food intake and promote energy expenditure by modulating sympathetic outputs to the periphery [3].

Macroautophagy (hereafter autophagy) maintains cellular homeostasis by sequestering cytosolic cargo within autophagosomes and delivering these to lysosomes for degradation [4]. The ubiquitin E1-like ligase, Atg7 initiates membrane elongation by mediating LC3 (microtubule-associated light chain 3) and Atg5-12 conjugation [4]. A well-known inhibitor of autophagy is the mammalian target of rapamycin (mTOR) [4]. Hypothalamic mTOR regulates food intake [5] suggesting that central mTOR might mediate these effects in part by modulating autophagy. In fact, autophagy in AgRP neurons has been shown to control food intake and energy balance [6]. As autophagy declines with ageing [7] it is plausible that decreased hypothalamic autophagy might contribute to the metabolic dysregulation observed with age. Recent studies in yeast [8–10] and in mammalian systems, including osteoclasts [11] and immune cells [12], have revealed new unconventional roles for autophagy proteins in the secretion of cellular proteins. These findings have led us to explore the possibility of similar autophagosome-dependent mechanisms in the nutrient-sensing hypothalamic neurons that might mediate nutrient-driven peptide processing and secretion. Here, we hypothesize that autophagic components contribute to POMC processing to generate α-MSH, and that reduced autophagy in POMC neurons promotes adiposity in part from decreased α-MSH availability. The hypothesis was tested in POMC neuron-selective *atg7*-deficient mice (knockout (KO)), and during reduced autophagy in normally aged mice.

RESULTS AND DISCUSSION

Loss of *atg7* in POMC neurons promotes adiposity

To test whether autophagy in hypothalamic POMC neurons is required for energy balance, we generated KO mice by crossing $Atg7^{F/F}$ mice with rodents that expressed *cre* in POMC neurons. Selective loss of autophagy in POMC neurons was confirmed by colocalization of cre with POMC (Fig 1A), and by demonstrating the absence of Atg7 (Fig 1B) and accumulation of autophagy substrate p62 in POMC neurons from KO mice (Fig 1C). KO mice did not display increased neuronal apoptosis as determined by TdT-mediated dUTP nick end labelling staining (Fig 1D) or POMC

[1]Department of Medicine,
[2]Institute for Aging Research,
[3]Department of Molecular Pharmacology,
[4]Diabetes Research Center, and
[5]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, USA
*These authors contributed equally to this work
+Corresponding author. Tel: +1 718 430 4118; Fax: +1 718 430 8557;
E-mail: rajat.singh@einstein.yu.edu

Received 20 September 2011; revised 16 December 2011; accepted 16 December 2011; published online 17 January 2012

Regulation of lipogenesis by cyclin-dependent kinase 8–mediated control of SREBP-1

Xiaoping Zhao,[1,2] Daorong Feng,[1,3] Qun Wang,[4] Arian Abdulla,[1,2] Xiao-Jun Xie,[4] Jie Zhou,[5] Yan Sun,[6] Ellen S. Yang,[1,2] Lu-Ping Liu,[7] Bhavapriya Vaitheesvaran,[1] Lauren Bridges,[4] Irwin J. Kurland,[1] Randy Strich,[8] Jian-Quan Ni,[7] Chenguang Wang,[5] Johan Ericsson,[9] Jeffrey E. Pessin,[1,3] Jun-Yuan Ji,[4] and Fajun Yang[1,2]

[1]Department of Medicine, Division of Endocrinology, Diabetes Research and Training Center, [2]Department of Developmental and Molecular Biology, and [3]Department of Molecular Pharmacology, Albert Einstein College of Medicine, New York, New York, USA. [4]Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center, College Station, Texas, USA. [5]Department of Cancer Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, USA. [6]Department of Geriatrics, Zhongshan Hospital of Fudan University, Shanghai, China. [7]Gene Regulation Laboratory and Tsinghua Fly Center, Tsinghua University, Beijing, China. [8]Department of Molecular Biology, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey, USA. [9]Conway Institute, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland.

Altered lipid metabolism underlies several major human diseases, including obesity and type 2 diabetes. However, lipid metabolism pathophysiology remains poorly understood at the molecular level. Insulin is the primary stimulator of hepatic lipogenesis through activation of the SREBP-1c transcription factor. Here we identified cyclin-dependent kinase 8 (CDK8) and its regulatory partner cyclin C (CycC) as negative regulators of the lipogenic pathway in *Drosophila*, mammalian hepatocytes, and mouse liver. The inhibitory effect of CDK8 and CycC on de novo lipogenesis was mediated through CDK8 phosphorylation of nuclear SREBP-1c at a conserved threonine residue. Phosphorylation by CDK8 enhanced SREBP-1c ubiquitination and protein degradation. Importantly, consistent with the physiologic regulation of lipid biosynthesis, CDK8 and CycC proteins were rapidly downregulated by feeding and insulin, resulting in decreased SREBP-1c phosphorylation. Moreover, overexpression of CycC efficiently suppressed insulin and feeding–induced lipogenic gene expression. Taken together, these results demonstrate that CDK8 and CycC function as evolutionarily conserved components of the insulin signaling pathway in regulating lipid homeostasis.

Introduction

Dysregulation of lipid metabolism is closely associated with major human diseases such as obesity, type 2 diabetes, and cardiovascular disease (1–4). However, the physiologic and pathophysiologic regulation of lipid metabolism is a highly complex and integrative process that remains poorly understood at the molecular level. Among the known lipogenic regulators, the SREBP transcription factors are pivotal activators of key enzymes responsible for hepatic biosynthesis of fatty acids and cholesterol (5–8) and play an important role in the development of fatty liver and dyslipidemia (9).

The three mammalian SREBP transcription factors, SREBP-1a, -1c, and -2, are synthesized as inactive precursors that are tethered to the ER membrane (10). Reduction of intracellular sterols results in the transportation of SREBP-2 to the Golgi, where it undergoes proteolytic maturation. Then, the N-terminal fragment of SREBP-2 translocates into the nucleus and activates transcription of target genes (11). Similarly, the two SREBP-1 isoforms, SREBP-1a and -1c, are also processed in the Golgi to generate the mature SREBP-1 protein (12, 13). Unlike SREBP-2, SREBP-1c is primarily activated by insulin (14). Both SREBP-1a and SREBP-1c proteins are produced by the same gene, *SREBF1*, by two distinct promoters and alternative splicing (15). Their amino acid sequences differ only at the very N-terminal end. The unique region of SREBP-1a is part of its transactivation domain (16). SREBP-1a and SREBP-1c have different expression profiles: SREBP-1a is highly expressed in proliferating cells, such as cancer cells, while SREBP-1c is the predominant form in normal cells, particularly hepatocytes (17).

CDK8 and its regulatory partner CycC have been reported as being subunits of the Mediator complexes in mammalian cells (18, 19). The mammalian Mediators are large protein complexes containing up to 30 distinct subunits, depending on starting materials and biochemical purification protocols, and play a critical role in bridging the signals from transcription factors to the basal transcription apparatus (20–22). Biochemical purifications have identified at least two groups of Mediator complexes, the small Mediator and the large Mediator, with the latter containing an extra submodule of CDK8, CycC, MED12, and MED13 (19). In chromatin-based in vitro transcription assays, the small Mediator can activate gene transcription, while the large Mediator is inactive (19). Interestingly, recent studies have established a role of CDK8-CycC in tumorigenesis (20). However, the in vivo functions and regulation of CDK8 and CycC are still poorly understood.

In this study, we identify CDK8 and CycC as key repressors for lipogenic gene expression, de novo lipogenesis, and lipid accumulation in *Drosophila* and mammals. This function of CDK8-CycC occurs through site-specific phosphorylation on nuclear SREBP-1c protein, resulting in rapid degradation of this central regulator of lipid metabolism. Since CDK8 and CycC are reduced upon feeding or by insulin, concomitant with increased levels of nuclear SREBP-1c protein, the CDK8-CycC complex appears to function downstream of the insulin signaling as a key regulator of de novo lipogenesis.

Authorship note: Xiaoping Zhao, Daorong Feng, and Qun Wang contributed equally to this work.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J Clin Invest.* 2012;122(7):2417–2427. doi:10.1172/JCI61462.

nature cell biology

IKKβ/NF-κB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes

Juxue Li[1,2,3], Yizhe Tang[1,2,3] and Dongsheng Cai[1,2,3,4]

Adult neural stem cells (NSCs) are known to exist in a few regions of the brain; however, the entity and physiological/disease relevance of adult hypothalamic NSCs (htNSCs) remain unclear. This work shows that adult htNSCs are multipotent and predominantly present in the mediobasal hypothalamus of adult mice. Chronic high-fat-diet feeding led to not only depletion but also neurogenic impairment of htNSCs associated with IKKβ/NF-κB activation. *In vitro* htNSC models demonstrated that their survival and neurogenesis markedly decreased on IKKβ/NF-κB activation but increased on IKKβ/NF-κB inhibition, mechanistically mediated by IKKβ/NF-κB-controlled apoptosis and Notch signalling. Mouse studies revealed that htNSC-specific IKKβ/NF-κB activation led to depletion and impaired neuronal differentiation of htNSCs, and ultimately the development of obesity and pre-diabetes. In conclusion, adult htNSCs are important for the central regulation of metabolic physiology, and IKKβ/NF-κB-mediated impairment of adult htNSCs is a critical neurodegenerative mechanism for obesity and related diabetes.

Adult neurogenesis, a once unconventional concept, is now an impelling topic in neuroscience. Recent research has shown that the adult central nervous system contains NSCs that generate neural cells, including neurons, astrocytes and oligodendrocytes[1–15]. Adult NSCs are present predominantly in the sub-ventricular zone (SVZ) of the forebrain and the sub-granular zone (SGZ) of the hippocampal dentate gyrus[1–15]. With regard to the hypothalamus, 5-bromodeoxyuridine (BrdU) labelling showed postnatal neurogenic activities in the hypothalamus of mice in CNTF-stimulated[16] or basal[17] conditions, and cell proliferation was reported[18] in the hypothalamus of mutant mice with agouti-related peptide (AGRP) neuronal degeneration. More recently, postnatal turnover of arcuate neurons and its impairment in obesity conditions was demonstrated[19], and, on the basis of observations of newborn pups or pre-adult young mice, it was reported[20] that tanycytes in the median eminence are important for postnatal hypothalamic neurogenesis. We are interested in understanding the identity and characteristics of adult htNSCs at post-development ages and their physiological actions and especially their disease relevance. It is known that environmental changes such as chronic high-fat-diet (HFD) feeding induce hypothalamic dysfunctions causing and promoting obesity and diabetes. Recent research revealed that the proinflammatory pathway involving IκB kinase β (IKKβ) and the downstream nuclear factor-κB (NF-κB) mediates HFD-induced hypothalamic inflammation, causing a metabolic syndrome[21–25]. It is of note that in addition to being an inflammatory regulator, IKKβ/NF-κB controls cell survival, growth, apoptosis and differentiation in a cell-specific manner[26–29]. Whereas IKKβ/NF-κB can be either pro-survival or anti-survival depending on the cell type and conditions[30,31], inflammatory changes such as those induced by brain microglia have been found to inhibit neurogenesis[32–38].

Metabolic physiological activities including feeding, body weight and glucose homeostasis are critically regulated by the mediobasal hypothalamus (MBH), which comprises the arcuate nucleus (ARC) and the ventral medial hypothalamic region. This regulation is primarily mediated by the balance between anorexigenic neurons expressing proopiomelanocortin (POMC) and orexigenic neurons expressing neuropeptide Y (NPY) and AGRP. In normal physiological conditions, increased systemic levels of nutrients and related hormones can act in the hypothalamus to activate the POMC neurons but inhibit the NPY/AGRP neurons. As a result, appetite is suppressed and energy expenditure is enhanced to maintain body weight and metabolic balance. However, under an obesity-prone environment such as chronic HFD feeding, POMC neurons are significantly impaired, exhibiting reduced sensitivities to hormones such as insulin and leptin, leading to the onset of central insulin and leptin resistance, which is known to be a critical neural mechanism for obesity and related

[1]Department of Molecular Pharmacology, Bronx, New York 10461, USA. [2]Diabetes Research and Training Center, Bronx, New York 10461, USA. [3]Institute of Aging, Albert Einstein College of Medicine, Bronx, New York 10461, USA.
[4]Correspondence should be addressed to D.C. (e-mail: dongsheng.cai@einstein.yu.edu)

Received 27 April 2012; accepted 19 July 2012; published online 2 September 2012; DOI: 10.1038/ncb2562

Cell Metabolism
Article

Distinct Hypothalamic Neurons Mediate Estrogenic Effects on Energy Homeostasis and Reproduction

Yong Xu,[1,2,5] Thekkethil P. Nedungadi,[3,5] Liangru Zhu,[1] Nasim Sobhani,[3] Boman G. Irani,[3] Kathryn E. Davis,[3] Xiaorui Zhang,[1] Fang Zou,[1] Lana M. Gent,[3] Lisa D. Hahner,[3] Sohaib A. Khan,[4] Carol F. Elias,[2] Joel K. Elmquist,[2] and Deborah J. Clegg[3,*]

[1]Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA
[2]Division of Hypothalamic Research
[3]Touchstone Diabetes Center
Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[4]Department of Cancer and Cell Biology, Vontz Center for Molecular Studies, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA
[5]These authors contributed equally to this work
*Correspondence: deborah.clegg@utsouthwestern.edu
DOI 10.1016/j.cmet.2011.08.009

SUMMARY

Estrogens regulate body weight and reproduction primarily through actions on estrogen receptor-α (ERα). However, ERα-expressing cells mediating these effects are not identified. We demonstrate that brain-specific deletion of ERα in female mice causes abdominal obesity stemming from both hyperphagia and hypometabolism. Hypometabolism and abdominal obesity, but not hyperphagia, are recapitulated in female mice lacking ERα in hypothalamic steroidogenic factor-1 (SF1) neurons. In contrast, deletion of ERα in hypothalamic pro-opiomelanocortin (POMC) neurons leads to hyperphagia, without directly influencing energy expenditure or fat distribution. Further, simultaneous deletion of ERα from both SF1 and POMC neurons causes hypometabolism, hyperphagia, and increased visceral adiposity. Additionally, female mice lacking ERα in SF1 neurons develop anovulation and infertility, while POMC-specific deletion of ERα inhibits negative feedback regulation of estrogens and impairs fertility in females. These results indicate that estrogens act on distinct hypothalamic ERα neurons to regulate different aspects of energy homeostasis and reproduction.

INTRODUCTION

Ovarian estrogens exert important antiobesity effects in women and female mammals. Lower levels of estrogens in postmenopausal women or in ovariectomized (OVX) animals are associated with obesity (Carr, 2003; Rogers et al., 2009). Estradiol-17β replacement in rodents prevents OVX-induced obesity by decreasing food intake and increasing energy expenditure (Gao et al., 2007). Hormone replacement therapy reverses the progression of obesity and metabolic dysfunctions in postmenopausal women (Wren, 2009). However, current hormone replacement therapy is often associated with increased prevalence of heart disease and breast cancer (Billeci et al., 2008). Because estrogens have both positive and negative effects on disease progression which are likely mediated by estrogen receptors (ERs) expressed in a variety of tissues, identification of the critical ERs and their sites of action is imperative in order to develop selective estrogen-based therapies which can selectively treat diseases associated with obesity.

Effects of estrogens on energy balance are primarily mediated by estrogen receptor-α (ERα), as women or female mice with mutations in the ERα gene display hyperadiposity (Heine et al., 2000; Okura et al., 2003), characteristically seen in postmenopausal women and OVX animals. However, the critical ERα sites that mediate estrogenic effects on energy homeostasis have not been identified. In the present study, we generated genetic mouse models with ERα selectively deleted in the central nervous system (CNS), in hypothalamic steroidogenic factor-1 (SF1) neurons, in pro-opiomelanocortin (POMC) neurons, or in both SF1 and POMC neurons, respectively. These models allowed us to identify ERα neuronal populations that regulate food intake, energy expenditure, fat distribution, and reproduction.

RESULTS

Loss of CNS ERα Impairs Multiple Aspects of Energy Homeostasis

Validation

To determine if CNS ERα is required for body weight control, we crossed mice carrying loxP-flanked ERα alleles ($ER\alpha^{lox/lox}$) to the Nestin-Cre transgenic mice. These crosses produced mice lacking ERα in most brain regions ($ER\alpha^{lox/lox}$/Nestin-Cre) and their control littermates ($ER\alpha^{lox/lox}$). Using immunohistochemistry, we demonstrated an almost complete absence of ERα in the hypothalamus (and other brain regions) in the $ER\alpha^{lox/lox}$/Nestin-Cre mice (see Figure S1 available online).

Increased Body Weight, Adiposity, and Visceral Fat Distribution

Compared to controls, both male and female $ER\alpha^{lox/lox}$/Nestin-Cre mice displayed significant increases in body weight

Cell Metabolism
Article

Ablation of Steroid Receptor Coactivator-3 Resembles the Human CACT Metabolic Myopathy

Brian York,[1,9] Erin L. Reineke,[1,9] Jørn V. Sagen,[2,3] Bryan C. Nikolai,[1] Suoling Zhou,[1] Jean-Francois Louet,[1] Atul R. Chopra,[1] Xian Chen,[1] Graham Reed,[4] Jeffrey Noebels,[4] Adekunle M. Adesina,[5] Hui Yu,[6] Lee-Jun C. Wong,[6] Anna Tsimelzon,[7] Susan Hilsenbeck,[7] Robert D. Stevens,[8] Brett R. Wenner,[8] Olga Ilkayeva,[8] Jianming Xu,[1] Christopher B. Newgard,[8,9] and Bert W. O'Malley[1,9,*]

[1]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA
[2]Institute of Medicine, University of Bergen, Bergen, Norway N-5021
[3]The Hormone Laboratory, Haukeland University Hospital, Bergen, Norway N-5021
[4]Department of Neurology, Baylor College of Medicine, Houston, TX 77030, USA
[5]Department of Pathology, Texas Children's Hospital, Houston, TX 77030, USA
[6]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA
[7]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX 77030, USA
[8]Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical School, Durham, NC 27704, USA
[9]These authors contributed equally to this work
*Correspondence: berto@bcm.edu
DOI 10.1016/j.cmet.2012.03.020

SUMMARY

Oxidation of lipid substrates is essential for survival in fasting and other catabolic conditions, sparing glucose for the brain and other glucose-dependent tissues. Here we show Steroid Receptor Coactivator-3 (SRC-3) plays a central role in long chain fatty acid metabolism by directly regulating carnitine/acyl-carnitine translocase (*CACT*) gene expression. Genetic deficiency of *CACT* in humans is accompanied by a constellation of metabolic and toxicity phenotypes including hypoketonemia, hypoglycemia, hyperammonemia, and impaired neurologic, cardiac and skeletal muscle performance, each of which is apparent in mice lacking SRC-3 expression. Consistent with human cases of CACT deficiency, dietary rescue with short chain fatty acids drastically attenuates the clinical hallmarks of the disease in mice devoid of SRC-3. Collectively, our results position SRC-3 as a key regulator of β-oxidation. Moreover, these findings allow us to consider platform coactivators such as the SRCs as potential contributors to syndromes such as CACT deficiency, previously considered as monogenic.

INTRODUCTION

During fasting or prolonged exercise, mitochondrial fatty acid metabolism is activated in skeletal muscle to protect glucose-dependent tissues such as the brain. This metabolic switch in skeletal muscle is controlled by tightly coordinated transcriptional and allosteric regulatory events that prime the mitochondrial β-oxidation machinery for utilization of lipid substrates. During periods of exertion when energy demand exceeds the mitochondrial capacity to deliver ATP, the muscle reverts to a less efficient anaerobic pathway, eventually depleting the body of glucose and forcing the cessation of exercise.

The mitochondrial β-oxidation system encompasses a set of at least 25 enzymes and transport proteins to facilitate highly efficient harvesting of energy from fatty acids (Eaton et al., 1996). To date, deficiencies in 22 of these proteins have been directly linked to a variety of human metabolic diseases (Moczulski et al., 2009). While there are several points of regulation for fatty acid metabolism, a critical step is the transport of fatty acids into the mitochondria. This process is governed by the carnitine transport system, where the enzymes carnitine palmitoyltransferase I and II (CPTI and CPTII) are functionally linked to the activity of CACT, making this translocase a central regulator of fatty acid metabolism (McGarry and Brown, 1997). Akin to other defects in β-oxidation, deficits in *CACT* gene expression or activity present as a constellation of clinical symptoms arising from energy depletion and toxicity of accumulating lipid species and other metabolites (Röschinger et al., 2000; Rubio-Gozalbo et al., 2004). Specifically, patients with CACT deficiency display elevated plasma acyl-carnitines (AC), hypoketotic hypoglycemia, hyperammonemia, ventricular arrhythmia, seizures, and progressive muscle weakness.

While the clinical manifestations of CACT deficiency have been well documented, mechanisms that control *CACT* gene expression remain poorly understood. Our current understanding is that metabolic adaptability of skeletal muscle is achieved through transcriptional networks that coordinate gene expression with fuel availability. Thus, transcription factors including PPARα, PPARδ, and FOXO along with coregulators such as PGC-1α regulate skeletal muscle fatty acid metabolism (Holloway et al., 2009). Another family of modulators with emergent influence on metabolic transcriptional responses are the Steroid Receptor Coactivators (SRCs) (York and O'Malley, 2010). The SRCs perform pleiotropic functions in diverse metabolic processes such as glycogenolysis, gluconeogenesis, dietary fat absorption and lipid storage (York and O'Malley, 2010).

Diabetologia (2011) 54:3093–3100
DOI 10.1007/s00125-011-2317-z

ARTICLE

Exenatide decreases hepatic fibroblast growth factor 21 resistance in non-alcoholic fatty liver disease in a mouse model of obesity and in a randomised controlled trial

S. L. Samson · P. Sathyanarayana · M. Jogi · E. V. Gonzalez · A. Gutierrez · R. Krishnamurthy · R. Muthupillai · L. Chan · M. Bajaj

Received: 12 June 2011 / Accepted: 26 August 2011 / Published online: 29 September 2011

Abstract
Aims/hypothesis Systemic fibroblast growth factor (FGF)21 levels and hepatic FGF21 production are increased in non-alcoholic fatty liver disease patients, suggesting FGF21 resistance. We examined the effects of exenatide on FGF21 in patients with type 2 diabetes and in a diet-induced mouse model of obesity (DIO).
Methods Type 2 diabetes mellitus patients (n=24) on diet and/or metformin were randomised (using a table of random numbers) to receive additional treatment consisting of pioglitazone 45 mg/day or combined therapy with pioglitazone (45 mg/day) and exenatide (10 μg twice daily) for 12 months in an open label parallel study at the Baylor Clinic.
Results Twenty-one patients completed the entire study and were included in the analysis. Pioglitazone treatment (n=10) reduced hepatic fat as assessed by magnetic resonance spectroscopy, despite a significant increase in body weight (Δ=3.7 kg); plasma FGF21 levels did not change (1.9±0.6 to 2.2±0.6 ng/ml [mean±SEM]). However, combined pioglitazone and exenatide therapy (n=11) was associated with a significant reduction of FGF21 levels (2.3±0.5 to 1.1±0.3 ng/ml) and a greater decrease in hepatic fat. Besides weight gain observed in the pioglitazone-treated patients, lower extremity oedema was observed as a side effect in two of the ten patients. Three patients who received pioglitazone and exenatide combination therapy complained of significant nausea that was self-limiting and did not require them to leave the study. In DIO mice, exendin-4 for 4 weeks significantly reduced hepatic triacylglycerol content, decreased hepatic FGF21 protein and mRNA, and enhanced phosphorylation of hepatic AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase, although no significant difference in weight and body fat was observed. Hepatic FGF21 correlated inversely with hepatic AMPK phosphorylation
Conclusions/interpretation In type 2 diabetes mellitus, combined pioglitazone and exenatide therapy is associated with a reduction in plasma FGF21 levels, as well as a greater decrease in hepatic fat than that achieved with pioglitazone therapy. In DIO mice, exendin-4 treatment reduces hepatic triacylglycerol and FGF21 protein, and enhances hepatic AMPK phosphorylation, suggesting an improvement of hepatic FGF21 resistance.

Trial registration number: ClinicalTrials.gov NCT 01432405
Funding: American Diabetes Association, the Ron MacDonald Foundation at St Luke's Episcopal Hospital, Amylin Pharmaceuticals, Eli-Lilly, NIH Molecular Medicine Scholars Training Grant, NIH Diabetes and Endocrinology Research Center (DERC) at Baylor College of Medicine, and the T.T. and W.F. Chao Foundation.

S.L. Samson and P. Sathyanarayana contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-011-2317-z) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

S. L. Samson · P. Sathyanarayana · E. V. Gonzalez · A. Gutierrez · L. Chan · M. Bajaj (✉)
Diabetes and Endocrinology Research Center,
Endocrinology Division, Baylor College of Medicine,
1709 Dryden Street,
Houston, TX 77030, USA
e-mail: mandeepbajaj@hotmail.com

S. L. Samson · M. Jogi · A. Gutierrez · L. Chan · M. Bajaj
Departments of Medicine, St Luke's Episcopal Hospital,
Houston, TX, USA

R. Krishnamurthy · R. Muthupillai
Department of Radiology, St Luke's Episcopal Hospital,
Houston, TX, USA

ARTICLE

doi:10.1038/nature10777

A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis

Pontus Boström[1], Jun Wu[1], Mark P. Jedrychowski[2], Anisha Korde[1], Li Ye[1], James C. Lo[1], Kyle A. Rasbach[1], Elisabeth Almer Boström[3], Jang Hyun Choi[1], Jonathan Z. Long[1], Shingo Kajimura[4], Maria Cristina Zingaretti[5], Birgitte F. Vind[6], Hua Tu[7], Saverio Cinti[5], Kurt Højlund[6], Steven P. Gygi[2] & Bruce M. Spiegelman[1]

Exercise benefits a variety of organ systems in mammals, and some of the best-recognized effects of exercise on muscle are mediated by the transcriptional co-activator PPAR-γ co-activator-1 α (PGC1-α). Here we show in mouse that PGC1-α expression in muscle stimulates an increase in expression of FNDC5, a membrane protein that is cleaved and secreted as a newly identified hormone, irisin. Irisin acts on white adipose cells in culture and *in vivo* to stimulate UCP1 expression and a broad program of brown-fat-like development. Irisin is induced with exercise in mice and humans, and mildly increased irisin levels in the blood cause an increase in energy expenditure in mice with no changes in movement or food intake. This results in improvements in obesity and glucose homeostasis. Irisin could be therapeutic for human metabolic disease and other disorders that are improved with exercise.

PGC1-α is a transcriptional co-activator that mediates many biological programs related to energy metabolism. Originally described as a co-activator of PPAR-γ that modulated expression of uncoupling protein 1 (UCP1) and thermogenesis in brown fat[1], it has also been shown to control mitochondrial biogenesis and oxidative metabolism in many cell types. PGC1-α is induced in muscle by exercise and stimulates many of the best-known beneficial effects of exercise in muscle: mitochondrial biogenesis, angiogenesis and fibre-type switching[2]. It also provides resistance to muscular dystrophy and denervation-linked muscular atrophy[3]. The health benefits of elevated muscle expression of PGC1-α may go beyond the muscle tissue itself. Transgenic mice with mildly elevated muscle PGC1-α are resistant to age-related obesity and diabetes and have a prolonged lifespan[4]. This suggests that PGC1-α stimulates the secretion of factors from skeletal muscle that affect the function of other tissues. Here we show that PGC1-α stimulates the expression of several muscle gene products that are potentially secreted, including FNDC5. The *Fndc5* gene encodes a type I membrane protein that is processed proteolytically to form a newly identified hormone secreted into the blood, termed irisin. Irisin is induced in exercise and activates profound changes in the subcutaneous adipose tissue, stimulating browning and UCP1 expression. Importantly, this causes a significant increase in total body energy expenditure and resistance to obesity-linked insulin resistance. Thus, irisin action recapitulates some of the most important benefits of exercise and muscle activity.

Muscle PGC1-α transgenics

Mice with transgenically increased PGC1-α in muscle are resistant to age-related obesity and diabetes[4], suggesting that these animals have a fundamental alteration in systemic energy balance. We therefore analysed the adipose tissue of the PGC1-α transgenic mice for expression of genes related to a thermogenic gene program and genes characteristic of brown fat development. There were no significant alterations in the expression of brown-fat-selective genes in the interscapular brown adipose tissue or in the visceral (epididymal) white adipose tissue (Fig. 1a). However, the subcutaneous fat layer (inguinal), a white adipose tissue that is particularly prone to 'browning' (that is, formation of multilocular, UCP1-positive adipocytes), had significantly increased levels of *Ucp1* and *Cidea* messenger RNAs (Fig. 1b). We also observed increased UCP1 protein levels and more UCP1-positive stained multilocular cells in transgenic mice compared to controls (Fig. 1c, d). There are recent reports that exercise causes a mild increase in the expression of a thermogenic gene program in the visceral adipose tissue, a depot that has minimal expression of these genes[5]. As it is the subcutaneous white adipose depot that has the greatest tendency to turn on a thermogenic gene program and alter the systemic energy balance of mice[6], we re-investigated this with regard to browning of the white adipose tissues in two types of exercise. Similar to what has been reported[5], a twofold increase in *Ucp1* mRNA expression was observed in the visceral, epididymal fat with 3 weeks of wheel running (Supplementary Fig. 1). However, a much larger change (approximately 25 fold) was seen in the same mice in the subcutaeneous inguinal fat depot. Similarly, a small increase in *Ucp1* mRNA expression was seen in the epididymal fat with repeated bouts of swimming in warm (32 °C) water (Supplementary Fig. 1); however a very large increase (65 fold) was observed in the inguinal white depot (Supplementary Fig. 1). Thus, muscle-specific expression of PGC1α drives browning of subcutaneous white adipose tissue, possibly recapitulating part of the exercise program.

Media from PGC1-α-expressing myocytes

The effect on browning of the adipose tissues from PGC1-α-expressing muscle could be due to direct muscle–fat signalling or to another,

[1]Dana-Farber Cancer Institute and Harvard Medical School, 3 Blackfan Circle, CLS Building, Floor 11, Boston, Massachusetts 02115, USA. [2]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA. [3]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA. [4]UCSF Diabetes Center and Department of Cell and Tissue Biology, University of California, San Francisco, California 94143, USA. [5]Department of Experimental and Clinical Medicine, Università Politecnica delle Marche, Electron Microscopy Unit-Azienda Ospedali Riuniti, Ancona 60020, Italy. [6]Diabetes Research Center, Department of Endocrinology, Odense University Hospital, DK-5000, Odense, Denmark. [7]LakePharma, Inc., 530 Harbor Blvd, Belmont, California 94002, USA.

ARTICLE

doi:10.1038/nature10986

A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism

Mark A. Herman[1], Odile D. Peroni[1], Jorge Villoria[1], Michael R. Schön[2], Nada A. Abumrad[3], Matthias Blüher[4], Samuel Klein[3] & Barbara B. Kahn[1]

The prevalence of obesity and type 2 diabetes is increasing worldwide and threatens to shorten lifespan. Impaired insulin action in peripheral tissues is a major pathogenic factor. Insulin stimulates glucose uptake in adipose tissue through the GLUT4 (also known as SLC2A4) glucose transporter, and alterations in adipose tissue GLUT4 expression or function regulate systemic insulin sensitivity. Downregulation of human and mouse adipose tissue GLUT4 occurs early in diabetes development. Here we report that adipose tissue GLUT4 regulates the expression of carbohydrate-responsive-element-binding protein (ChREBP; also known as MLXIPL), a transcriptional regulator of lipogenic and glycolytic genes. Furthermore, adipose ChREBP is a major determinant of adipose tissue fatty acid synthesis and systemic insulin sensitivity. We find a new mechanism for glucose regulation of ChREBP: glucose-mediated activation of the canonical ChREBP isoform (ChREBP-α) induces expression of a novel, potent isoform (ChREBP-β) that is transcribed from an alternative promoter. *ChREBP-β* expression in human adipose tissue predicts insulin sensitivity, indicating that it may be an effective target for treating diabetes.

Insulin resistance is a common complication of obesity and a major factor in the pathogenesis of type 2 diabetes and cardiovascular disease[1]. Adipose tissue contributes to the development of obesity-related insulin resistance through increased release of fatty acids, altered adipokine secretion, and/or macrophage infiltration and cytokine release[2,3]. Altered adipose tissue glucose metabolism is also an important cause of insulin resistance, and adipose tissue GLUT4, the major insulin-responsive glucose transporter, has a central role in systemic glucose metabolism[1,4,5]. In insulin-resistant states, GLUT4 is downregulated in adipose tissue, but not in muscle[1], the major site of insulin-stimulated glucose uptake. In addition, mice with adipose-specific GLUT4 overexpression (AG4OX) have improved glucose homeostasis[5] whereas adipose-specific GLUT4 knockout mice (AG4KO) have insulin resistance and type 2 diabetes[4]. We investigated how altering adipose tissue glucose flux regulates glucose homeostasis. We show that ChREBP, a glucose-responsive transcription factor that regulates fatty acid synthesis and glycolysis[6], is highly regulated by GLUT4 in adipose tissue and is a key determinant of systemic insulin sensitivity and glucose homeostasis. Also, ChREBP in adipose tissue is required for the improved glucose homeostasis resulting from increased adipose GLUT4 expression. GLUT4-mediated glucose uptake induces ChREBP, which activates adipose tissue *de novo* lipogenesis (DNL). The latter is associated with enhanced insulin sensitivity[7–10]. In obese humans, adipose *ChREBP* gene expression correlates with insulin sensitivity, suggesting that ChREBP protects against obesity-associated insulin resistance. In addition, we discovered a novel mechanism for glucose-regulated *ChREBP* expression involving a new isoform, *ChREBP-β*, which is expressed from an alternative promoter in a glucose- and ChREBP-dependent manner. In contrast, expression of the canonical *ChREBP-α* isoform is not regulated by glucose flux. However, glucose-induced ChREBP-α transcriptional activity increases *ChREBP-β* expression. Furthermore, expression of *ChREBP-β* is more highly regulated than *ChREBP-α* in adipose tissue in insulin-resistant states. Thus, activation of adipose ChREBP, and particularly ChREBP-β, may be a novel strategy for preventing and treating obesity-related metabolic dysfunction and type 2 diabetes (Supplementary Fig. 1).

Glucose regulates adipose tissue ChREBP

To understand the mechanisms by which adipocytes respond to changes in glucose flux, we analysed global gene expression in adipose tissue from AG4OX and AG4KO mice. Gene-set enrichment analysis[11] demonstrated coordinate upregulation of DNL enzymes in AG4OX mice (Supplementary Table 1), which we confirmed (Fig. 1a). DNL enzymes were downregulated in AG4KO mice (Fig. 1a). Therefore we investigated the expression of the two major transcription factors known to regulate DNL enzymes, sterol regulatory-element-binding-protein 1c (*SREBP-1c*; also known as isoform 1c of *SREBF1*) and *ChREBP*[6,12]. *ChREBP*, but not *SREBP-1c*, expression is increased 50% in AG4OX and decreased 44% in AG4KO adipose tissue compared to controls (Fig. 1b). SREBP-1c transcriptional activity is primarily determined by the accumulation of mature SREBP-1c in the nucleus[13]. However, the nuclear abundance of SREBP-1c is not increased in AG4OX adipose tissue (Supplementary Fig. 2a). Liver X receptors (LXR-α and LXR-β; also known as NR1H3 and NR1H2, respectively) can regulate the expression of both *ChREBP* and *SREBP-1c*[14,15], and DNL enzymes[16]. Expression of canonical LXR targets do not change (Supplementary Fig. 2b) in AG4OX or AG4KO adipose tissue, indicating that LXR activity is unaffected and is not driving the changes in *ChREBP* or DNL enzyme expression. In contrast, expression of *Rgs16* and *Txnip*, two ChREBP transcriptional targets[17–19] not known to be regulated by other lipogenic transcription factors, were reciprocally regulated in AG4OX and AG4KO mice (Supplementary Fig. 2c). In AG4KO and control mice (Fig. 1c), and also in 30 different mouse strains (Supplementary Fig. 3), adipose *ChREBP* expression strongly correlates with *GLUT4* expression. Expression of *ChREBP* transcriptional targets *FAS* (also known as *Fasn*) and *Acc1* (also known as

[1]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215, USA. [2]Städtisches Klinikum Karlsruhe, Clinic of Visceral Surgery, Karlsruhe 76133, Germany. [3]Center for Human Nutrition, Washington University School of Medicine, St Louis, Missouri 63110, USA. [4]Department of Medicine, University of Leipzig, Leipzig 04103, Germany.

Blood Glucose Control in Type 1 Diabetes With a Bihormonal Bionic Endocrine Pancreas

Steven J. Russell, md, phd[1]
Firas H. El-Khatib, phd[2]
David M. Nathan, md[1]
Kendra L. Magyar, msn, np[1]
John Jiang, bs[2]
Edward R. Damiano, phd[2]

OBJECTIVE—To test whether safe and effective glycemic control could be achieved in type 1 diabetes using a bihormonal bionic endocrine pancreas driven by a continuous glucose monitor in experiments lasting more than two days and including six high-carbohydrate meals and exercise as challenges to glycemic control.

RESEARCH DESIGN AND METHODS—Six subjects with type 1 diabetes and no endogenous insulin secretion participated in two 51-h experiments. Blood glucose was managed with a bionic endocrine pancreas controlling subcutaneous delivery of insulin and glucagon with insulin pumps. A partial meal-priming bolus of insulin (0.035 units/kg/meal, then 0.05 units/kg per meal in repeat experiments) was administered at the beginning of each meal (on average 78 ± 12 g of carbohydrates per meal were consumed). Plasma glucose (PG) control was evaluated with a reference quality measurement on venous blood every 15 min.

RESULTS—The overall mean PG was 158 mg/dL, with 68% of PG values in the range of 70–180 mg/dL. There were no significant differences in mean PG between larger and smaller meal-priming bolus experiments. Hypoglycemia (PG <70 mg/dL) was rare, with eight incidents during 576 h of closed-loop control (0.7% of total time). During 192 h of nighttime control, mean PG was 123 mg/dL, with 93% of PG values in the range of 70–180 mg/dL and only one episode of mild hypoglycemia (minimum PG 62 mg/dL).

CONCLUSIONS—A bihormonal bionic endocrine pancreas achieved excellent glycemic control with minimal hypoglycemia over the course of 2 days of continuous use despite high-carbohydrate meals and exercise. A trial testing a wearable version of the system under free-living conditions is justified.

Development of a fully or semiautomated device that achieves glycemic levels demonstrated to reduce long-term complications (1–4) while lowering the risk for hypoglycemia (5) and reducing patient burden has long been a goal in the treatment of type 1 diabetes and would improve quality of life for people with type 1 diabetes. We previously demonstrated the feasibility of safe and effective bihormonal therapy with subcutaneous insulin and glucagon directed by a computer algorithm using frequently sampled venous plasma glucose (PG) in sedentary subjects over the course of 27 h (6). In the same study we also compared the accuracy and reliability of three commercially available continuous glucose monitors (CGMs) in each subject. Based on these results and preclinical studies in diabetic pigs, we hypothesized that glycemic control could be achieved in humans with type 1 diabetes using glucose values from one of these CGMs as the sole input to the controller. Here, we report the results of a study testing this hypothesis in experiments more than 2 days in length that included six high-carbohydrate meals and a period of exercise as challenges to glycemic control. Subcutaneous dosing of glucagon and insulin was controlled by an algorithm requiring only the subject weight for initialization.

RESEARCH DESIGN AND METHODS

Subjects

The protocol was approved by the Massachusetts General Hospital (MGH) and Boston University Human Research Committees and all participants gave written informed consent. At baseline, subjects were required to be 18 years of age or older, have had type 1 diabetes for at least 1 year, and be treated with an insulin pump. In addition, glycated hemoglobin A_{1c} (HbA_{1c}) had to be ≤9.0%, BMI had to be 20–35 kg/m^2, daily insulin requirement was ≤1 units/kg/day, and a peak stimulated C-peptide level had to be ≤0.1 nmol/L after a mixed meal tolerance test. Other criteria are detailed in the Supplementary Data.

Closed-loop glucose control system

Insulin and glucagon were administered under closed-loop control (Supplementary Fig. 1) using control algorithms similar to those previously described (6). The only input signal was data from the Freestyle Navigator (Abbott Diabetes Care), an interstitial fluid CGM approved by the United States Food and Drug Administration (FDA). Insulin dosing was controlled by a customized model predictive control algorithm incorporating a pharmacokinetic model for insulin lispro that assumed a t_{max} of 65 min. Glucagon dosing was controlled by a customized proportional derivative algorithm. Insulin lispro and glucagon (Eli Lilly) were administered subcutaneously by OmniPod patch pumps (Insulet). With the exception of a weight-based partial meal-priming bolus, delivered at the beginning of each meal, the system was fully

From the [1]Diabetes Unit and Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; and the [2]Department of Biomedical Engineering, Boston University, Boston, Massachusetts.
Corresponding author: Edward R. Damiano: edamiano@bu.edu.
Received 12 January 2012 and accepted 12 June 2012.
DOI: 10.2337/dc12-0071. Clinical trial reg. no. NCT01161862, clinicaltrials.gov
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc12-0071/-/DC1.
Dr. Russell and Dr. El-Khatib share equal responsibility for this work.

Diabetes Care Publish Ahead of Print, published online August 24, 2012

nature genetics

Generation of functional insulin-producing cells in the gut by *Foxo1* ablation

Chutima Talchai[1–3], Shouhong Xuan[4], Tadahiro Kitamura[5], Ronald A DePinho[6] & Domenico Accili[1,2]

Restoration of regulated insulin secretion is the ultimate goal of therapy for type 1 diabetes. Here, we show that, unexpectedly, somatic ablation of *Foxo1* in Neurog3$^+$ enteroendocrine progenitor cells gives rise to gut insulin-positive (Ins$^+$) cells that express markers of mature β cells and secrete bioactive insulin as well as C-peptide in response to glucose and sulfonylureas. Lineage tracing experiments showed that gut Ins$^+$ cells arise cell autonomously from *Foxo1*-deficient cells. Inducible *Foxo1* ablation in adult mice also resulted in the generation of gut Ins$^+$ cells. Following ablation by the β-cell toxin streptozotocin, gut Ins$^+$ cells regenerate and produce insulin, reversing hyperglycemia in mice. The data indicate that Neurog3$^+$ enteroendocrine progenitors require active Foxo1 to prevent differentiation into Ins$^+$ cells. *Foxo1* ablation in gut epithelium may provide an approach to restore insulin production in type 1 diabetes.

A longstanding goal of regenerative medicine is the identification of genetic, cellular and biochemical pathways governing the generation of insulin-producing β cells, with a view toward enlisting them in ongoing cellular replacement efforts in individuals with type 1 diabetes[1,2]. The process by which primitive endodermal precursors adopt an endocrine fate has been examined in detail[3]. A key step seems to be the formation of Neurog3-expressing (Neurog3$^{+)}$ cells that go on to differentiate into all known pancreatic islet cell types[4–6]. Of note, Neurog3$^+$ endocrine progenitors are not restricted to the pancreas but are found in the stomach and intestine, where they give rise to most cells in the enteroendocrine system, the largest endocrine organ in the body[7,8].

Despite their common endodermal origin, pancreatic and gut Neurog3$^+$ endocrine progenitors share few if any properties, giving rise to cell types that produce distinct peptide hormones and have remarkably different developmental fates and lifespans[9]. Pancreatic endocrine progenitors are formed during embryonic development and do not arise again in the adult organ[10], except under special circumstances[11]. Enteroendocrine progenitors instead continually arise from gut stem cells and contribute to the repopulation of the high-turnover enteroendocrine population[8]. The distinct features of these two cell populations dovetail with the classical theory of positional specification, whereby partly committed progenitor cells acquire position-dependent properties that dictate specific fates[12]. It is unclear how gut Neurog3$^+$ progenitors are restricted to the enteroendocrine versus the pancreatic endocrine fate. Understanding this mechanism would allow investigators to explore the use of gut epithelium as a source for cell replacement therapy in insulin-dependent diabetes, given the self-renewing nature of this tissue.

Foxo transcription factors integrate hormonal and nutrient cues with the cell transcriptional response to regulate diverse cellular processes[13]. In addition to their metabolic functions, Foxo proteins inhibit terminal differentiation in multiple cell types[14–16]. In myoblasts, Foxo1 can affect cell fate decisions in a Notch-dependent manner, leading to the generation of different muscle fiber types[17]. In the pancreas, Foxo1 has an important role in regulating endocrine cell mass[18–20] and β-cell responses to oxidative stress[21,22]. Of note, Foxo1 is coexpressed with Neurog3 in embryonic pancreas, but *Foxo1* ablation does not affect the generation of different pancreatic endocrine cell types[18]. In cultures of human fetal pancreatic epithelium, *FOXO1* knockdown increases the number of NEUROG3$^+$ cells[23]. In contrast, virtually nothing is known about the functional properties of Foxo1-expressing cells in the gut. In this study, we use a combination of genetic and cellular approaches to characterize these cells.

RESULTS

Foxo1 ablation expands *Neurog3*$^+$ enteric progenitors

Foxo1 is expressed in gut epithelial cells, including most Neurog3$^+$ enteroendocrine progenitors (**Supplementary Fig. 1a**). To investigate its role in this cell type, we generated mice with somatic deletion of *Foxo1* in Neurog3$^+$ cells[8] (Neurog3-Cre–driven *Foxo1* knockouts; NKO). To assess Cre-mediated recombination, we intercrossed NKO and *Neurog3-Gfp* transgenic mice (**Supplementary Fig. 1b**). In control studies, we determined that GFP immunoreactivity colocalized with endogenous Neurog3 immunoreactivity (**Supplementary Fig. 1c**). In NKO mice, Foxo1 was no longer detectable in GFP-labeled cells (**Supplementary Fig. 1b**), and *Foxo1* mRNA levels were decreased by >80% in flow-sorted cells from *Neurog3-Gfp* animals,

[1]Naomi Berrie Diabetes Center, Columbia University Medical Center, New York, New York, USA. [2]Department of Medicine, College of Physicians & Surgeons of Columbia University, New York, New York, USA. [3]Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok, Thailand. [4]Department of Genetics and Development, College of Physicians & Surgeons of Columbia University, New York, New York, USA. [5]Metabolic Signal Research Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan. [6]Department of Cancer Biology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA. Correspondence should be addressed to D.A. (da230@columbia.edu).

Received 30 November 2011; accepted 7 February 2012; published online 11 March 2012; doi:10.1038/ng.2215

Cell

Brown Remodeling of White Adipose Tissue by SirT1-Dependent Deacetylation of Pparγ

Li Qiang,[1] Liheng Wang,[1,6] Ning Kon,[2,6] Wenhui Zhao,[2] Sangkyu Lee,[4] Yiying Zhang,[3] Michael Rosenbaum,[3] Yingming Zhao,[4] Wei Gu,[2] Stephen R. Farmer,[5] and Domenico Accili[1,*]
[1]Naomi Berrie Diabetes Center, Department of Medicine
[2]Institute of Cancer Genetics, Department of Pathology
[3]Division of Molecular Genetics, Department of Pediatrics
College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA
[4]Ben May Department for Cancer Research, University of Chicago, Chicago, IL 60637, USA
[5]Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118, USA
[6]These authors contributed equally to this work
*Correspondence: da230@columbia.edu
http://dx.doi.org/10.1016/j.cell.2012.06.027

SUMMARY

Brown adipose tissue (BAT) can disperse stored energy as heat. Promoting BAT-like features in white adipose (WAT) is an attractive, if elusive, therapeutic approach to staunch the current obesity epidemic. Here we report that gain of function of the NAD-dependent deacetylase SirT1 or loss of function of its endogenous inhibitor Deleted in breast cancer-1 (Dbc1) promote "browning" of WAT by deacetylating peroxisome proliferator-activated receptor (Ppar)-γ on Lys268 and Lys293. SirT1-dependent deacetylation of Lys268 and Lys293 is required to recruit the BAT program coactivator Prdm16 to Pparγ, leading to selective induction of BAT genes and repression of visceral WAT genes associated with insulin resistance. An acetylation-defective Pparγ mutant induces a brown phenotype in white adipocytes, whereas an acetylated mimetic fails to induce "brown" genes but retains the ability to activate "white" genes. We propose that SirT1-dependent Pparγ deacetylation is a form of selective Pparγ modulation of potential therapeutic import.

INTRODUCTION

Obesity and its comorbidities pose a growing therapeutic challenge (Wang et al., 2011). White adipose tissue (WAT) is the main "storage site" of excess energy, primarily in the form of triglycerides. In addition, a functionally and morphologically distinct adipocyte subset—whose dense mitochondrial, innervation, and vascular content earned it the moniker of "brown" adipose tissue (BAT)—dissipates energy as heat (nonshivering thermogenesis). Brown adipocytes uncouple mitochondrial electron transport from ATP synthesis to a greater extent than other cells by permeabilizing the inner mitochondrial membrane to allow inter-membrane proton to leak back into the mitochondrial matrix, primarily through uncoupling protein-1 (Ucp1), but also through other mitochondrial proteins (Ravussin and Galgani, 2011). Promoting BAT function has therapeutic potential to combat obesity (Farmer, 2009). But its limited amount and activity in humans are unlikely to offset the positive energy balance associated with excessive WAT deposition (Virtanen and Nuutila, 2011).

As an alternative strategy to increase energy expenditure and prevent weight gain, we investigated mechanisms that confer BAT-like features onto WAT, thus remodeling the latter from an energy storage into an energy-disposal site (Kozak, 2010). The metabolic benefits of this conversion include prevention of diet-induced obesity and increased insulin sensitivity (Seale et al., 2011). Browning of rodent WAT can be brought about by hormones and cytokines, such as Irisin (Boström et al., 2012) and Fgf21 (Fisher et al., 2012), as well as by transcriptional modulation through Prdm16 (Seale et al., 2011), FoxC2 (Cederberg et al., 2001), RIP140 (Powelka et al., 2006), 4E-BP1 (Tsukiyama-Kohara et al., 2001), TIF2 (Picard et al., 2002), pRb and p107 (Scimè et al., 2005). However, there is an unmet need for strategies that would translate these mechanisms into the clinic.

Activation of the nuclear receptor Pparγ by thiazolidinediones (TZDs) can also induce a brown-like phenotype in white adipocytes by promoting expression of brown adipocyte-specific genes (brown genes) and suppressing visceral WAT genes (white genes) (Vernochet et al., 2009). The mechanism of this "browning" effect is unclear, and is unlikely to be clinically applicable without further modulation, in view of the adverse effects associated with TZD use (Kim-Muller and Accili, 2011).

Activation of the NAD^+-dependent deacetylase SirT1 by small molecules, calorie restriction or exercise promotes mitochondrial biogenesis and activities (Cantó et al., 2009; Milne et al., 2007), raising the possibility that SirT1 regulates BAT functions. Furthermore, SirT1 gain-of-function mimics the

Cell

FoxO1 Target Gpr17 Activates AgRP Neurons to Regulate Food Intake

Hongxia Ren,[1] Ian J. Orozco,[2] Ya Su,[3] Shigetomo Suyama,[4] Roger Gutiérrez-Juárez,[3] Tamas L. Horvath,[4] Sharon L. Wardlaw,[1] Leona Plum,[1] Ottavio Arancio,[2] and Domenico Accili[1,*]
[1]Berrie Diabetes Center and Department of Medicine
[2]Taub Institute and Department of Pathology and Cell Biology
Columbia University, New York, NY 10032, USA
[3]Diabetes Research and Training Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[4]Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06519, USA
*Correspondence: da230@columbia.edu
DOI 10.1016/j.cell.2012.04.032

SUMMARY

Hypothalamic neurons expressing Agouti-related peptide (AgRP) are critical for initiating food intake, but druggable biochemical pathways that control this response remain elusive. Thus, genetic ablation of insulin or leptin signaling in AgRP neurons is predicted to reduce satiety but fails to do so. FoxO1 is a shared mediator of both pathways, and its inhibition is required to induce satiety. Accordingly, FoxO1 ablation in AgRP neurons of mice results in reduced food intake, leanness, improved glucose homeostasis, and increased sensitivity to insulin and leptin. Expression profiling of flow-sorted FoxO1-deficient AgRP neurons identifies G-protein-coupled receptor Gpr17 as a FoxO1 target whose expression is regulated by nutritional status. Intracerebroventricular injection of Gpr17 agonists induces food intake, whereas Gpr17 antagonist cangrelor curtails it. These effects are absent in *Agrp-Foxo1* knockouts, suggesting that pharmacological modulation of this pathway has therapeutic potential to treat obesity.

INTRODUCTION

The prevalence of obesity has grown at an alarming rate, reaching pandemic proportions. One-third of U.S. adults are obese, leading to increased numbers of patients affected by the comorbidities of obesity, such as cardiovascular disease, type 2 diabetes, respiratory disorders, and cancer (Wang et al., 2011). Considering the staggering cost and serious challenges to public health associated with this condition, identification of biochemical pathways that can be effectively and safely targeted for obesity therapy has acquired new urgency.

Hypothalamic neurons expressing Agouti-related peptide (AgRP) have been directly implicated in promoting feeding. AgRP neuron activation by either light-gated Channelrhodopsin-2 or ligand-activated G proteins rapidly increases food intake (Aponte et al., 2011; Krashes et al., 2011). Conversely, acute ablation of AgRP neurons in adulthood causes cessation of feeding and results in starvation (Luquet et al., 2005). Despite the established functional importance of AgRP neurons in feeding behavior, genetic analyses of specific hormonal pathways that impinge on this process have yielded limited and occasionally conflicting results. For example, transgenic overexpression of *Agrp* results in obesity, and acute intracerebroventricular (ICV) delivery of AgRP or neuropeptide Y (NPY), another peptide produced in AgRP neurons, elicits hyperphagia (Levine et al., 2004; Ollmann et al., 1997; Rossi et al., 1998). However, mice with loss of *Agrp*, *Npy*, or both exhibit no feeding or body weight phenotypes and maintain a normal response to starvation (Qian et al., 2002).

The key anorexigenic hormones, insulin and leptin, inhibit AgRP neurons, raising their activation threshold (Könner et al., 2007; Takahashi and Cone, 2005). Surprisingly, though, genetic ablation of insulin receptor in AgRP neurons has no effect on energy homeostasis, even as it impairs insulin-mediated suppression of hepatic glucose production (Könner et al., 2007). Similarly, inactivation of leptin receptors in AgRP neurons results in a mild increase of body weight without affecting feeding behavior and energy balance (van de Wall et al., 2008). Thus, neither pathway appears to exert obligate control over AgRP-neuron-dependent feeding and energy homeostasis.

The goal of this study was twofold: (1) to solve the apparent paradox of the absence of food intake abnormalities following ablation of leptin or insulin receptors and (2) to identify alternative pathways that regulate AgRP-neuron-dependent food intake. To this end, we adopted a two-pronged strategy. As transcription factor FoxO1 integrates both leptin and insulin signaling (Kim et al., 2006; Kitamura et al., 2006), it is plausible that greater effects in AgRP neurons might be engendered by FoxO1 ablation. Therefore, we knocked out FoxO1 in AgRP neurons with the intent to bring about a metabolic and energy balance phenotype that recapitulated constitutive insulin and leptin signaling in this cell type. We found that mice lacking FoxO1 in AgRP neurons are lean, eat less, and show improved glucose homeostasis as well as increased insulin/leptin sensitivity. Having achieved this goal, we sought to identify FoxO1-dependent pathways that modulate energy homeostasis and can be

A Prospective Study of the Associations Between Treated Diabetes and Cancer Outcomes

Hsin-Chieh Yeh, phd[1,2]
Elizabeth A. Platz, scd, mph[2,3]
Nae-Yuh Wang, phd[1,4]
Kala Visvanathan, mbbs, fracp, mhs[2,3]
Kathy J. Helzlsouer, md, mhs[2,3,5]
Frederick L. Brancati, md, mhs[1,2]

OBJECTIVE—To quantify the association of treated diabetes with cancer incidence and cancer mortality as well as cancer case fatality and all-cause mortality in adults who subsequently develop cancer and to calculate attributable fractions due to diabetes on various cancer outcomes.

RESEARCH DESIGN AND METHODS—Prospective data on 599 diabetic and 17,681 nondiabetic adults from the CLUE II (Give Us a Clue to Cancer and Heart Disease) cohort in Washington County, Maryland, were analyzed. Diabetes was defined by self-reported use of diabetes medications at baseline. Cancer incidence was ascertained using county and state cancer registries. Mortality data were obtained from death certificates.

RESULTS—From 1989 to 2006, 116 diabetic and 2,365 nondiabetic adults developed cancer, corresponding to age-adjusted incidence of 13.25 and 10.58 per 1,000 person-years, respectively. Adjusting for age, sex, education, BMI, smoking, hypertension treatment, and high cholesterol treatment using Cox proportional hazards regression, diabetes was associated with a higher risk of incident cancer (hazard ratio 1.22 [95% CI 0.98–1.53]) and cancer mortality (1.36 [1.02–1.81]). In individuals who developed cancer, adults with diabetes had a higher risk of cancer case fatality (1.34 [1.002–1.79]) and all-cause mortality (1.61 [1.29–2.01]). For colorectal, breast, and prostate cancers, the attributable fractions resulting from diabetes were larger for cancer fatality and mortality than cancer incidence.

CONCLUSIONS—In this prospective cohort, diabetes appears to exert a greater influence downstream on the risk of mortality in people with cancer than on upstream risk of incident cancer.

Diabetes Care 35:113–118, 2012

Although previous cohort studies have examined the influence of diabetes on particular aspects of cancer outcomes (1–9), none have sought to quantify the impact of diabetes across the full continuum of cancer control, from cancer development to survival after a cancer diagnosis. We, therefore, analyzed prospective data from CLUE II (Give Us a Clue to Cancer and Heart Disease), a community-based, cohort study in Washington County, Maryland, to test the hypothesis that preexisting, treated diabetes would predict *1*) cancer incidence and cancer mortality in people at risk for cancer and *2*) cancer case fatality and all-cause mortality in adults who subsequently develop cancer. Because data on cancer incidence and mortality were available in the same population, we further calculated the attributable fractions (AFs) to isolate effects of diabetes on cancer incidence from its effects on cancer case fatality and death from all causes. We hypothesized that diabetes would exert a stronger influence on downstream risk of mortality in people with cancer than upstream on the development of incident cancer.

RESEARCH DESIGN AND METHODS

—In 1989, a local campaign against cancer and heart disease named CLUE II was conducted in Washington County, Maryland. Mobile trailers were stationed in a wide variety of locations in the county in an effort to give all segments of the community an opportunity to participate. At baseline, written informed consent was obtained, and a short questionnaire on demographics and medical history were administered to all participants. A total of 32,894 individuals, nearly one-third of the adult population of Washington County at that time, took part in this study. To reduce the likelihood of a case subject not being identified through the cancer registries, the analysis cohort was limited to 25,076 CLUE II participants who lived in Washington County at baseline. We further excluded participants aged <30 years (n = 5,470) or with history of invasive cancer at baseline (n = 1,360; individuals could be excluded for more than one reason). The final study sample consisted of 18,280 adults aged ≥30 years without a history of cancer at baseline.

Diabetes status

Individuals were classified as having diabetes if they reported taking antidiabetes medications in the last 48 h on the baseline questionnaire.

Cancer incidence and mortality assessment

Cancer cases in the CLUE II cohort have been identified through linkage of the cohort participants with the Washington County Cancer Registry and, since 1992, with the Maryland Cancer Registry. For all-cause mortality, we identified deaths among cohort members via searches of the National Death Index, Maryland death certificates, reviews of obituaries of the local newspaper, and reports by next of kin. Death certificates were reviewed to determine underlying cause of death.

From the [1]Department of Medicine, Johns Hopkins University, Baltimore, Maryland; the [2]Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland; the [3]Department of Oncology, Johns Hopkins University, Baltimore, Maryland; the [4]Department of Biostatistics, Johns Hopkins University, Baltimore, Maryland; and the [5]Prevention and Research Center, Weinberg Center for Women's Health and Medicine, Mercy Medical Center, Baltimore, Maryland.
Corresponding author: Hsin-Chieh Yeh, hyeh1@jhmi.edu.
Received 8 February 2011 and accepted 5 October 2011.
DOI: 10.2337/dc11-0255
The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Reproductive Tissues Maintain Insulin Sensitivity in Diet-Induced Obesity

Sheng Wu, Sara Divall, Fredric Wondisford, and Andrew Wolfe

Reproductive dysfunction is associated with obesity. We previously showed that female mice with diet-induced obesity (DIO) exhibit infertility and thus serve as a model of human polycystic ovary syndrome (PCOS). We postulated that differential insulin signaling of tissues leads to reproductive dysfunction; therefore, a comparison of insulin signaling in reproductive tissues and energy storage tissues was performed. Pituitary-specific insulin receptor knockout mice were used as controls. High-fat diet–induced stress, which leads to insulin resistance, was also investigated by assaying macrophage infiltration and phosphorylated Jun NH_2-terminal kinase (pJNK) signaling. In lean mice, reproductive tissues exhibited reduced sensitivity to insulin compared with peripheral metabolic tissues. However, in obese mice, where metabolic tissues exhibited insulin resistance, the pituitary and ovary maintained insulin sensitivity. Pituitaries responded to insulin through insulin receptor substrate (IRS)2 but not IRS1, whereas in the ovary, both IRS1 and IRS2 were activated by insulin. Macrophage infiltration and pJNK signaling were not increased in the pituitary or ovary of lean mice relative to DIO mice. The lack of inflammation and cytokine signaling in the pituitary and ovary in DIO mice compared with lean mice may be one of the reasons that these tissues remained insulin sensitive. Retained sensitivity of the pituitary and ovary to insulin may contribute to the pathophysiology of PCOS. ***Diabetes*** **61:114–123, 2012**

Secretion of luteinizing hormone and follicle-stimulating hormone stimulates the maturation, development, and function of the gonads and, ultimately, through regulation of gonadal steroid hormone secretion, regulates reproduction. While nutritional deprivation inhibits reproductive function, it is becoming clear that nutritional excess can also impair reproductive function. Recently, the response of the reproductive axis to nutritional excess has gained attention because of the rise in obesity and its associated diseases, such as type 2 diabetes, metabolic syndrome, and polycystic ovary syndrome (PCOS) (1), all of which are associated with reproductive dysfunction. PCOS is defined as hyperandrogenism with oligo/amenorrhea and accounts for >75% of cases of anovulatory infertility. PCOS is marked by an increase in luteinizing hormone pulsatility, often accompanied by high baseline luteinizing hormone levels, ovarian overproduction of testosterone, polycystic ovaries, and peripheral insulin resistance. The pathophysiologic mechanisms underlying the reproductive and metabolic derangements in PCOS have yet to be delineated. We recently revealed a role for insulin signaling in the pituitary in a mouse model of obesity-induced reproductive dysfunction and hyperandrogenism (2).

While insulin resistance in the energy-storing tissues has been extensively studied, the effects of chronic hyperinsulinemia and obesity in tissues of the female reproductive axis have been more superficially addressed. Analysis of the insulin receptor substrate (IRS)2 knockout (KO) mouse has confirmed the importance of the IRS2 scaffolding protein for normal reproductive function (3); yet, the role that IRS1 and -2 play in the function of reproductive tissues in obesity has not been explored. Therefore, an analysis of the insulin-signaling pathways in the pituitary and ovary in lean and diet-induced obesity (DIO) mice was performed in this study. We demonstrate that the mechanisms that induce insulin resistance at the level of IRS2 are absent in the pituitary and ovary and that retained insulin sensitivity in reproductive tissues results in elevated insulin signaling in hyperinsulinemic conditions such as obesity.

RESEARCH DESIGN AND METHODS

Animals and diets. Mice were maintained on a mixed background: CD1/129SvJ/C57BL6. Pituitary-specific insulin receptor KO mice (PITIRKO) were produced by our group as described previously (2). αGSU-Cre-negative littermates were used as controls (wild type [WT]). Female PITIRKO mice and controls were fed a high-fat diet (HFD) or regular chow as described previously (2). Mice used in this study were female and were maintained with food and water ad libitum under a 14-h/10-h dark/light cycle. Procedures were approved by the Johns Hopkins Animal Care and Use Committee. Experiments were conducted with 5.5- to 6.5-month-old female mice (lean and DIO): lean WT (30.6 ± 1.0 g), lean PITIRKO (29.6 ± 1.8 g), WT DIO (44.3 ± 6.9 g), and PITIRKO-DIO (43.4 ± 6.4 g). Mice were tested for estrous cyclicity, and WT DIO mice were acyclic as reported previously (2).

Hormonal assays. Mice were fasted overnight, and blood was collected from mice via mandibular bleed at 10 A.M. All samples were collected by 10:30 A.M. Insulin, leptin, and glucagon were measured with the Milliplex Map Mouse Serum Adipokine Panel (Millipore, Billerica, MA), and insulin-like growth factor-I (IGF-I) was measured with a single-plex IGF-I panel (Millipore) on a Luminex 200IS platform (Luminex Corporation, Austin, TX). All samples were performed in one plate. The intra-assay coefficient of variation for each assay was between 5 and 9%.

Glucose and insulin tolerance tests. Mice were fasted overnight and injected intraperitoneally with 2 g/kg body wt dextrose. Glucose was measured from tail blood at the times indicated using a One Touch Ultra glucometer. After insulin injection (Lilly, Indianapolis, IN) in lean and WT DIO mice, glucose was measured as described above.

Insulin-signaling assay. Mice were fasted overnight, and insulin or 0.9% saline was injected intraperitoneally with different doses. Tissues were collected at 10, 15, 30, or 45 min after insulin injection and snap-frozen in liquid nitrogen. Protein was obtained and measured as described previously (2). To quantify the levels of phosphorylated AKT (pAKT), phosphorylated extracellular signal–related kinase (pERK), and AKT for different tissues of each individual animal after insulin or saline injection, Bio-Plex Phosphoprotein Detection Multiplex assays were used (Bio-Rad Laboratories, Hercules, CA. For quantification of phosphorylated Tyr (pTyr)-IRS1 (or total IRS1) levels in cell lysates, Milliplex Map Phospho IRS1 Mapmates (or Total IRS1 Mapmates) kits were used. Protein (10 μg) from each tissue was loaded into a 96-well microplate. Tissues from four to eight mice were measured independently and were not

From the Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland.
Corresponding author: Sheng Wu, swu24@jhmi.edu.
Received 10 July 2011 and accepted 11 October 2011.
DOI: 10.2337/db11-0956
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-0956/-/DC1.

Does Genetic Ancestry Explain Higher Values of Glycated Hemoglobin in African Americans?

Nisa M. Maruthur,[1,2] W.H. Linda Kao,[1,2,3] Jeanne M. Clark,[1,2,3] Frederick L. Brancati,[1,2,3] Ching-Yu Cheng,[3,4,5,6] James S. Pankow,[7] and Elizabeth Selvin[1,2,3]

OBJECTIVE—Glycated hemoglobin (HbA_{1c}) values are higher in African Americans than whites, raising the question of whether classification of diabetes status by HbA_{1c} should differ for African Americans. We investigated the relative contribution of genetic ancestry and nongenetic factors to HbA_{1c} values and the effect of genetic ancestry on diabetes classification by HbA_{1c} in African Americans.

RESEARCH DESIGN AND METHODS—We performed a cross-sectional analysis of data from the community-based Atherosclerosis Risk in Communities (ARIC) Study. We estimated percentage of European genetic ancestry (PEA) for each of the 2,294 African Americans without known diabetes using 1,350 ancestry-informative markers. HbA_{1c} was measured from whole-blood samples and categorized using American Diabetes Association diagnostic cut points (<5.7, 5.7–6.4, and ≥6.5%).

RESULTS—PEA was inversely correlated with HbA_{1c} (adjusted $r = -0.07$; $P < 0.001$) but explained <1% of its variance. Age and socioeconomic and metabolic factors, including fasting glucose, explained 13.8% of HbA_{1c} variability. Eleven percent of participants were classified as having diabetes; adjustment for fasting glucose decreased this to 4.4%. Additional adjustment for PEA did not significantly reclassify diabetes status (net reclassification index = 0.034; $P = 0.94$) nor did further adjustment for demographic, socioeconomic, and metabolic risk factors.

CONCLUSIONS—The relative contribution of demographic and metabolic factors far outweighs the contribution of genetic ancestry to HbA_{1c} values in African Americans. Moreover, the impact of adjusting for genetic ancestry when classifying diabetes by HbA_{1c} is minimal after taking into account fasting glucose levels, thus supporting the use of currently recommended HbA_{1c} categories for diagnosis of diabetes in African Americans. ***Diabetes* 60:2434–2438, 2011**

From the [1]Division of General Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; the [2]Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, Maryland; the [3]Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland; the [4]Department of Epidemiology and Public Health and Department of Ophthalmology, National University of Singapore, Singapore; the [5]Singapore Eye Research Institute, Singapore; the [6]Centre for Quantitative Medicine, Duke-NUS Graduate Medical School, Singapore; and the [7]Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, Minnesota.

Corresponding authors: W.H. Linda Kao, wkao@jhsph.edu; Nisa M. Maruthur, maruthur@jhmi.edu.

Received 8 March 2011 and accepted 20 June 2011.

DOI: 10.2337/db11-0319

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-0319/-/DC1.

Glycated hemoglobin (HbA_{1c}) values are significantly higher in African Americans compared with whites even after adjustment for fasting blood glucose (1–4). Whether this racial difference in HbA_{1c} reflects true differences in hyperglycemia or differences in biologic determinants of HbA_{1c} unrelated to hyperglycemia is controversial (5–7), especially in the context of the American Diabetes Association recommendation to use HbA_{1c} ≥6.5% for diagnosis of diabetes (8).

Self-reported African American race is associated with many socioeconomic factors that influence health (9), particularly diabetes risk (10). Genetically derived ancestry can be used to partially deconstruct race as it places each individual on a continuous spectrum of race as opposed to grouping all individuals into one racial group. Therefore, our main objective was to determine the contribution of genetic ancestry to HbA_{1c} in self-reported African Americans. Genetic ancestry may be associated with HbA_{1c} through direct biological effects unrelated to hyperglycemia or indirectly through social and demographic determinants of hyperglycemia (11,12). Because epidemiologic studies report higher HbA_{1c} values in African Americans compared with whites independent of their fasting glucose (1–4), we examine the ancestral genetic contribution to HbA_{1c} after accounting for fasting glucose levels. We hypothesized that *1*) percentage of European ancestry (PEA) explains only a small proportion of the variability in HbA_{1c} in African Americans; *2*) PEA and HbA_{1c} are associated with similar social and biologic factors; and *3*) PEA does not significantly alter diabetes classification by HbA_{1c} independent of fasting glucose levels.

RESEARCH DESIGN AND METHODS

Study population. We included 2,294 African American participants without known diabetes and with a complete set of covariates of interest presenting to visits 1 and 2 of the Atherosclerosis Risk in Communities (ARIC) Study, an ongoing prospective cohort study of adults from four U.S. communities who were 45–64 years of age at the baseline visit in 1987–1989 (13).

Estimation of percentage of European ancestry from ancestry-informative markers. We included single nucleotide polymorphisms (SNPs) whose frequencies differ significantly between Caucasian and African ancestral populations, ancestry-informative markers (AIMs), to estimate the PEA among African Americans, an admixed population (14). The race-specific frequency of each SNP in the AIM panel was estimated using West African and European samples to provide a Bayesian prior for ancestral allele frequencies (14).

Genotyping methods for estimating genetic ancestry in African Americans in the ARIC Study have been described previously (14). In brief, genotyping was performed on stored DNA from visit 1 using the Illumina BeadLab platform (15) at the Center for Inherited Disease Research (Johns Hopkins University). Evaluation of 218,461 blind duplicate genotypes yielded a mismatch rate of 0.1% (14). We used standard filters for quality control of individual SNP genotyping. Samples were excluded for duplicity, low call rate, lack of sex concordance, or excess heterozygosity. We used ANCESTRYMAP software to estimate PEA for each participant (14). Additional detail regarding study population and genotyping is provided in the Supplementary Data.

The DA VINCI Study: Phase 2 Primary Results of VEGF Trap-Eye in Patients with Diabetic Macular Edema

Diana V. Do, MD,[1] Ursula Schmidt-Erfurth, MD,[2] Victor H. Gonzalez, MD,[3] Carmelina M. Gordon, MD,[4] Michael Tolentino, MD,[5] Alyson J. Berliner, MD, PhD,[6] Robert Vitti, MD, MBA,[5] Rene Rückert, MD,[7] Rupert Sandbrink, MD, PhD,[7,8] David Stein, BS,[6] Ke Yang, PhD,[6] Karola Beckmann, MSc,[7] Jeff S. Heier, MD[9]

Purpose: To determine whether different doses and dosing regimens of intravitreal vascular endothelial growth factor (VEGF) Trap-Eye are superior to focal/grid photocoagulation in eyes with diabetic macular edema (DME).

Design: Multicenter, randomized, double-masked, phase 2 clinical trial.

Participants: A total of 221 diabetic patients with clinically significant macular edema involving the central macula.

Methods: Patients were assigned to 1 of 5 treatment regimens: 0.5 mg VEGF Trap-Eye every 4 weeks; 2 mg VEGF Trap-Eye every 4 weeks; 2 mg VEGF Trap-Eye for 3 initial monthly doses and then every 8 weeks; 2 mg VEGF Trap-Eye for 3 initial monthly doses and then on an as-needed (PRN) basis; or macular laser photocoagulation. Assessments were completed at baseline and every 4 weeks thereafter.

Main Outcome Measures: Mean change in visual acuity and central retinal thickness (CRT) at 24 weeks.

Results: Patients in the 4 VEGF Trap-Eye groups experienced mean visual acuity benefits ranging from +8.5 to +11.4 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters versus only +2.5 letters in the laser group ($P \leq 0.0085$ for each VEGF Trap-Eye group vs. laser). Gains from baseline of 0+, 10+, and 15+ letters were seen in up to 93%, 64%, and 34% of VEGF Trap-Eye groups versus up to 68%, 32%, and 21% in the laser group, respectively. Mean reductions in CRT in the 4 VEGF Trap-Eye groups ranged from −127.3 to −194.5 μm compared with only −67.9 μm in the laser group ($P = 0.0066$ for each VEGF Trap-Eye group vs. laser). VEGF Trap-Eye was generally well tolerated. Ocular adverse events in patients treated with VEGF Trap-Eye were generally consistent with those seen with other intravitreal anti-VEGF agents.

Conclusions: Intravitreal VEGF Trap-Eye produced a statistically significant and clinically relevant improvement in visual acuity when compared with macular laser photocoagulation in patients with DME.

Financial Disclosure(s): Proprietary or commercial disclosure may be found after the references.
Ophthalmology 2011;118:1819–1826 © 2011 by the American Academy of Ophthalmology.

Diabetic macular edema (DME) is the most common vision-threatening manifestation of diabetic retinopathy. The population-based Wisconsin Epidemiologic Study of Diabetic Retinopathy reported 28% prevalence of DME 20 years after the diagnosis of type 1 or type 2 diabetes,[1] and the 10-year incidence of DME varies between 20% and 40% depending on age, diabetes type, and severity of diabetes.[2] The prevalence is projected to increase as the prevalence of diabetes mellitus increases from 180 million people worldwide to 300 million by the year 2025.[3]

Phosphorylation of tight junction proteins and disorganization of the blood–retina–barrier are the key events in the pathophysiology of DME,[4,5] to which hypoxia-triggered vascular endothelial growth factor (VEGF) release contributes significantly.[6] Intravitreal injection of VEGF has been shown to produce all findings of diabetic retinopathy, including microaneurysms, macular edema, and retinal neovascularization.[7,8] Correspondingly, intravitreal VEGF levels are elevated in patients with DME.[9] The importance of VEGF is underscored by the efficacy of anti-VEGF drugs in reducing swelling of the retina and improving vision in patients with DME. Recent prospective, randomized studies have demonstrated the efficacy of intravitreal injections of ranibizumab, a humanized monoclonal antibody that binds all isoforms of VEGF-A.[10,11] Comparable results were reported for bevacizumab, the complete antibody with almost identical binding sites to VEGF-A as ranibizumab, in interventional studies or case series.[12,13]

VEGF Trap-Eye (Regeneron Pharmaceuticals, Inc., Tarrytown, New York, NY, and Bayer Healthcare Pharmaceuticals, Berlin, Germany) is a 115-kDA recombinant fusion protein consisting of the VEGF binding domains of human VEGF receptors 1 and 2 fused to the Fc domain of human immunoglobulin-G1.[14] Animal studies have demonstrated

ISSN 0161-6420/11/$–see front matter
doi:10.1016/j.ophtha.2011.02.018

DIABETES

Myocardial Infarction Triggers Chronic Cardiac Autoimmunity in Type 1 Diabetes

Raju V. S. R. K. Gottumukkala,[1]* HuiJuan Lv,[1]* Lizbeth Cornivelli,[1] Amy J. Wagers,[1,2,3] Raymond Y. Kwong,[4] Roderick Bronson,[5] Garrick C. Stewart,[4] P. Christian Schulze,[4,6] William Chutkow,[4] Howard A. Wolpert,[1] Richard T. Lee,[3,4] Myra A. Lipes[1†]

Patients with type 1 diabetes (T1D) suffer excessive morbidity and mortality after myocardial infarction (MI) that is not fully explained by the metabolic effects of diabetes. Acute MI is known to trigger a profound innate inflammatory response with influx of mononuclear cells and production of proinflammatory cytokines that are crucial for cardiac repair. We hypothesized that these same pathways might exert "adjuvant effects" and induce pathological responses in autoimmune-prone T1D hosts. Here, we show that experimental MI in nonobese diabetic mice, but not in control C57BL/6 mice, results in a severe post-infarction autoimmune (PIA) syndrome characterized by destructive lymphocytic infiltrates in the myocardium, infarct expansion, sustained cardiac autoantibody production, and T helper type 1 effector cell responses against cardiac (α-)myosin. PIA was prevented by inducing tolerance to α-myosin, demonstrating that immune responses to cardiac myosin are essential for this disease process. Extending these findings to humans, we developed a panel of immunoassays for cardiac autoantibody detection and found autoantibody positivity in 83% post-MI T1D patients. We further identified shared cardiac myosin autoantibody signatures between post-MI T1D patients and nondiabetic patients with myocarditis, which were absent in post-MI type 2 diabetic patients, and confirmed the presence of myocarditis in T1D by cardiac magnetic resonance imaging techniques. These data provide experimental and clinical evidence for a distinct post-MI autoimmune syndrome in T1D. Our findings suggest that PIA may contribute to worsened post-MI outcomes in T1D and highlight a role for antigen-specific immunointervention to selectively block this pathway.

INTRODUCTION

Over the past few decades, new knowledge about basic mechanisms underlying the pathogenesis of cardiovascular disease (CVD) has led to aggressive pharmacological and interventional therapies and a major decline in mortality from myocardial infarction (MI) in the general population (*1*). Despite this progress, CVD accounts for 65 to 70% of deaths in individuals with T1D (*2*, *3*) and incurs a ~13-fold increase in age-adjusted mortality rates in T1D patients compared to the nondiabetic population (*3*). This excess mortality has shown essentially no improvement over the past 30 years, despite improved outcomes from other diabetes complications, in particular, renal failure (*4*), which has long been considered the primary driver of CVD mortality in T1D (*5*, *6*). Although chronic hyperglycemia has been established as a key mediator of CVD risk in T1D (*7*), the mechanisms accounting for the excessive post-MI mortality are poorly understood. Although numerous factors related to diabetes have been implicated, none have been unique to T1D (*2*).

T1D is an autoimmune disorder caused by T lymphocyte–mediated destruction of the pancreatic β cells ("insulitis") (*8*). Once established, insulitis can be detected indirectly by screening serum for autoantibodies to islet antigens. The β cell specificity of this autoimmune attack has been attributed to specific alleles of major histocompatibility complex (MHC) class II, most notably human leukocyte antigen (HLA)–DQ8 (hereafter, DQ8). However, non-MHC genes that are associated with more broad-spectrum defects in immunological tolerance are also required and are thought to underlie the clustering of other autoimmune disorders in individuals with T1D (*9*). Environmental factors are also critical for the development of T1D, and it has been assumed that in genetically susceptible individuals, an inflammatory trigger—presumably, an infection or other cause of β cell injury—is required for disease expression.

Inflammation plays a key role in the early stages of tissue repair after MI (*10*, *11*). After acute MI, signals are generated that trigger the influx of neutrophils, macrophages, and dendritic cells into the infarct zone (*12*, *13*), along with the release of proinflammatory cytokines such as tumor necrosis factor–α (TNF-α), interleukin-1β (IL-1β), and IL-6 (*11*). Although these innate immune responses are crucial for repairing the damaged heart, these same cytokines and signals from necrotic cells are particularly potent maturation factors for dendritic cells, transforming them into highly immunogenic antigen-presenting cells capable of activating adaptive immune responses (*14*, *15*). However, there has been substantial debate about whether endogenous ("danger") signals generated by tissue damage can by themselves—in the absence of adjuvant or microbial stimuli—fully activate adaptive immune responses (*16*, *17*). It has been postulated that in tissue injury settings, the released self-antigens should not be recognized as "foreign" because high-avidity T cells specific for these self-antigens would normally have been deleted during thymic negative selection, a major barrier against autoimmunity.

We and others have shown that "humanized" transgenic nonobese diabetic (NOD) mice expressing DQ8, instead of endogenous murine

[1]Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA. [2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA. [3]Harvard Stem Cell Institute, Cambridge, MA 02138, USA. [4]Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [5]Department of Microbiology and Immunology, Harvard Medical School, Boston, MA 02115, USA. [6]Division of Cardiology, Columbia University Medical Center, New York, NY 10032, USA.
*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: myra.lipes@joslin.harvard.edu

Cold but not sympathomimetics activates human brown adipose tissue in vivo

Aaron M. Cypess[a], Yih-Chieh Chen[b], Cathy Sze[a], Ke Wang[b], Jeffrey English[b], Onyee Chan[a], Ashley R. Holman[a], Ilan Tal[b], Matthew R. Palmer[b], Gerald M. Kolodny[b], and C. Ronald Kahn[a,1]

[a]Section of Integrative Physiology and Metabolism, Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215; and [b]Division of Nuclear Medicine, Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215

Contributed by C. Ronald Kahn, May 9, 2012 (sent for review March 17, 2012)

As potential activators of brown adipose tissue (BAT), mild cold exposure and sympathomimetic drugs have been considered as treatments for obesity and diabetes, but whether they activate the same pathways is unknown. In 10 healthy human volunteers, we found that the sympathomimetic ephedrine raised blood pressure, heart rate, and energy expenditure, and increased multiple circulating metabolites, including glucose, insulin, and thyroid hormones. Cold exposure also increased blood pressure and energy expenditure, but decreased heart rate and had little effect on metabolites. Importantly, cold increased BAT activity as measured by ^{18}F-fluorodeoxyglucose PET-CT in every volunteer, whereas ephedrine failed to stimulate BAT. Thus, at doses leading to broad activation of the sympathetic nervous system, ephedrine does not stimulate BAT in humans. In contrast, mild cold exposure stimulates BAT energy expenditure with fewer other systemic effects, suggesting that cold activates specific sympathetic pathways. Agents that mimic cold activation of BAT could provide a promising approach to treating obesity while minimizing systemic effects.

metabolism | thermogenesis | respiratory quotient | norepinephrine | white adipose tissue

Brown adipose tissue (BAT) is a type of fat that consumes calories to generate heat. Multiple recent studies have shown that adult humans have functional BAT that can be activated in response to cold exposure in a process called nonshivering thermogenesis (1–4). In both small and large population studies (1, 2, 4, 5), there is an inverse correlation between BAT activity and obesity, suggesting that activating BAT, through pharmacological, environmental, or potentially nutritional interventions, could become a therapeutic means to treat obesity and diabetes. Indeed, human BAT energy expenditure may be a critical counterbalance to the weight gain and metabolic dysregulation caused by excess energy storage in white adipose tissue.

Human BAT has a high density of both nerves and blood vessels (6), providing two general approaches to activate BAT. Based on studies in rodents, it is known that the sensation of cold by the skin and body core sends signals via peripheral neurons to the spinal cord and then up to the preoptic area of the hypothalamus for processing. From the hypothalamus, some signals go to the cerebral cortex for conscious thermal perception and localization, and others go to premotor neurons in the rostral raphe pallidus of the brainstem, projecting to neurons of the peripheral sympathetic nervous system (SNS) (reviewed in ref. 7). Ultimately, postganglionic SNS nerves release norepinephrine to activate BAT via induction of uncoupling protein-1, the tissue-specific protein that allows BAT to generate heat by uncoupling aerobic respiration from the generation of ATP.

Because the endogenous pathways by cold exposure are complex and indirect, an attractive alternative for stimulation of BAT has been the use of pharmacological agents. As norepinephrine itself has too many adverse effects on the cardiovascular system, drugs that bind to the relatively fat-specific β3-adrenergic receptor agonists have been developed in attempt to stimulate BAT, but these have had little success to date in humans (8–11). Given the increasing health burden of the obesity and diabetes pandemics, it is vital to develop novel approaches to increase energy expenditure and induce weight loss through BAT. A critical first phase is to demonstrate the effectiveness of different environmental and pharmacologic methods. In this study we compared the ability of mild cold exposure and the sympathomimetic drug ephedrine to stimulate BAT in 10 healthy volunteers. Ephedrine was chosen because it is comparatively safe yet still achieves broad activation of the SNS; it has been used for decades to increase energy expenditure and achieve weight loss in humans (12); in addition, ephedrine has been shown at very high doses to increase BAT glucose uptake in rodents (13). We find that although these two methods both use the SNS to increase thermogenesis, at similar levels of energy expenditure and cardiovascular response, only cold exposure appreciably stimulates BAT. In fact, the pathways of BAT activation are specific and not mimicked by generalized pharmacological activation of the SNS.

Results

Effects of Ephedrine and Cold Exposure on Vital Signs and Energy Expenditure. For each volunteer (Table 1), we compared the average systolic blood pressure (BP), diastolic BP, and heart rate for two time intervals: during the hour before intervention (four measures) and then from 45 to 90 min afterward (four measures). Paired *t* tests showed that there was no change in any of these parameters after saline injection ($P > 0.05$). Repeated-measures ANOVA showed that treatment condition has a significant effect on vital signs ($P < 0.001$). As expected, ephedrine at 1 mg/kg induced significant increases in systolic BP ($P < 0.001$), diastolic BP ($P = 0.002$), and heart rate ($P = 0.015$). Cold also increased systolic ($P = 0.002$) and diastolic BP ($P < 0.001$), but decreased heart rate ($P = 0.040$) (Fig. 1*A*). Of note, the female volunteers had lower height, body surface area, percent lean mass, and total lean mass, but there was no significant sexual dimorphism seen in any of the other anthropometric parameters, vital signs, laboratory values, bioenergetics, or measures of BAT function.

Neither basal metabolic rate (BMR) nor respiratory quotient (RQ) changed after saline injection ($P > 0.05$). The mean BMR was 1,448 kcal/d. In contrast, the metabolic rate increased by 136 kcal/d, or 9.0%, after ephedrine treatment ($P = 0.01$) and 79 kcal/d, or 5.5%, after cold exposure by ($P = 0.033$). These were not significantly different from each other ($P > 0.05$) (Fig. 1*B*). RQ decreased after both ephedrine treatment ($P = 0.003$) and cold exposure ($P = 0.029$), and again there was no difference between the effects of ephedrine and cold exposure ($P > 0.05$) (Fig. 1*C*).

Author contributions: A.M.C. and C.R.K. designed research; A.M.C., Y-C.C., C.S., K.W., J.E., A.R.H., M.R.P., and G.M.K. performed research; I.T. contributed new reagents/analytic tools; A.M.C., Y-C.C., C.Z., K.W., O.C., M.R.P., and G.M.K. analyzed data; and A.M.C. and C.R.K. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. E-mail: c.ronald.kahn@joslin.harvard.edu.

Cell Stem Cell
Brief Report

Short-Term Calorie Restriction Enhances Skeletal Muscle Stem Cell Function

Massimiliano Cerletti,[2,3] Young C. Jang,[2,3] Lydia W.S. Finley,[4] Marcia C. Haigis,[4] and Amy J. Wagers[1,2,3,*]
[1]Howard Hughes Medical Institute
[2]Department of Stem Cell and Regenerative Biology, Harvard University, Harvard Stem Cell Institute, 7 Divinity Avenue, Cambridge, MA 02138, USA
[3]Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA
[4]Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: amy_wagers@harvard.edu
DOI 10.1016/j.stem.2012.04.002

SUMMARY

Calorie restriction (CR) extends life span and ameliorates age-related pathologies in most species studied, yet the mechanisms underlying these effects remain unclear. Using mouse skeletal muscle as a model, we show that CR acts in part by enhancing the function of tissue-specific stem cells. Even short-term CR significantly enhanced stem cell availability and activity in the muscle of young and old animals, in concert with an increase in mitochondrial abundance and induction of conserved metabolic and longevity regulators. Moreover, CR enhanced endogenous muscle repair and CR initiated in either donor or recipient animals improved the contribution of donor cells to regenerating muscle after transplant. These studies indicate that metabolic factors play a critical role in regulating stem cell function and that this regulation can influence the efficacy of recovery from injury and the engraftment of transplanted cells.

Calorie restriction (CR) is a dietary intervention that extends life span and delays, prevents, or reduces the severity of age-related pathologies in many species and tissues (Ahmet et al., 2005; Hursting et al., 2003; Russell and Kahn, 2007). Indeed, age-matched animals maintained on a reduced-calorie diet throughout life show fewer malignancies, enhanced cognitive and motor function, and a lower incidence of diabetes, as compared to control animals allowed food ad libitum. Prior studies in the rodent hematopoietic system (Ertl et al., 2008) suggest that lifelong calorie restriction may maintain stem cell function, which normally declines with age, but whether short-term CR in otherwise healthy young animals might enhance the function of tissue stem cells has not been addressed. Moreover, the effect of CR in recipient animals on the efficiency of stem cell engraftment remains unknown. Answers to both of these questions may help to improve the targeting and transplant of stem cells for therapy and illuminate normal metabolic regulators of tissue stem cell function and the age-related factors that typically impair this function (Rossi et al., 2008).

To assess the effects of short-term CR on tissue stem cell activity, we focused on skeletal muscle, a well-characterized tissue that normally undergoes robust repair in response to injury. Muscle regenerative activity is mediated by a specialized population of precursor cells known as satellite cells (Mauro, 1961), which reside adjacent to myofibers. Satellite cells are activated by muscle injury to divide and differentiate, regenerating damaged tissue and restoring muscle function (reviewed in Wagers and Conboy, 2005). The satellite cell pool contains self-renewing muscle stem cells, which can be isolated using antibody staining and fluorescence-activated cell sorting (FACS). A number of different marker combinations have been used for satellite cell isolation (Kuang et al., 2007; Montarras et al., 2005; Sacco et al., 2008; Sherwood et al., 2004; Tanaka et al., 2009); here, we identify satellite cells as $CD45^-Sca1^-Mac1^-CXCR4^+\beta1\text{-integrin}^+$ myofiber-associated cells (Figure 1A; Figure S2A available online), based on our previous analyses demonstrating that this marker combination yields a substantial enrichment of satellite cells that matches or exceeds that seen with other approaches (Montarras et al., 2005; Sacco et al., 2008). In particular, > 95% of sorted $CD45^-Sca1^-Mac1^-CXCR4^+\beta1\text{-integrin}^+$ cells stain for the canonical satellite cell transcription factor Pax7 (Figure 1D and Cerletti et al., 2008a), and < 5% express MyoD (Cerletti et al., 2008a). $CD45^-Sca1^-Mac1^-CXCR4^+\beta1\text{-integrin}^+$ satellite cells also coexpress other reported satellite cell markers, including CD34 (100% CD34+; data not shown), Syndecan-4 (100% Syndecan-4+; Cerletti et al., 2008a), M-cadherin (100% M-cadherin+; Cerletti et al., 2008a), and alpha7-integrin (95% alpha7-integrin+; Jang et al., 2011). Most importantly, however, $CD45^-Sca1^-Mac1^-CXCR4^+\beta1\text{-integrin}^+$ cells exhibit robust, clonal myogenic activity in vitro and in vivo (Cerletti et al., 2008a, 2008b), replenish the satellite cell compartment upon intramuscular transplantation (Cerletti et al., 2008a), and are completely devoid of fibrogenic or adipogenic potential as assessed by in vitro differentiation and transplant assays (Hettmer et al., 2011; Schulz et al., 2011; Tan et al., 2011). Thus, $CD45^-Sca1^-Mac1^-CXCR4^+\beta1\text{-integrin}^+$ cells display phenotypic and functional properties consistent with a highly enriched population of adult muscle satellite cells.

To examine the effect of CR on the overall frequency and myogenic activity of skeletal muscle stem cells, we purified these cells from young C57BL/6 mice, raised initially on control diet and then switched to CR at 2 months of age, or from aged

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.™

PREVENTING CHRONIC DISEASE
PUBLIC HEALTH RESEARCH, PRACTICE, AND POLICY

ORIGINAL RESEARCH

Intervention Mapping as a Guide for the Development of a Diabetes Peer Support Intervention in Rural Alabama

Andrea Cherrington, MD, MPH; Michelle Y. Martin, PhD; Michaela Hayes, MPH; Jewell H. Halanych, MD, MPH; Mary Annette Wright, PhD; Susan J. Appel, PhD; Susan J. Andreae, MPH; Monika Safford, MD

Suggested citation for this article: Cherrington A, Martin MY, Hayes M, Halanych JH, Wright MA, Appel SJ, et al. Intervention mapping as a guide for the development of a diabetes peer support intervention in rural Alabama. Prev Chronic Dis 2012;9:110053. DOI: http://dx.doi.org/10.5888/pcd9.110053.

PEER REVIEWED

Abstract

Introduction

Peer support is a promising strategy for the reduction of diabetes-related health disparities; however, few studies describe the development of such strategies in enough detail to allow for replication. The objective of this article is to describe the development of a 1-year peer support intervention to improve diabetes self-management among African American adults with diabetes in Alabama's Black Belt.

Methods

We used principles of intervention mapping, including literature review, interviews with key informants, and a discussion group with community health workers, to guide intervention development. Qualitative data were combined with behavioral constructs and principles of diabetes self-management to create a peer support intervention to be delivered by trained peer advisors. Feedback from a 1-month pilot was used to modify the training and intervention.

Results

The resulting intervention includes a 2-day training for peer advisors, who were each paired with 3 to 6 clients. A one-on-one in-person needs assessment begins an intensive intervention phase conducted via telephone for 8 to 12 weeks, followed by a maintenance phase of at least once monthly contacts for the remainder of the intervention period. A peer support network and process measures collected monthly throughout the study supplement formal data collection points at baseline, 6 months, and 12 months.

Discussion

Intervention mapping provided a useful framework for the development of culturally relevant diabetes peer support intervention for African Americans living in Alabama's Black Belt. The process described could be implemented by others in public health to develop or adapt programs suitable for their particular community or context.

Introduction

The prevalence of type 2 diabetes is increasing (1). In the United States, the disease disproportionately affects minority populations, particularly African Americans in the rural South (2). Proper diabetes self-management can lead to improved glycemic control, blood pressure, and lipid levels, and can mitigate the negative health effects of comorbid conditions (3,4). However, the demands of self-management behaviors (eg, diet modification, physical activity, medication adherence, self-monitoring of blood glucose levels) can be difficult to balance and are influenced by socioeconomic, cultural, and psychosocial factors such as lack of social support, self-efficacy, coping skills, and increased barriers to self-care (2,5,6). Residents of rural areas face additional barriers to diabetes self-management, including limited access to health care services, providers, and education programs; high rates of poverty; low levels of health literacy; and increased distances from social networks (7).

These conditions are widespread in Alabama's Black Belt region. Named for its dark soil and agricultural history, the

J Appl Physiol 112: 688–693, 2012.
First published December 15, 2011; doi:10.1152/japplphysiol.00843.2011.

HIGHLIGHTED TOPIC | *Physiology and Pathophysiology of Physical Inactivity*

Aerobic exercise training conserves insulin sensitivity for 1 yr following weight loss in overweight women

Gordon Fisher,[1] Gary R. Hunter,[2] and Barbara A. Gower[1]

Departments of [1]Nutrition Sciences and [2]Human Studies, University of Alabama-Birmingham, Birmingham, Alabama

Submitted 6 July 2011; accepted in final form 11 December 2011

Fisher G, Hunter GR, Gower BA. Aerobic exercise training conserves insulin sensitivity for 1 yr following weight loss in overweight women. *J Appl Physiol* 112: 688–693, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00843.2011.—The objectives of this study were to *1*) identify the independent effects of exercise (aerobic or resistance training) and weight loss on whole body insulin sensitivity and *2*) determine if aerobic or resistance training would be more successful for maintaining improved whole body insulin sensitivity 1 yr following weight loss. Subjects were 97 healthy, premenopausal women, body mass index (BMI) 27–30 kg/m^2. Following randomized assignment to one of three groups, diet only, diet + aerobic, or diet + resistance training until a BMI <25 kg/m^2 was achieved, body composition, fat distribution, and whole body insulin sensitivity were determined at baseline, in the weight reduced state, and at 1-yr follow up. The whole body insulin sensitivity index (S_I) was determined using a frequently sampled intravenous glucose tolerance test. Results of repeated-measures ANOVA indicated a significant improvement in S_I following weight loss. However, there were no group or group×time interactions. At 1-yr follow up, there were no significant time or group interactions for S_I; however, there was a significant group×time interaction for S_I. Post hoc analysis revealed that women in the aerobic training group showed a significant increased S_I from weight reduced to 1-yr follow up ($P < 0.05$), which was independent of intra-abdominal adipose tissue and %fat. No significant differences in S_I from weight reduced to 1-yr follow up were observed for diet only or diet + resistance groups. Additionally, multiple linear regression analysis revealed that change in whole body insulin sensitivity from baseline to 1-yr follow up was independently associated with the change in $\dot{V}O_{2max}$ from baseline to 1-yr follow up ($P < 0.05$). These results suggest that long-term aerobic exercise training may conserve improvements in S_I following weight loss and that maintaining cardiovascular fitness following weight loss may be important for maintaining improvements in S_I.

diet; cardiovascular fitness; visceral fat

OBESITY AND PHYSICAL INACTIVITY are associated with decreased insulin sensitivity and the development of Type 2 diabetes (15, 20). It has been reported that the more overweight or obese an individual is, the more likely they are to be insulin resistant (6). It is well recognized that weight loss achieved through caloric restriction and/or exercise can improve insulin sensitivity in obese and overweight individuals (5, 7, 8, 26, 27). The improvements in insulin sensitivity following caloric restriction appear to be attributable to changes in body composition (2, 7), whereas the improvement in glucose tolerance and/or insulin sensitivity following exercise training appear to be attributable to changes within the contracting muscle via both a short-term insulin-independent mechanism and long-term insulin-dependent mechanism (9, 11, 25, 30). Therefore, the effects of weight loss and exercise training are often thought to have a synergistic effect on insulin sensitivity.

Weight loss interventions are largely successful at inducing weight loss; the usual course of weight loss interventions show that weight is rapidly lost at the onset of therapy, and the greatest loss occurs 6-mo following treatment (13). However, it has also been shown that approximately one-third of those that successfully lose weight will regain weight within 1 yr following treatment (29). Given that modest weight loss is associated with reduced incidence of Type 2 diabetes (18, 19), hypertension (21), dyslipidemia (14), and improved control of diabetes (23), it is important to assess the effect of different treatments on weight loss and associated risk factors associated with metabolic and cardiovascular diseases.

Current literature regarding the independent effects of exercise on improvement in insulin sensitivity has yielded equivocal results. Some studies suggest that exercise in conjunction with diet provides an additive effect on insulin sensitivity (3, 5, 24), whereas others do not (12, 26). Furthermore, a recent study by Thomas et al. (28) used a novel study design to induce weight regain following weight loss and showed that exercise training preserved several of the improvements in metabolic syndrome parameters that improved following weight loss, demonstrating an independent effect of exercise on these variables even in the presence of weight regain (28). To date, we are unaware of any well-controlled randomized studies that have assessed the independent effects of diet with and without exercise training on whole body insulin sensitivity for 1 yr following weight loss. Therefore, the objectives of this study were to *1*) identify the independent effects of exercise (aerobic or resistance training) and weight loss on whole body insulin sensitivity and *2*) determine if diet with or without aerobic or resistance training would be more successful for maintaining whole body insulin sensitivity during a 1-yr follow-up period.

METHODS

Subjects. The study group comprised 213 healthy premenopausal women who volunteered for, and enrolled in, an ongoing study designed to examine metabolic factors that predispose women to weight gain. The sample size included in this study was 97 women from the previously mentioned parent study. This sample size included those subjects that adhered to the diet and exercise requirements for the weight loss phase and remained in the study throughout the 1-yr follow-up assessment. Importantly, there were no significant

Address for reprint requests and other correspondence: Gordon Fisher, Univ. of Alabama at Birmingham, Dept. of Nutrition Sciences, 427 Webb Bldg., 1675 Univ. Blvd., Birmingham, AL 35294-3360 (e-mail: grdnfs@uab.edu).

Reduced Carbohydrate Diet to Improve Metabolic Outcomes and Decrease Adiposity in Obese Peripubertal African American Girls

*Krista Casazza, *Michelle Cardel, *Akilah Dulin-Keita, *Lynae J. Hanks, *Barbara A. Gower, *Anna L. Newton, and †Stephenie Wallace

ABSTRACT

Objective: Obesity prevalence among African American (AA) girls is higher than that in other groups. Because typical energy-restriction obesity treatment strategies have had limited success, alterations in macronutrient composition may effectively improve metabolic outcomes in this population and affect future body composition trajectories. The objective was to evaluate the efficacy of a moderately restricted carbohydrate (CHO) versus a standard CHO diet on weight/fat loss and metabolic parameters in overweight/obese AA girls ages 9 to 14 years.

Methods: A total of 26 AA girls (ranging from 92nd body mass index percentile and above) were assigned to either a reduced- (SPEC: 42% energy from CHO, n = 12) or a standard- (STAN: 55% of energy from CHO, n = 14) CHO diet (protein held constant) for 16 weeks. All of the meals were provided and clinically tailored to meet the estimated energy requirements (resting energy expenditure × 1.2 in eucaloric phase and resting energy expenditure × 1.2 − 1000 kcal in energy deficit phase). The first 5 weeks encompassed a eucaloric phase evaluating metabolic changes in the absence of weight change. The subsequent 11 weeks were hypocaloric (1000 kcal/day deficit) to promote weight/fat loss. Meal tests were performed during the eucaloric phase for metabolic analyses. Dual-energy x-ray absorptiometry was used to evaluate body composition.

Results: Both groups experienced reductions in weight/adiposity, but the difference did not reach significance. The solid meal test indicated improved glucose/insulin homeostasis on the SPEC diet up to 3 hours postingestion. In addition, significantly lower triglycerides ($P < 0.001$) were observed on the SPEC diet.

Conclusions: Dietary CHO reduction favorably influences metabolic parameters but did not result in greater weight/fat loss relative to a standard diet in obese AA girls. Future research is needed to determine long-term effectiveness of a reduced CHO diet on glucose and insulin homeostasis and how it may apply to weight maintenance/fat loss during development alone and/or in combination with additional weight loss/metabolic improvement strategies.

Received September 10, 2010; accepted October 10, 2011.

From the *Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, and the †Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.

Address correspondence and reprint requests to Krista Casazza, PhD, RD, Department of Nutrition Sciences, Webb 415, 1530 3rd Ave S, University of Alabama at Birmingham, Birmingham, AL 35294-3360 (e-mail: kristac@uab.edu).

The present study was funded by grant NIH K99 DK083333, Thrasher Research Fund, University of Alabama Center for Women's Reproductive Health P/F Grant, CA-47888, NIH P60DK079626, P30 DK056336.

The authors report no conflicts of interest.

DOI: 10.1097/MPG.0b013e31823df207

Key Words: carbohydrate metabolism, diet, obesity, puberty, race

(*JPGN* 2012;54: 336–342)

In addition to the increased probability of developing obesity in adulthood, excessive body fat accrual during the developmental years is linked with earlier onset of chronic diseases (1) and, because of greater exposure, more adverse metabolic health outcomes (2–4). This trend is particularly salient in African American (AA) girls, who are disproportionately burdened by obesity as well as risk factors for the future progression to type 2 diabetes mellitus and cardiovascular diseases when compared with other racial/ethnic groups (5). Identification of strategies to limit excess adipose tissue acquisition and prevent metabolic perturbations early in the life course, particularly among AA girls, is essential.

Adipose tissue deposition/accumulation is influenced by multiple metabolic pathways in which nutrition plays a central role (2,6,7). Thus, dietary modification strategies are commonly undertaken to control weight and fat accumulation in both overweight/obese adults and children (6–10). Carbohydrate (CHO), the main dietary component affecting insulin action, affects the postprandial metabolic response contributing to weight and body composition changes. For example, in comparison to a low-CHO meal, the response to a meal high in CHO results in an augmented metabolic cascade of events (increased serum glucose and insulin, and subsequent increased lipogenesis and glycogenesis) (11). The relatively more rapid absorption of glucose associated with this type of meal challenges homeostatic mechanisms, complicating the transition from the postprandial to the postabsorptive state. For example, hypoglycemia is more likely to occur following consumption of a high-CHO meal, triggering counterregulatory hormone pathways, thereby influencing satiety cues (12). In addition, the proportion of CHO influences fuel oxidation and energy expenditure, reflecting a shift from fat to glucose oxidation and concomitant downregulation of energy expended at rest (12,13). Thus, high CHO intake can perturb metabolic pathways involved in fuel metabolism and may permit unfavorable body tissue compartmentalization and be conducive to impaired insulin homeostasis (14).

The response to a high-CHO diet may be further amplified in an individual with inherently high insulin secretion (eg, AA girls), particularly during sensitive times of growth and development (eg, puberty), further increasing the potential for excess fat deposition (15,16). We previously reported higher insulin secretion in AA girls and greater adipose tissue deposition (17). Accordingly, a dietary approach that minimizes the insulin-related altered/exaggerated cascade of events following meal ingestion, implemented during a critical period for adipose tissue deposition, may be beneficial in improving metabolic outcomes and facilitating weight loss. The

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 15, pp. 12241–12249, April 6, 2012

Noggin Is Novel Inducer of Mesenchymal Stem Cell Adipogenesis

IMPLICATIONS FOR BONE HEALTH AND OBESITY[*] [S]

Received for publication, August 12, 2011, and in revised form, February 7, 2012 Published, JBC Papers in Press, February 20, 2012, DOI 10.1074/jbc.M111.293613

Anandi Sawant[‡], Diptiman Chanda[‡], Tatyana Isayeva[‡], George Tsuladze[‡], W. T. Garvey[§], and Selvarangan Ponnazhagan[‡1]

From the Departments of [‡]Pathology and [§]Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama 35294

Background: Besides inhibiting osteoblast differentiation of MSC, noggin may induce adipogenesis.
Results: Noggin induces adipogenic differentiation of MSC via a novel mechanism. Individuals with high BMI have elevated circulating noggin levels in plasma.
Conclusion: Noggin regulates both osteoblast and adipocyte differentiation of MSC and, hence, is a master regulator of MSC plasticity.
Significance: Noggin may be a novel biomarker for obesity.

Noggin is a glycosylated-secreted protein known so far for its inhibitory effects on bone morphogenetic protein (BMP) signaling by sequestering the BMP ligand. We report here for the first time a novel mechanism by which noggin directly induces adipogenesis of mesenchymal stem cells independently of major human adipogenic signals through C/EBPδ, C/EBPα and peroxisome proliferator-activated receptor-γ. Evaluation of a possible mechanism for noggin-induced adipogenesis of mesenchymal stem cells identified the role of Pax-1 in mediating such differentiation. The relevance of elevated noggin levels in obesity was confirmed in a preclinical, immunocompetent mouse model of spontaneous obesity and in human patients with higher body mass index. These data clearly provide a novel role for noggin in inducing adipogenesis and possibly obesity and further indicates the potential of noggin as a therapeutic target to control obesity.

Ever since obesity was recognized as a major health problem, considerable efforts have been invested in identifying its causes, which affects individuals all over the world. A variety of factors play a role in obesity, thus making it a complex health issue to address. The earlier belief that obesity is caused by uncoupling of energy intake and expenditure has been further delineated in the last decade at a molecular level (1, 2). Consequently, roles of various adipokines such as adiponectin, leptin, and hormones like testosterone, adrenalin, and thyroid hormones in promoting obesity have been established (3). However, not much information exists on the key molecular mechanism(s) that triggers adipogenesis.

Adipocytes originate from multipotent mesenchymal stem cells (MSC), which also give rise to other lineages including osteoblasts, chondrocytes, and myocytes (4). Within the bone marrow, the differentiation of MSC into either osteoblasts or adipocytes is delicately balanced and influenced by several growth factors. The processes of osteoblastogenesis and adipogenesis are reciprocally associated (5). The balance is tilted toward adipogenesis with age and in several bone diseases with progressive bone loss such as osteoporosis. MSC from osteoporosis patients have increased adipogenic potential as shown by increased PPAR-γ[2] levels, which is a major transcription factor for adipocyte differentiation (6).

Studies using murine models of obesity and aging clearly show decreased osteoblastogenesis and increased adipogenic potential of bone marrow MSC (7, 8). *Ex vivo* cultures of MSC from these mice showed increased numbers of fully differentiated marrow adipocytes compared with age-matched control (8). Both these mice models also exhibit decreased osteoblast numbers and functions (7, 8). Decreased osteoblast function in such models was not due to impaired levels of bone morphogenetic protein (BMP), which is known to induce osteoblast differentiation and function, but was rather due to increased noggin levels. Noggin is a glycoprotein that was discovered for its ability to induce secondary axis formation in *Xenopus* (9). Classically, noggin is well known as a potent inhibitor of BMPs and thus of osteoblast differentiation (10, 11). Very little is known about other functions of noggin, if any. As the processes of osteoblastogenesis and adipogenesis are strikingly interdependent, we hypothesized that in addition to sequestering BMP from interacting with its receptor, noggin may have a dual role in inducing adipogenesis and consequently obesity.

* This work was supported, in whole or in part, by National Institutes of Health Grants AR050251, AR060948 (to S. P.), DK083562 (to W. T. G.), and DK038765 (to W. T. G.). This work was also supported by the University of Alabama at Birmingham Diabetes Research and Training Center Grant P60 DK079626 (to W. T. G.).
[S] This article contains supplemental Figs. S1–S5.
[1] To whom correspondence should be addressed: Dept. of Pathology, 701 19th St. South, LHRB 505, The University of Alabama at Birmingham, Birmingham, AL 35294-0007. Tel.: 205-934-6731; Fax: 205-975–9927; E-mail: pons@uab.edu.

[2] The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; MSC, mesenchymal stem cell; Pax-1, Paired box gene-1; BMP, bone morphogenetic protein; ADM, adipocyte differentiation medium; GPDH, glycerol-3-phosphate dehydrogenase; micro-CT, micro-computed tomography; BMI, body mass index.

Race Differences in the Association of Oxidative Stress With Insulin Sensitivity in African- and European-American Women

Gordon Fisher[1], Jessica A. Alvarez[1], Amy C. Ellis[1], Wesley M. Granger[2], Fernando Ovalle[3], Chiara Dalla Man[4], Claudio Cobelli[4] and Barbara A. Gower[1]

Excessive metabolism of glucose and/or fatty acids may impair insulin signaling by increasing oxidative stress. The objective of this study was to examine the association between insulin sensitivity and protein carbonyls, a systemic marker of oxidative stress, in healthy, nondiabetic women, and to determine if the relationship differed with race. Subjects were 25 African-Americans (AA, BMI 28.4 ± 6.2 kg/m^2, range 18.8–42.6 kg/m^2; age 33.1 ± 13.5 years, range 18–58 years) and 28 European-Americans (EA, BMI 26.2 ± 5.9 kg/m^2, range 18.7–48.4 kg/m^2; age 31.6 ± 12.4 years, range 19–58 years). Insulin sensitivity was determined using an intravenous glucose tolerance test incorporating [6,6-$^2H_2$]-glucose, and a two-compartment mathematical model. Multiple linear regression results indicated that insulin sensitivity was inversely associated with protein carbonyls in AA (standardized regression coefficient –0.47, $P < 0.05$) but not EA (0.01, $P = 0.945$), after adjusting for %body fat. In contrast, %body fat was significantly and positively associated with insulin sensitivity in EA (–0.54, $P < 0.01$) but not AA (–0.24, $P = 0.196$). Protein carbonyls were associated with free fatty acids (FFA) in AA ($r = 0.58$, $P < 0.01$) but not EA ($r = –0.11$, $P = 0.59$). When subjects were divided based on median levels of fasting glucose and FFA, those with higher glucose/FFA concentrations had a significantly greater concentration of circulating protein carbonyls compared to those with lower glucose/FFA concentrations ($P < 0.05$). These results suggest that oxidative stress independently contributes to insulin sensitivity among AA women. Further, this association in AA may be mediated by circulating FFA and/or glucose.

Obesity (2012) **20,** 972–977. doi:10.1038/oby.2011.355

INTRODUCTION

Type 2 diabetes is associated with both insulin resistance and decreased insulin secretion (1–3). There is considerable evidence demonstrating that hyperglycemia and/or elevated free fatty acids (FFA) may increase the production of reactive oxygen species (ROS) (4,5). An increase in glucose/FFA-induced production of ROS may create a persistent imbalance between the formation and adequate removal (via antioxidant defenses) of ROS, leading to oxidative stress. Convincing evidence, both *in vivo* (6–8) and *in vitro* (9,10), has shown that oxidative stress may play a critical role in the pathogenesis of type 2 diabetes.

Elevated ROS production without a concomitant increase in scavenging from antioxidant defense mechanisms can alter the redox balance within the cell, leading to oxidative damage to proteins, lipids, and nucleic acids. The mitochondria respiratory chain is thought to represent the major source of ROS formation (11); however, NADPH oxidases are also known to contribute to ROS production (12). A few recent clinical trials have shown associations between systemic oxidative stress and insulin resistance, evaluated by homeostasis model assessment, in both diabetic and prediabetic individuals (13,14). The mechanism through which ROS elicits its deleterious effects on insulin signaling is thought to be the activation of multiple serine/threonine kinase pathways (15,16). Several studies have shown that oxidative stress is often present before diabetic complications become clinically evident (5,17); therefore it is important to assess the relationship between oxidative stress and insulin sensitivity in nondiabetic individuals.

Oxidative stress has been implicated in the etiology of several chronic diseases, including type 2 diabetes, atherosclerosis, hypertension, and cancer (12,18–21). It has also been well documented that African-Americans (AA) are at a disproportionately higher risk for developing many of these oxidative stress-related conditions (20,22,23). Whether oxidative stress plays a larger role in determining insulin sensitivity within populations at elevated risk for type 2 diabetes, such as AA,

[1]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA; [2]Department of Clinical and Diagnostic Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA; [3]Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA; [4]Department of Information Engineering, Padova University, Padova, Italy. Correspondence: Gordon Fisher (grdnfs@uab.edu)

Received 6 July 2011; accepted 20 October 2011; advance online publication 15 December 2011. doi:10.1038/oby.2011.355

Body Fat and Racial Genetic Admixture Are Associated With Aerobic Fitness Levels in a Multiethnic Pediatric Population

Amanda L. Willig[1,2], Gary R. Hunter[3], Krista Casazza[1], Douglas C. Heimburger[4], T. Mark Beasley[5] and Jose R. Fernandez[1,4]

Aerobic fitness and adiposity are each independently associated with health outcomes among children, although the relationship between these two variables is unclear. Our objectives were to evaluate (i) the association of adiposity with aerobic fitness using objectively measured levels of percent body fat, compared to BMI as a percentile proxy for adiposity while controlling for genetic admixture, and (ii) the congruence of BMI categories with high and low body fat categories of objectively measured percent body fat. Participants were 232 African-American (AA), European-American (EA), and Hispanic-American (HA) children aged 7–12 years (Tanner stage <3). Aerobic fitness was measured via a submaximal indirect calorimetry treadmill test (VO_{2-170}), and physical activity levels with accelerometry. Genetic admixture estimates were obtained using 140 genetic ancestry informative markers to estimate European, African, and Amerindian admixture. Fat mass was determined using dual-energy x-ray absorptiometry (DXA). Children were classified into a low body fat group (<25% in males, <30% in females) or a high body fat group based on their percent body fat; children were also categorized according to BMI percentile. Children in the low body fat group had significantly higher aerobic fitness ($P < 0.05$) regardless of BMI percentile classification. Higher African genetic admixture was associated with lower aerobic fitness ($P < 0.05$), while physical activity was positively associated with fitness ($P < 0.01$). In conclusion, aerobic fitness levels differ by percent body fat and genetic admixture irrespective of BMI classification, and such differences should be taken into account when evaluating outcomes of health interventions.

Obesity (2011) **19,** 2222–2227. doi:10.1038/oby.2011.109

INTRODUCTION

Aerobic fitness has been inversely associated with risk for several chronic diseases in both children and adolescents (1–3). Lower body fat levels are also indicative of decreased chronic disease risk, and an inverse association of fitness with body fat levels has been noted (4). Increased aerobic fitness may therefore be an important outcome of interventions designed to reduce the frequency of high body fat, insulin resistance, and elevated blood pressure among children. Studies that measure fitness levels among children often utilize gender- and age-adjusted BMI (weight (kg)/height (m^2)) percentiles to recruit participants classified as overweight/obese, in which a BMI ≥85th percentile is considered overweight, and BMI ≥95th percentile categorized as obese (5). However, a higher pediatric BMI is often caused by greater amounts of both lean and fat mass, and the actual contribution of excess adiposity to aerobic fitness may be masked if BMI cut points are used to estimate excess adiposity (6,7). Recent studies have shown that not all children classified as overweight or obese present with excess body fat (8); however, no studies have evaluated whether this discrepancy could influence outcomes in aerobic fitness.

Recent investigations have also identified racial/ethnic differences in aerobic fitness, as well as in the relationship between BMI and body fat (4,9–11). Racial/ethnic differences in fitness as measured by maximum oxygen consumption (VO_{2-max}) among African-American (AA) and European-American (EA) children and adults are well documented, with a lower VO_{2-max} in AA independent of body weight (9,12). Other studies have indicated that Hispanic-American (HA) children may present with aerobic fitness levels similar to or lower than those of EA children (10,11). Additionally, traditional cut points for overweight and obese classification may represent different levels

[1]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA; [2]Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA; [3]Department of Human Studies, University of Alabama at Birmingham, Birmingham, Alabama, USA; [4]Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA; [5]Section on Statistical Genetics, Department of Biostatistics, University of Alabama at Birmingham, Birmingham, Alabama, USA. Correspondence: Amanda L. Willig (mandyrd@uab.edu)

Received 22 September 2010; accepted 22 March 2011; published online 5 May 2011. doi:10.1038/oby.2011.109

Cell

Adipocyte NCoR Knockout Decreases PPARγ Phosphorylation and Enhances PPARγ Activity and Insulin Sensitivity

Pingping Li,[1] WuQiang Fan,[1] Jianfeng Xu,[1] Min Lu,[1] Hiroyasu Yamamoto,[2] Johan Auwerx,[2] Dorothy D. Sears,[1] Saswata Talukdar,[1] DaYoung Oh,[1] Ai Chen,[1] Gautam Bandyopadhyay,[1] Miriam Scadeng,[3] Jachelle M. Ofrecio,[1] Sarah Nalbandian,[1] and Jerrold M. Olefsky[1,*]

[1]Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

[2]Laboratory of Integrative and Systems Physiology, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

[3]Department of Radiology, University of California, San Diego, CA 92098, USA

*Correspondence: jolefsky@ucsd.edu

DOI 10.1016/j.cell.2011.09.050

SUMMARY

Insulin resistance, tissue inflammation, and adipose tissue dysfunction are features of obesity and Type 2 diabetes. We generated adipocyte-specific Nuclear Receptor Corepressor (NCoR) knockout (AKO) mice to investigate the function of NCoR in adipocyte biology, glucose and insulin homeostasis. Despite increased obesity, glucose tolerance was improved in AKO mice, and clamp studies demonstrated enhanced insulin sensitivity in liver, muscle, and fat. Adipose tissue macrophage infiltration and inflammation were also decreased. PPARγ response genes were upregulated in adipose tissue from AKO mice and CDK5-mediated PPARγ ser-273 phosphorylation was reduced, creating a constitutively active PPARγ state. This identifies NCoR as an adaptor protein that enhances the ability of CDK5 to associate with and phosphorylate PPARγ. The dominant function of adipocyte NCoR is to transrepress PPARγ and promote PPARγ ser-273 phosphorylation, such that NCoR deletion leads to adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity, phenocopying the TZD-treated state.

INTRODUCTION

The adipocyte uses well regulated transcriptional programs to adapt to environmental inputs through storage of calories as triglycerides and secretion of adipokines and other factors (Rosen and Spiegelman, 2006). PPARγ is a key factor controlling the importance of adipose tissue in whole-body glucose metabolism (Evans et al., 2004; Lehrke and Lazar, 2005; Saltiel and Olefsky, 1996; Sugii et al., 2009; Tontonoz and Spiegelman, 2008). PPARγ is a member of the nuclear hormone receptor (NR) family and is highly enriched in adipose tissue, where it plays a critical role in adipocyte differentiation, insulin sensitivity, and adipokine/cytokine secretion (Evans et al., 2004; Imai et al., 2004; Rangwala and Lazar, 2004; Tontonoz and Spiegelman, 2008). Although its endogenous ligand is poorly understood, PPARγ is the molecular target for the thiazolidinedione (TZD) class of insulin-sensitizing drugs used to treat type 2 diabetes.

Transcriptional control by NRs, including PPARγ and others, depends on multiprotein coregulatory complexes (Feige and Auwerx, 2007; Fowler and Alarid, 2004; Hermanson et al., 2002). In general, corepressor complexes are recruited to NRs in the absence of ligand, whereas coactivator complexes are recruited to NRs in the presence of agonists (Lonard and O'Malley, 2005). Coactivators and corepressors modulate gene transcription by a variety of mechanisms including histone acetylation, chromatin remodeling, and direct interactions with basal transcription complexes (Collingwood et al., 1999). There are several coactivators, such as CBP, PGC1α, and CRTC2, that are known to play important roles in metabolic control (Handschin and Spiegelman, 2008; Revilla and Granja, 2009; Wang et al., 2010). However, the role and underlying mechanisms of corepressor function in metabolic tissues remains unclear. Two major NR corepressors are the silencing mediator of retinoid and thyroid hormone receptors (SMRT) and the nuclear receptor corepressor (NCoR) (Chen and Evans, 1995; Horlein et al., 1995). It has been shown that downregulation of SMRT and NCoR expression in 3T3-L1 cells leads to enhanced adipocyte differentiation, in part through increased PPARγ transcriptional activity (Yu et al., 2005). However, their role in adipogenesis, adipocyte function, and glucose metabolism in vivo remains uncertain. Since whole body NCoR deletion is embronically lethal (Jepsen et al., 2000), we generated adipocyte-specific NCoR knockout (AKO) mice to assess the role of this corepressor in glucose metabolism, insulin sensitivity, and adipogenesis. We show that AKO mice develop increased adiposity on HFD relative to WT controls. Despite this increase in obesity, the AKO animals exhibit enhanced systemic insulin sensitivity, improved glucose tolerance, and decreased adipose tissue inflammation. Taken together, these features phenocopy the effects of systemic TZD treatment.

Molecular Cell
Article

A Protective Strategy against Hyperinflammatory Responses Requiring the Nontranscriptional Actions of GPS2

M. Dafne Cardamone,[1,6] Anna Krones,[1] Bogdan Tanasa,[1,4] Havilah Taylor,[1] Laura Ricci,[5] Kenneth A. Ohgi,[1] Christopher K. Glass,[2,3] Michael G. Rosenfeld,[1,2,*] and Valentina Perissi[2,6,*]
[1]Howard Hughes Medical Institute
[2]Department of Medicine, Division of Endocrinology and Metabolism
[3]Department of Cellular and Molecular Medicine
School of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[4]Graduate Program, Kellogg School of Science and Technology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
[5]Center for Molecular Systems Biology, University of Torino, 10124 Torino, Italy
[6]Present address: Boston University School of Medicine, Biochemistry Department, 72 East Concord Street, Boston, MA 02118, USA
*Correspondence: mrosenfeld@ucsd.edu (M.G.R.), vperissi@bu.edu (V.P.)
DOI 10.1016/j.molcel.2012.01.025

SUMMARY

The association between hyperinflammatory states and numerous diseases is widely recognized, but our understanding of the molecular strategies that have evolved to prevent uncontrolled activation of inflammatory responses remains incomplete. Here, we report a critical, nontranscriptional role of GPS2 as a guardian against hyperstimulation of the TNF-α-induced gene program. GPS2 cytoplasmic actions are required to specifically modulate RIP1 ubiquitylation and JNK activation by inhibiting TRAF2/Ubc13 enzymatic activity. In vivo relevance of GPS2 anti-inflammatory role is confirmed by inhibition of TNF-α target genes in macrophages and by improved insulin signaling in the adipose tissue of *aP2-GPS2* transgenic mice. As the nontranscriptional role is complemented by GPS2 functioning as positive and negative cofactor for nuclear receptors, in vivo overexpression also results in elevated circulating level of Resistin and development of hepatic steatosis. Together, these studies define GPS2 as a molecular guardian required for precise control of inflammatory responses involved in immunity and homeostasis.

INTRODUCTION

Preventing the hyperstimulation of inflammatory responses is a key requirement for normal homeostasis, which can be achieved by different strategies, including regulating the production of inflammatory cytokines, restricting the response to basal stimuli, and ensuring the proper termination of the activated signaling cascade. Excessive or prolonged activation of proinflammatory signaling pathways has been implicated in the pathogenesis of several human diseases, including autoimmune disorders, neurodegenerative diseases, and cancer (Amor et al., 2010; Grivennikov et al., 2010). Chronic inflammation has also been linked with obesity, insulin resistance, and development of type 2 diabetes, and convincing evidence has indicated that local production of proinflammatory cytokines, including TNF-α, leads to the disruption of the insulin response in adipose tissue and peripheral organs (Hotamisligil, 2006; Lee and Pratley, 2005). Proinflammatory signals, together with elevated levels of FFA and ROS, activate a series of stress-induced serine kinases, including JNK and IKKβ, which play a key role in the development of insulin resistance as indicated by the improvement in insulin sensitivity associated with their chemical inhibition or with the genetic disruption of JNK (Hirosumi et al., 2002; Sabio and Davis, 2010).

Activation of JNK and IKKβ kinases in response to inflammatory stimuli is achieved via the coordinated actions of a large number of signaling proteins and enzymatic activities. In the case of TNF-α, trimerization of liganded TNFR1 leads to the formation of a membrane-associated complex including TRADD, TRAF2, RIP1, and cIAP1/2, with one TRAF2 trimer required for the recruitment of each cIAP2 molecule (Chen and Goeddel, 2002; Zheng et al., 2010). Overall, the enzymatic activities of TRAF2, cIAP1, and Ubc13 are together required for the polyubiquitination of RIP1, which was initially thought to act as a general scaffold for the assembly of TAK1/TAB1/TAB2 and IKKα/IKKβ/NEMO complexes. However, this simple model has been recently revised, and in the emerging picture, there is a significant separation between the activation of JNK and IKKβ in terms of the adaptor proteins, the enzymatic activities, and the type of ubiquitin chains utilized in the activation cascade (Bianchi and Meier, 2009; Deribe et al., 2010; Liu and Chen, 2010). Because of the importance of avoiding uncontrolled stimulation of inflammatory responses, each of the signaling steps has to be tightly regulated. While some inhibitory strategies have been uncovered, including factors such as A20 (TNFAIP3) and CYLD that negatively regulate the ubiquitylation status of key signaling molecules (Bhoj and Chen, 2009; Sun, 2008), the

nature genetics

A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance

Recent genome-wide association studies have described many loci implicated in type 2 diabetes (T2D) pathophysiology and β-cell dysfunction but have contributed little to the understanding of the genetic basis of insulin resistance. We hypothesized that genes implicated in insulin resistance pathways might be uncovered by accounting for differences in body mass index (BMI) and potential interactions between BMI and genetic variants. We applied a joint meta-analysis approach to test associations with fasting insulin and glucose on a genome-wide scale. We present six previously unknown loci associated with fasting insulin at $P < 5 \times 10^{-8}$ in combined discovery and follow-up analyses of 52 studies comprising up to 96,496 non-diabetic individuals. Risk variants were associated with higher triglyceride and lower high-density lipoprotein (HDL) cholesterol levels, suggesting a role for these loci in insulin resistance pathways. The discovery of these loci will aid further characterization of the role of insulin resistance in T2D pathophysiology.

In contrast to recent progress in the discovery of genetic variants underlying T2D pathophysiology and β-cell function, the understanding of the genetic basis of insulin resistance remains limited[1]. Partly because early case-control studies of T2D were designed to maximize the likelihood of detecting variants that directly increase T2D risk rather than those that affect risk through the mediation of adiposity, most of the associated loci discovered in these studies mapped to genes related to β-cell dysfunction[2]. More recently, we have shown that the genetic architectures of quantitative indices of β-cell function and of insulin resistance differ markedly: given the same individuals, sample sizes and biochemical measurements, we described a larger number of signals for β-cell function than for insulin resistance[3,4]. Although this observation is consistent with the higher reported heritability of insulin secretion compared to resistance, overall heritability estimates of insulin resistance in individuals of European ancestry of 25–44% suggest that many loci remain to be discovered and that new strategies are required for their identification[5].

Obesity is an important determinant of insulin resistance[6]. It was postulated that adiposity might modulate the genetic determinants of insulin resistance and contribute to the heterogeneity of T2D etiology. It has been shown that the heritability of insulin resistance increases with higher BMI[7], and some candidate gene studies have observed that genetic effect size varies with adiposity level[8–10], findings that are compatible with the presence of an underlying interaction between BMI and genetic variants for insulin resistance. Furthermore, the adipokine hormones and proinflammatory cytokines that are produced by adipose tissue can influence insulin signaling via diverse mechanisms[11,12], and these processes may interact with genetic variants influencing insulin resistance pathways. Therefore, to identify variants associated with insulin resistance, it may also be important to account for gene variant by BMI interaction, which would allow for the potential for adiposity levels to perturb the physiological milieu in which genetic variants in insulin signaling pathways operate. Adiposity may also hinder the identification of genetic variants influencing insulin resistance by introducing variance in the outcome that is not attributable to genetic variation[5], suggesting that adjustment for adiposity *per se* may be necessary.

A joint test that investigates the association between an outcome and a genetic variant, while allowing for possible effect modification by an environmental variable, has been proposed[13]. Moreover, a statistical method was developed that extends this joint test to a meta-analysis context[14]. This enabled us to simultaneously test both the main genetic effect, adjusted for BMI, and potential interaction between each genetic variant and BMI. This joint meta-analysis (JMA) approach can provide increased power for detecting genetic loci when underlying interaction effects are suspected but unknown[13], and, notably, as shown in simulation studies, this approach does not reduce power to detect the main genetic effects in the absence of interaction[14]. Within the Meta-Analyses of Glucose- and Insulin-related traits Consortium (MAGIC), we implemented this approach and performed a genome-wide JMA to search for SNPs significantly associated with glycemic traits, while simultaneously adjusting for BMI and allowing for interaction with BMI. Using this method, we successfully identified loci that are associated with fasting insulin levels at genome-wide significance levels.

RESULTS

Study overview

As a first phase, we conducted a discovery genome-wide JMA of the main effects of SNPs and of SNP by BMI (SNP × BMI) interaction for four diabetes-related quantitative traits: fasting insulin levels, fasting glucose levels and surrogate measures of β-cell function

A full list of authors and affiliations appears at the end of the paper.

Received 7 July 2011; accepted 13 April 2012; published online 13 May 2012; doi:10.1038/ng.2274

Cell Metabolism
Short Article

PPARγ agonists Induce a White-to-Brown Fat Conversion through Stabilization of PRDM16 Protein

Haruya Ohno,[1] Kosaku Shinoda,[1] Bruce M. Spiegelman,[2,*] and Shingo Kajimura[1,*]
[1]UCSF Diabetes Center and Department of Cell and Tissue Biology, University of California, San Francisco, 35 Medical Center Way, San Francisco, CA 94143-0669, USA
[2]Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA
*Correspondence: bruce_spiegelman@dfci.harvard.edu (B.M.S.), skajimura@diabetes.ucsf.edu (S.K.)
DOI 10.1016/j.cmet.2012.01.019

SUMMARY

Brown adipose tissue dissipates energy through heat and functions as a defense against cold and obesity. PPARγ ligands have been shown to induce the browning of white adipocytes; however, the underlying mechanisms remain unclear. Here, we show that PPARγ ligands require full agonism to induce a brown fat gene program preferentially in subcutaneous white adipose. These effects require expression of PRDM16, a factor that controls the development of classical brown fat. Depletion of PRDM16 blunts the effects of the PPARγ agonist rosiglitazone on the induced brown fat gene program. Conversely, PRDM16 and rosiglitazone synergistically activate the brown fat gene program in vivo. This synergy is tightly associated with an increased accumulation of PRDM16 protein, due in large measure to an increase in the half-life of the protein in agonist treated cells. Identifying compounds that stabilize PRDM16 protein may represent a plausible therapeutic pathway for the treatment of obesity and diabetes.

INTRODUCTION

White adipose tissue (WAT) functions to store excess energy as triglycerides, whereas brown adipose tissue (BAT) is specialized to dissipate chemical energy as heat. The developmental and transcriptional control of BAT has received much attention over the last several years, mainly because of its potential role in the defense against obesity and obesity-associated disorders (reviewed in [Enerbäck, 2010]). Recent findings using ^{18}fluoro-labeled 2-deoxy-glucose positron emission tomography (^{18}FDG-PET) scanning have shown clearly that most if not all normal adult humans have distinct brown fat deposits. The thermogenic activity of this tissue correlates inversely with overall adiposity, raising the possibility that variation in the amount or activity of BAT may contribute to the propensity for weight gain in humans (Cypess et al., 2009; Nedergaard et al., 2007; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Yoneshiro et al., 2011). Recent research has also identified several dominant transcriptional regulators of brown adipocyte development and function, including peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α), FoxC2 (Forkhead box C2) and PRDM16 (PRD1-BF-1-RIZ1 homologous domain containing protein-16) (reviewed in [Kajimura et al., 2010]) Genetic loss of PGC1α and PRDM16 in mice clearly interferes with the function or/and development of BAT. Detailed knowledge of these pathways may offer promising opportunities to manipulate BAT in vivo for therapeutic regimens to counteract obesity and type 2 diabetes.

It is now clear that two different types of brown adipocytes exist, and these have distinct developmental origins. Classical brown adipocytes residing in the interscapular and perirenal regions develop from myoblastic-like *Myf5*-positive precursors (Seale et al., 2008) that differentiate into brown adipocytes through the action of transcriptional regulators PRDM16 and C/EBPβ (Kajimura et al., 2009; Seale et al., 2008). Furthermore, global gene expression analyses indicate that classical interscapular brown fat precursors have a gene profile overlapping that of skeletal muscle cells (Timmons et al., 2007). On the other hand, pockets of a second, distinct type of UCP1-positive adipocytes are found sporadically in WAT of adult animals that have been exposed to chronic cold or β-adrenergic agonists. These inducible brown-like adipocytes (beige or brite cells) possess many of the biochemical and morphological characteristics of classical brown adipocytes, including the presence of multilocular lipid droplets (Frontini and Cinti, 2010). However, they arise from a non-*Myf5* cell lineage and, hence, have distinct origins from the classical brown adipocytes. Indeed, it has been shown previously that epididymal WAT-derived brite cells that are induced by rosiglitazone do not express myocyte-enriched genes (Petrovic et al., 2010). In addition, adipogenic Sca-1$^+$/CD45$^-$/Mac1$^-$progenitors from different adipose depots showed unique molecular signatures (Schulz et al., 2011).

Because the emergence of inducible-brown adipocytes in WAT is associated with a protection against obesity and metabolic diseases in rodent models (Cederberg et al., 2001; Leonardsson et al., 2004; Seale et al., 2011), an important challenge is to understand the molecular mechanisms by which environmental cues stimulate the development of these beige/brite cells. In this regard, it has been shown that activation of PPARγ by synthetic ligands induces a brown fat-like gene program in WAT (Fukui et al., 2000; Petrovic et al., 2010; Rong et al., 2007; Sell et al., 2004; Tai et al., 1996; Vernochet et al., 2009; Wilson-Fritch et al., 2004). Mechanistically, these drugs function by directly binding to and activating PPARγ and PPAR-response elements (PPREs) on the promoter and/or enhancer of brown

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 2 DECEMBER 2012 | DOI: 10.1038/NCHEMBIO.1136

Whole-organism screening for gluconeogenesis identifies activators of fasting metabolism

Philipp Gut[1–5]*, Bernat Baeza-Raja[6], Olov Andersson[1–5,12], Laura Hasenkamp[1–5], Joseph Hsiao[1–5], Daniel Hesselson[1–5,12], Katerina Akassoglou[6], Eric Verdin[7,8], Matthew D Hirschey[7–11] & Didier Y R Stainier[1–5,12]*

Improving the control of energy homeostasis can lower cardiovascular risk in metabolically compromised individuals. To identify new regulators of whole-body energy control, we conducted a high-throughput screen in transgenic reporter zebrafish for small molecules that modulate the expression of the fasting-inducible gluconeogenic gene *pck1*. We show that this *in vivo* strategy identified several drugs that affect gluconeogenesis in humans as well as metabolically uncharacterized compounds. Most notably, we find that the translocator protein ligands PK 11195 and Ro5-4864 are glucose-lowering agents despite a strong inductive effect on *pck1* expression. We show that these drugs are activators of a fasting-like energy state and, notably, that they protect high-fat diet–induced obese mice from hepatosteatosis and glucose intolerance, two pathological manifestations of metabolic dysregulation. Thus, using a whole-organism screening strategy, this study has identified new small-molecule activators of fasting metabolism.

Cardiovascular disease is the primary cause of death worldwide[1]. Metabolic disorders such as obesity and type 2 diabetes, which constitute major risk factors for cardiovascular pathogenesis[2], lead to a poor control of energy homeostasis. The resulting chronic hyperlipidemia and hyperglycemia can further contribute to insulin resistance and the loss of metabolic flexibility toward changes in nutrient availability. Ultimately, aberrant lipid accumulation occurs in several organs and has deleterious consequences[3,4]. Current drug discovery strategies focus on molecules that can function on diverse pathogenic modalities of metabolic disease by shifting the impaired energy control of a metabolically ill individual toward that of a metabolically healthy person[5,6]. Despite vigorous academic and industrial efforts, discovery and development of new lead compounds for the amelioration of metabolic diseases remain ineffective. The slow progress reflects complex inter-organ feedback circuits that regulate the behavior and metabolic management of energy intake, storage and expenditure. In addition, biotransformation and xenobiotic defense of different organs influence the pharmacodynamics and pharmacokinetics of chemical agents in a living organism. These complexities set high bars for extrapolating drug-target interactions *in vitro* to pharmacological actions *in vivo*, and many drugs that are identified in target-based screening approaches ultimately fail[7,8]. Taking these challenges into consideration, it becomes evident that developing rapid, cost-efficient and translational small-molecule discovery and metabolic profiling technologies in whole organisms is necessary to identify effective therapeutics.

Here, we report on an innovative drug discovery strategy in larval zebrafish for the identification of metabolically active drugs with potential therapeutic function. Zebrafish have proven to be a powerful model for phenotype-based small-molecule screening that can be translated into mammalian pharmacology[9]. In addition, fundamental principles of energy homeostasis are evolutionarily conserved in metazoans[10]. We leveraged zebrafish yolk consumption to pharmacologically profile pathways of energy control; when yolk-derived carbohydrates become depleted, zebrafish initiate a gluconeogenic program to match glucose demand for organ function and organismal survival. This gluconeogenic 'feeding-to-fasting' switch can be monitored in thousands of larvae per experimental day without additional variables such as an external nutrient supply. At this stage, zebrafish larvae have developed functional organs, and they are still small enough to be assayed in a high-throughput 96-well-plate format. Furthermore, zebrafish can be easily treated with drugs, and their transparency allows direct microscopic evaluation of organ morphology as well as general health.

On the basis of these characteristics, we developed and applied a drug discovery approach that rapidly interrogates small molecules on their ability to interfere with gluconeogenesis and its homeostatic control. For fast quantitative and visual monitoring of gluconeogenesis, we generated transgenic bioluminescence and fluorescence reporter zebrafish using the cytosolic phosphoenolpyruvate carboxykinase (*pck1*) promoter. *pck1* is a prototypical fasting responsive gene: when dietary carbohydrates are low after prolonged fasting, *pck1* is induced transcriptionally, and its protein product catalyzes a regulatory step in gluconeogenesis by mediating the conversion of tricarboxylic acid cycle–derived oxaloacetate to phosphoenolpyruvate and carbon dioxide in the liver

[1]Department of Biochemistry and Biophysics, University of California–San Francisco, San Francisco, California, USA. [2]Program in Developmental and Stem Cell Biology, University of California–San Francisco, San Francisco, California, USA. [3]Program in Genetics and Human Genetics, University of California–San Francisco, San Francisco, California, USA. [4]The Diabetes Center, University of California–San Francisco, San Francisco, California, USA. [5]Institute for Regeneration Medicine and Liver Center, University of California–San Francisco, San Francisco, California, USA. [6]The Gladstone Institute of Neurological Disease, San Francisco, California, USA. [7]The Gladstone Institute of Virology and Immunology, San Francisco, California, USA. [8]Department of Medicine, University of California–San Francisco, San Francisco, California, USA. [9]Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, North Carolina, USA. [10]Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA. [11]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina, USA. [12]Present addresses: Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden (O.A.); Garvan Institute of Medical Research, Diabetes and Obesity Research Program, Darlinghurst, New South Wales, Australia (D.H.); Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany (D.Y.R.S.). *e-mail: philipp.gut@ucsf.edu or didier.stainier@mpi-bn.mpg.de

Research Resource: RNA-Seq Reveals Unique Features of the Pancreatic β-Cell Transcriptome

Gregory M. Ku,* Hail Kim,* Ian W. Vaughn, Matthew J. Hangauer, Chang Myung Oh, Michael S. German, and Michael T. McManus

Diabetes Center (G.M.K., I.W.V., M.J.H., H.K., C.M.O., M.S.G., M.T.M.); Division of Endocrinology and Metabolism, Department of Medicine (G.M.K., M.S.G.); and Department of Microbiology and Immunology (M.T.M.), University of California, San Francisco, California 94143; and Graduate School of Medical Science and Engineering (H.K., C.M.O.), KAIST, Daejeon, South Korea

The pancreatic β-cell is critical for the maintenance of glycemic control. Knowing the compendium of genes expressed in β-cells will further our understanding of this critical cell type and may allow the identification of future antidiabetes drug targets. Here, we report the use of next-generation sequencing to obtain nearly 1 billion reads from the polyadenylated RNA of islets and purified β-cells from mice. These data reveal novel examples of β-cell-specific splicing events, promoter usage, and over 1000 long intergenic noncoding RNA expressed in mouse β-cells. Many of these long intergenic noncoding RNA are β-cell specific, and we hypothesize that this large set of novel RNA may play important roles in β-cell function. Our data demonstrate unique features of the β-cell transcriptome. **(*Molecular Endocrinology* 26: 1783–1792, 2012)**

The pancreatic β-cell is the body's main source of insulin. The devastating metabolic consequences of the β-cell loss or dysfunction seen in diabetes mellitus highlight the critical role of these cells in nutrient metabolism. The ability to match insulin production to physiological needs results from the β-cell's unique transcriptional program. Yet, no studies have defined β-cell transcriptional landscapes with a high resolution, either in diseased or healthy primary β-cells.

Some studies have described transcriptional profiles of β-cells and pancreatic islets using oligonucleotide arrays (1, 2) and, more recently, massively parallel signature sequencing (3). However, oligonucleotide array studies are limited to the detection of sequences that are already printed on the arrays, whereas unbiased massively parallel signature sequencing is limited by sheer throughput. Next-generation mRNA sequencing (mRNA-seq) addresses these shortcomings (4) and has not yet been applied to primary β-cells.

The ability of mRNA-seq to detect low-abundance, novel transcripts has resulted in the identification of a novel class of RNA, long intergenic noncoding RNA (lincRNA). These RNA are greater than 200 nucleotides in length and do not encode proteins. Thousands of distinct lincRNA loci have been described in the mouse and human genomes (5, 6). Although the biological functions of only a few have been explored, lincRNA regulate diverse processes including epigenetic silencing, apoptosis, alternative splicing, and protein translation (reviewed in Ref. 7).

Here, we describe a high-resolution analysis of pancreatic β-cells, providing a new view of the β-cell transcriptome with an unprecedented level of specificity, sensitivity, and breadth. In addition to β-cell-specific gene expression, we also delineate β-cell-specific promoter use, alternative splicing, and a comprehensive inventory of novel β-cell-specific lincRNA.

Materials and Methods

Islet isolation and cell sorting

Islets from 16- to 20-wk-old mouse insulin promoter (MIP)-green fluorescent protein (GFP) mice were isolated by the Uni-

ISSN Print 0888-8809 ISSN Online 1944-9917
Printed in U.S.A.

doi: 10.1210/me.2012-1176 Received May 7, 2012. Accepted July 16, 2012.
First Published Online August 21, 2012

* G.M.K. and H.K. contributed equally to this work.

Abbreviations: FACS, Fluorescence-activated cell sorting; FPKM, fragments per kilobase of transcript per million mapped reads; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; lincRNA, long intergenic noncoding RNA; MIP, mouse insulin promoter; mRNA-seq, mRNA sequencing; qPCR, quantitative PCR; UTR, untranslated regions.

Diabetologia (2013) 56:391–400
DOI 10.1007/s00125-012-2753-4

ARTICLE

Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial

K. C. Herold · S. E. Gitelman · S. M. Willi · P. A. Gottlieb · F. Waldron-Lynch · L. Devine · J. Sherr · S. M. Rosenthal · S. Adi · M. Y. Jalaludin · A. W. Michels · J. Dziura · J. A. Bluestone

Received: 15 June 2012 / Accepted: 7 September 2012 / Published online: 21 October 2012

Abstract
Aims/hypothesis Type 1 diabetes results from a chronic autoimmune process continuing for years after presentation. We tested whether treatment with teplizumab (a Fc receptor non-binding anti-CD3 monoclonal antibody), after the new-onset period, affects the decline in C-peptide production in individuals with type 1 diabetes.
Methods In a randomised placebo-controlled trial we treated 58 participants with type 1 diabetes for 4–12 months with teplizumab or placebo at four academic centres in the USA. A central randomisation centre used computer generated tables to allocate treatments. Investigators, patients, and caregivers were blinded to group assignment. The primary outcome was a comparison of C-peptide responses to a mixed meal after 1 year. We explored modification of treatment effects in subgroups of patients.
Results Thirty-four and 29 subjects were randomized to the drug and placebo treated groups, respectively. Thirty-one and 27, respectively, were analysed. Although the primary outcome analysis showed a 21.7% higher C-peptide response in the teplizumab-treated group (0.45 vs 0.371; difference, 0.059 [95% CI 0.006, 0.115]nmol/l) (p=0.03), when corrected for baseline imbalances in HbA_{1c} levels, the C-peptide levels in the teplizumab-treated group were 17.7% higher (0.44 vs 0.378; difference, 0.049 [95% CI 0, 0.108]nmol/l, p=0.09). A greater proportion of placebo-treated participants lost detectable C-peptide responses at 12 months (p=0.03). The teplizumab group required less exogenous insulin (p<0.001) but treatment differences in HbA_{1c} levels were not observed. Teplizumab was well tolerated. A subgroup analysis showed that treatment benefits were larger in younger individuals and those with HbA_{1c} <6.5% at

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2753-4) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

K. C. Herold (✉) · F. Waldron-Lynch · L. Devine
Departments of Immunobiology and Internal Medicine,
Yale University,
300 George St, 353E,
New Haven, CT 06511, USA
e-mail: Kevan.herold@yale.edu

S. E. Gitelman · S. M. Rosenthal · S. Adi · J. A. Bluestone
Departments of Pediatrics and Internal Medicine,
University of California at San Francisco,
San Francisco, CA, USA

S. M. Willi · M. Y. Jalaludin
Department of Pediatrics, Children's Hospital of Philadelphia,
Philadelphia, PA, USA

P. A. Gottlieb · A. W. Michels
Department of Internal Medicine,
University of Colorado Anschutz Medical Campus,
Aurora, CO, USA

J. Sherr
Department of Pediatrics, Yale University,
New Haven, CT, USA

J. Dziura
Department of Emergency Medicine, Yale University,
New Haven, CT, USA

ARTICLE

Genetic Risk Factors for Type 2 Diabetes: A *Trans*-Regulatory Genetic Architecture?

Steven C. Elbein,[1,7,8] Eric R. Gamazon,[2,7] Swapan K. Das,[1] Neda Rasouli,[3,4] Philip A. Kern,[5,6] and Nancy J. Cox[2,*]

To date, 68 loci have been associated with type 2 diabetes (T2D) or glucose homeostasis traits. We report here the results of experiments aimed at functionally characterizing the SNPs replicated for T2D and glucose traits. We sought to determine whether these loci were associated with transcript levels in adipose, muscle, liver, lymphocytes, and pancreatic β-cells. We found an excess of *trans*, rather than *cis*, associations among these SNPs in comparison to what was expected in adipose and muscle. Among transcripts differentially expressed (FDR < 0.05) between muscle or adipose cells of insulin-sensitive individuals and those of insulin-resistant individuals (matched on BMI), *trans*-regulated transcripts, in contrast to the *cis*-regulated ones, were enriched. The paucity of *cis* associations with transcripts was confirmed in a study of liver transcriptome and was further supported by an analysis of the most detailed transcriptome map of pancreatic β-cells. Relative to location- and allele-frequency-matched random SNPs, both the 68 loci and top T2D-associated SNPs from two large-scale genome-wide studies were enriched for *trans* eQTLs in adipose and muscle but not in lymphocytes. Our study suggests that T2D SNPs have broad-reaching and tissue-specific effects that often extend beyond local transcripts and raises the question of whether patterns of *cis* or *trans* transcript regulation are a key feature of the architecture of complex traits.

Introduction

To date, at least 68 regions have shown replicated association with type 2 diabetes (T2D; MIM 125853), fasting or post-challenge glucose levels, or insulin sensitivity.[1–3] These variants are all common SNPs; with just a few exceptions, the disease-associated SNPs are themselves noncoding—they reside in either intronic or intergenic regions—and are not in linkage disequilibrium with known nonsynonymous SNPs. Hence, few of the T2D- and glucose-homeostasis-associated SNPs are likely to alter protein function; rather, they are expected to alter gene regulation and/or splicing.[4,5] Because many of the T2D- and glucose-homeostasis-associated SNPs are also associated with measures of insulin secretion (such measures are often surrogate, as in HOMA-B or insulin response to oral glucose),[6] most have been thought to act primarily to alter pancreatic β-cell function.[7] Nonetheless, the specific transcripts reported to be implicated by GWAS findings are often at a considerable distance from the disease-associated SNP. In rare cases do functional data implicate any particular gene or transcript in the disease association. Furthermore, many of the transcripts near T2D-associated SNPs are widely expressed, including in the diabetes-relevant tissues liver, adipose, and muscle. Thus, a much broader impact on T2D susceptibility is plausible, and the actual molecular and physiological explanations for these well-replicated associations remain uncertain.

The use of expression quantitative trait (eQTL) mapping has recently elucidated and expanded on GWAS findings.[8,9] For example, a recent eQTL study in liver showed that some implicated transcripts for type 1 diabetes (MIM 222100) were probably incorrect.[8] A recent study of celiac disease (MIM 212750)[10] reported that 52% of disease-associated SNPs were also associated with *cis*-regulated eQTLs in blood cells. Nicolae et al.[11] reported that SNPs associated with traits from the Wellcome Trust Case Control Consortium were more likely to be eQTLs (e.g., particularly *cis* eQTLs in Crohn disease [MIM 266600]) in transformed lymphocytes, supporting the use of surrogate tissues to identify functional transcripts. Nica et al.[12] reported similar findings. These studies did not specifically address the question of whether the SNPs reproducibly associated with T2D and related traits are more likely to be eQTLs than randomly selected SNPs of similar frequency.[11] Hence, functional data explaining the association of SNPs with T2D and glucose homeostasis are lacking. Furthermore, the tacit assumption in suggesting that these disease-associated SNPs act through specific transcripts near T2D- and glucose-homeostasis[2,3,13]-associated SNPs is without supportive data.

Although our results provide some support for the regulation of transcripts near GWAS SNPs by *cis*-acting eQTLs and more generally support a role of these SNPs in tissues other than pancreatic β-cells, our analyses suggest a much broader role for GWAS SNPs in controlling distal

[1]Section on Endocrinology and Metabolism, Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157; [2]Section of Genetic Medicine, Department of Medicine, University of Chicago, Chicago, IL; [3]Division of Endocrinology, Metabolism, and Diabetes, University of Colorado, Denver, Aurora, CO 80045; [4]Eastern Colorado Veterans Health Care System, Denver, CO 80220; [5]Division of Endocrinology, Department of Internal Medicine, University of Kentucky School of Medicine, Lexington, KY 40536; [6]The Barnstable Brown Diabetes and Obesity Center, University of Kentucky School of Medicine, Lexington, KY 40536
[7]These authors contributed equally to this work.
[8]Our colleague and friend Steven C. Elbein passed away unexpectedly during the preparation of this manuscript.
*Correspondence: ncox@bsd.uchicago.edu
http://dx.doi.org/10.1016/j.ajhg.2012.08.002.

OPEN ACCESS Freely available online

PLoS one

Altered Islet Composition and Disproportionate Loss of Large Islets in Patients with Type 2 Diabetes

German Kilimnik[1], Billy Zhao[1], Junghyo Jo[2], Vipul Periwal[2], Piotr Witkowski[3], Ryosuke Misawa[3], Manami Hara[1]*

1 Department of Medicine, The University of Chicago, Chicago, Illinois, United States of America, **2** Laboratory of Biological Modeling, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, United States of America, **3** Department of Surgery, The University of Chicago, Chicago, Illinois, United States of America

Abstract

Human islets exhibit distinct islet architecture with intermingled alpha- and beta-cells particularly in large islets. In this study, we quantitatively examined pathological changes of the pancreas in patients with type 2 diabetes (T2D). Specifically, we tested a hypothesis that changes in endocrine cell mass and composition are islet-size dependent. A large-scale analysis of cadaveric pancreatic sections from T2D patients ($n = 12$) and non-diabetic subjects ($n = 14$) was carried out combined with semi-automated analysis to quantify changes in islet architecture. The method provided the representative islet distribution in the whole pancreas section that allowed us to examine details of endocrine cell composition in individual islets. We observed a preferential loss of large islets (>60 μm in diameter) in T2D patients compared to non-diabetic subjects. Analysis of islet cell composition revealed that the beta-cell fraction in large islets was decreased in T2D patients. This change was accompanied by a reciprocal increase in alpha-cell fraction, however total alpha-cell area was decreased along with beta-cells in T2D. Delta-cell fraction and area remained unchanged. The computer-assisted quantification of morphological changes in islet structure minimizes sampling bias. Significant beta-cell loss was observed in large islets in T2D, in which alpha-cell ratio reciprocally increased. However, there was no alpha-cell expansion and the total alpha-cell area was also decreased. Changes in islet architecture were marked in large islets. Our method is widely applicable to various specimens using standard immunohistochemical analysis that may be particularly useful to study large animals including humans where large organ size precludes manual quantitation of organ morphology.

Citation: Kilimnik G, Zhao B, Jo J, Periwal V, Witkowski P, et al. (2011) Altered Islet Composition and Disproportionate Loss of Large Islets in Patients with Type 2 Diabetes. PLoS ONE 6(11): e27445. doi:10.1371/journal.pone.0027445

Editor: Sookja Chung, The University of Hong Kong, Hong Kong

Received August 1, 2011; **Accepted** October 17, 2011; **Published** November 15, 2011

Funding: The study is supported by United States of America Public Health Service Grant DK-081527, DK-072473, DK-042086 and DK-20595 to the University of Chicago Diabetes Research and Training Center (Animal Models Core), a gift from the Kovler Family Foundation (to MH); and the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (to JJ and VP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mhara@midway.uchicago.edu

Introduction

Type 2 diabetes (T2D) is a metabolic disease caused by a relative lack of insulin- mediated control of glucose homeostasis. The gradual progression of T2D hampers determination of the precise onset of the disease. The diagnosis is currently only confirmed when a patient develops chronic hyperglycemia, which is recognized by the patient with various symptoms such as excessive thirst, frequent urination, fatigue, blurred vision, and weight loss. As the onset of T2D is poorly defined, this silent aspect of disease progression has been noted as one of the major obstacles for the treatment of T2D. Levetan et al. have reported that 40% of hospitalized patients with T2D were undiagnosed [1]. It has been estimated that most patients with T2D are not diagnosed until ~10 years after the disease onset [2].

The unclear pathogenesis leads to incomplete understanding of the disease. While it is well-known that type 1 diabetes results from selective autoimmune destruction of pancreatic beta-cells, there is still considerable debate over the degree of beta-cell loss in T2D in relation to beta-cell dysfunction [3–5], which has important clinical implications regarding the treatment of the disease including possible regenerative therapies. The challenge of developing *in vivo* imaging modalities of beta-cell mass in humans is well recognized, and pancreas biopsy is not an option. Therefore, we reasoned that it is critical to obtain maximal information from available autopsy specimens. In fact, it is only recently that cadaveric pancreas specimens have been made more accessible to the scientific community, owing in part to the clinical success of islet transplantation, in which the assessment of the donor pancreas and islet quality is an important issue to improve the clinical outcomes.

Recent immunohistochemistry studies of the human pancreas have demonstrated the distinct islet architecture with more alpha-cells intermingled with beta-cells in humans compared to rodents with the central core of beta-cells with less alpha-cells residing in the periphery [6–8]. In interspecies comparative studies, we have shown that such drastic morphological changes occur selectively in large islets (>50–100 μm in diameter) in humans, and similar changes are also observed in mice under conditions of an increased demand for insulin such as pregnancy, obesity and diabetes [9,10]. In large animals, including humans, a proportionate increase in the pancreas size, islet number and total islet mass compensates for

Sleep Disturbances and Their Relationship to Glucose Tolerance in Pregnancy

SIRIMON REUTRAKUL, MD, CDE[1,2]
NAUSHEEN ZAIDI, MD[3]
KRISTEN WROBLEWSKI, MS[4]
HELEN H. KAY, MD[3]
MAHMOUD ISMAIL, MD[3]
DAVID A. EHRMANN, MD[1,2]
EVE VAN CAUTER, PHD[1,2]

OBJECTIVE—To explore relationships among sleep disturbances, glucose tolerance, and pregnancy outcomes.

RESEARCH DESIGN AND METHODS—Four validated sleep questionnaires were administered to 169 pregnant women at the time of 50-g oral glucose tolerance testing (OGTT) during the second trimester. Pregnancy outcomes were analyzed in 108 women with normal glucose tolerance (NGT).

RESULTS—Of the participants, 41% had excessive daytime sleepiness (Epworth Sleepiness Scale [ESS] >8); 64% had poor sleep quality; 25% snored frequently; 29% had increased risk of sleep-disordered breathing (SDB); 52% experienced short sleep (SS); 19% had both increased SDB risk and SS (SDB/SS); and 14% had daytime dysfunction. Reported sleep duration inversely correlated with glucose values from 50-g OGTT ($r = -0.21$, $P < 0.01$). Each hour of reduced sleep time was associated with a 4% increase in glucose levels. Increased likelihood of gestational diabetes mellitus (GDM) was found in subjects with increased SDB risk (odds ratio 3.0 [95% CI 1.2–7.4]), SS (2.4 [1.0–5.9]), SDB/SS (3.4 [1.3–8.7]), and frequent snoring (3.4 [1.3–8.8], after adjustment for BMI). Among NGT subjects, preterm delivery was more frequent in those with increased ESS ($P = 0.02$), poor sleep quality ($P = 0.02$), and SS ($P = 0.03$). Neonatal intensive care unit admissions were associated with increased ESS ($P = 0.03$), SDB/SS ($P = 0.03$), and daytime dysfunction ($P < 0.01$) in mothers.

CONCLUSIONS—Pregnant women experience significant sleep disturbances that are associated with increased risk of GDM and unfavorable pregnancy outcomes. Pregnant women with increased SDB risk, frequent snoring, and sleep duration of <7 h/night have increased risk of developing GDM.

Diabetes Care 34:2454–2457, 2011

Sleep-disordered breathing (SDB) is present in 24% of men and 9% of women in the U.S. population (1) and has been linked to insulin resistance and type 2 diabetes (2–5). Recent studies reveal that SDB is present in up to 86% of patients with type 2 diabetes (6,7). SDB severity has been associated with poorer glucose control (6).

Decreases in both duration and quality of sleep are common in pregnant women as a result of hormonal and physical factors (8,9). Collectively, these disorders have been termed pregnancy-associated sleep disorders by the International Classification of Sleep Disorders (10).

Prospective studies show that SDB symptoms increase during pregnancy (11). SDB in pregnancy has been associated with preeclampsia, intrauterine growth retardation, and preterm delivery (12,13). A few recent studies using questionnaires that variably assess snoring, SDB symptoms, and/or sleep duration report an association between short sleep (SS) and/or frequent snoring and glucose intolerance and gestational diabetes mellitus (GDM) (14–16).

We used four validated sleep questionnaires to obtain a comprehensive evaluation of sleep duration and quality and assess associations with glucose tolerance and pregnancy outcomes.

RESEARCH DESIGN AND METHODS

—Pregnant adult women scheduled to undergo a 50-g oral glucose tolerance test (OGTT) during the second trimester of gestation were invited to participate. Exclusion criteria were history of pre-GDM; sleep disorders; severe pulmonary, cardiac, or renal diseases; steroid use; substance abuse; current neurologic or psychiatric disorders; use of prescription or over-the-counter medications known to affect sleep or glucose metabolism; cigarette smoking; significant alcohol or caffeine consumption; recent travel across time zones; and shift work. Written informed consent was obtained. The study was approved by the institutional review board of the University of Chicago.

Age, ethnicity, prepregnancy BMI, current weight, height, and medical and family history were recorded. Subjects completed four standardized questionnaires: the Epworth Sleepiness Scale (ESS), which assesses daytime somnolence (normal score ≤8) (17); the Berlin Sleep Questionnaire, which assesses SDB risk (18); the Pittsburgh Sleep Quality Index (PSQI) to assess sleep during the past month (normal score ≤5) (19); and the Nocturia, Nocturnal Enuresis, and Sleep-Interruption Questionnaire (20).

Subjects with a 1-h glucose value <140 mg/dL post 50-g glucose were considered to have normal glucose tolerance (NGT). If the value was ≥140 mg/dL, they underwent a 100-g OGTT to formally confirm or exclude GDM (21). Subjects whose 1-h glucose value was ≥200 mg/dL post 50-g glucose challenge were diagnosed as having GDM without further testing.

From the [1]Section of Adult and Pediatric Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Chicago, Chicago, Illinois; the [2]Sleep, Metabolism, and Health Center, Department of Medicine, University of Chicago, Chicago, Illinois; the [3]Section of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, University of Chicago, Chicago, Illinois; and the [4]Department of Health Studies, University of Chicago, Chicago, Illinois.
Corresponding author: Sirimon Reutrakul, sirimon_reutrakul@rush.edu.
Received 25 April 2011 and accepted 11 August 2011.
DOI: 10.2337/dc11-0780

nature medicine

Leptin action through hypothalamic nitric oxide synthase-1–expressing neurons controls energy balance

Rebecca L Leshan[1,2,4,5], Megan Greenwald-Yarnell[1,3,5], Christa M Patterson[1], Ian E Gonzalez[1] & Martin G Myers Jr[1–3]

Few effective measures exist to combat the worldwide obesity epidemic[1], and the identification of potential therapeutic targets requires a deeper understanding of the mechanisms that control energy balance. Leptin, an adipocyte-derived hormone that signals the long-term status of bodily energy stores, acts through multiple types of leptin receptor long isoform (LepRb)-expressing neurons (called here LepRb neurons) in the brain to control feeding, energy expenditure and endocrine function[2–4]. The modest contributions to energy balance that are attributable to leptin action in many LepRb populations[5–9] suggest that other previously unidentified hypothalamic LepRb neurons have key roles in energy balance. Here we examine the role of LepRb in neuronal nitric oxide synthase (NOS1)-expressing LebRb (LepRb^{NOS1}) neurons that comprise approximately 20% of the total hypothalamic LepRb neurons. *Nos1^{cre}*-mediated genetic ablation of LepRb (*Lepr^{Nos1KO}*) in mice produces hyperphagic obesity, decreased energy expenditure and hyperglycemia approaching that seen in whole-body LepRb-null mice. In contrast, the endocrine functions in *Lepr^{Nos1KO}* mice are only modestly affected by the genetic ablation of LepRb in these neurons. Thus, hypothalamic LepRb^{NOS1} neurons are a key site of action of the leptin-mediated control of systemic energy balance.

Commensurate with the diverse processes controlled by leptin, specialized types of LepRb neurons are located in multiple brain regions that are involved in the regulation of systemic energy balance, including the brainstem, midbrain and hypothalamus[10–13]. Knockdown or deletion of LepRb in the hindbrain interferes with satiation, although these alterations have only a slight affect on body adiposity[8,9]. Within the midbrain ventral tegmental area and substantia nigra, a subset of dopamine neurons contain LepRb; leptin action through these neurons contributes minimally to body weight control but does have a role in dopamine-mediated behaviors, including those that are linked to anxiety[14–17]. Midbrain serotonin neurons, although initially reported to have a key role in leptin action, neither express LepRb nor contribute to leptin action[18,19]. In contrast, genetic ablation of hypothalamic LepRb produces a profound metabolic phenotype, showing the key role of hypothalamic LepRb signaling in leptin action[20].

Within the hypothalamus, the specific set(s) of LepRb neurons that are responsible for the control of energy balance by leptin are not completely defined. Direct leptin action through the proopiomelanocortin (Pomc)-expressing neurons (Pomc neurons) of the hypothalamic arcuate nucleus (ARC) and through the ARC agouti-related peptide (Agrp)-expressing neurons (Agrp neurons) and steroidogenic factor-1 (Sf-1)-expressing neurons of the ventromedial hypothalamic nucleus contributes only modestly to the overall energy balance[5–7]. LepRb neurons in the lateral hypothalamic area, including those that contain neurotensin, mediate the action of leptin on orexin neurons and the mesolimbic dopamine system, but genetic deletion of LepRb from these neurons only modestly increases adiposity[21–23]. Thus, the identity of the hypothalamic LepRb neurons that are responsible for the majority of leptin-mediated regulation of energy balance is unclear.

LepRb^{NOS1} neurons comprise a relatively small population of LepRb neurons and are primarily restricted to the hypothalamus, where they are distributed in areas in which they are poised to affect output from the paraventricular hypothalamic nucleus (signaling by this nucleus mediates much of the hypothalamic control of energy balance)[24–26]. To study LepRb^{NOS1} neurons, we inserted an internal ribosome entry site (IRES) plus the coding sequences for Cre recombinase into the 3′ untranslated region of *Nos1* in mice to promote Nos1-restricted Cre expression (resulting in *Nos1^{cre}* mice) (**Fig. 1a**). We bred *Nos1^{cre}* mice to mice from the Cre-dependent ROSA26–enhanced GFP (eGFP) reporter line, generating *Nos1^{eGFP}* mice. The immunoreactivity of eGFP overlapped with the immunoreactivity of Nos1 in the soma of the *Nos1^{eGFP}* mice in all regions examined (data not shown).

We next examined the leptin-stimulated induction of phosphorylated signal transducer and activator-3 (pStat3), which reveals neurons containing functional LepRb (ref. 27), and its colocalization with eGFP immunoreactivity in *Nos1^{eGFP}* mice. We also evaluated the colocalization of Nos1 immunoreactivity with eGFP immunoreactivity in *Lepr^{eGFP}* mice[12] that express eGFP in their LepRb neurons (**Supplementary Figs. 1** and **2**). As has been previously suggested by the colocalization of NADPH diaphorase with leptin-stimulated

[1]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA. [2]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, USA. [3]Department of Neuroscience Graduate Program, University of Michigan, Ann Arbor, Michigan, USA. [4]Present address: Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, New York, USA. [5]These authors contributed equally to this work. Correspondence should be addressed to M.G.M. (mgmyers@umich.edu).

Received 8 February; accepted 9 March; published online 22 April 2012; doi:10.1038/nm.2724

The 10-Year Cost-Effectiveness of Lifestyle Intervention or Metformin for Diabetes Prevention

An intent-to-treat analysis of the DPP/DPPOS

THE DIABETES PREVENTION PROGRAM RESEARCH GROUP*

OBJECTIVE—The Diabetes Prevention Program (DPP) and its Outcomes Study (DPPOS) demonstrated that either intensive lifestyle intervention or metformin could prevent type 2 diabetes in high-risk adults for at least 10 years after randomization. We report the 10-year within-trial cost-effectiveness of the interventions.

RESEARCH DESIGN AND METHODS—Data on resource utilization, cost, and quality of life were collected prospectively. Economic analyses were performed from health system and societal perspectives.

RESULTS—Over 10 years, the cumulative, undiscounted per capita direct medical costs of the interventions, as implemented during the DPP, were greater for lifestyle ($4,601) than metformin ($2,300) or placebo ($769). The cumulative direct medical costs of care outside the DPP/DPPOS were least for lifestyle ($24,563 lifestyle vs. $25,616 metformin vs. $27,468 placebo). The cumulative, combined total direct medical costs were greatest for lifestyle and least for metformin ($29,164 lifestyle vs. $27,915 metformin vs. $28,236 placebo). The cumulative quality-adjusted life-years (QALYs) accrued over 10 years were greater for lifestyle (6.81) than metformin (6.69) or placebo (6.67). When costs and outcomes were discounted at 3%, lifestyle cost $10,037 per QALY, and metformin had slightly lower costs and nearly the same QALYs as placebo.

CONCLUSIONS—Over 10 years, from a payer perspective, lifestyle was cost-effective and metformin was marginally cost-saving compared with placebo. Investment in lifestyle and metformin interventions for diabetes prevention in high-risk adults provides good value for the money spent.

Diabetes Care **35:723–730, 2012**

Intensive lifestyle and metformin interventions can delay or prevent progression from impaired glucose tolerance (IGT) to type 2 diabetes (1–3). The Diabetes Prevention Program (DPP), a randomized controlled clinical trial, demonstrated that compared with the placebo intervention (placebo), the intensive lifestyle intervention (lifestyle) reduced the incidence of type 2 diabetes by 58%, and the metformin intervention (metformin) reduced the incidence of type 2 diabetes by 31% over 2.8 years (3). The Diabetes Prevention Program Outcomes Study (DPPOS) is a long-term follow-up of the DPP participants to investigate whether the delay in the development of diabetes observed during the DPP is sustained and to assess the long-term effects of the interventions on health.

The DPPOS has followed participants for an additional 7 years during which time participants in lifestyle and metformin were encouraged to continue those interventions, and all participants were offered a group lifestyle intervention (4). The incidence of diabetes during the 10-year average follow-up after randomization was reduced by 34% in those initially randomized to lifestyle and 18% in those initially randomized to metformin compared with placebo (4). Previously, we reported the resource utilization and costs of care in the DPP (5), and the cost per quality-adjusted life-year (QALY) gained over the 3-year timeframe of the randomized controlled clinical trial (6). We also used 3-year DPP data and a computer model to simulate the longer-term cost-effectiveness of the interventions. Although we (7) and others (8,9) suggested that lifestyle would be cost-effective or even cost-saving over the long term, one analysis suggested that it might be too expensive for health plans or a national program to implement (10). In this report, we present a within trial intent-to-treat analysis spanning the combined 10-year DPP/DPPOS timeframe to assess the longer-term cost-effectiveness of lifestyle and metformin for diabetes prevention. All of the DPPOS clinical centers as well as the DPP Coordinating Center had institutional review board approvals. All participants gave written informed consent.

RESEARCH DESIGN AND METHODS

Interventions

DPP. The DPP enrolled 3,234 participants with IGT and fasting hyperglycemia who were at least 25 years of age and had BMI of 24 kg/m^2 or higher (22 kg/m^2 in Asian Americans) (3). Enrollment began in July 1996 and ended in May 1999. Mean age was 51 years of age and mean BMI was 34.0 kg/m^2 (3). Sixty-eight percent were women, and forty-five percent were members of minority groups (3).

Corresponding author: Diabetes Prevention Program Coordinating Center, dppmail@biostat.bsc.gwu.edu.
Received 3 August 2011 and accepted 15 December 2011.
DOI: 10.2337/dc11-1468. Clinical trial reg. nos. NCT00038727 (DPPOS) and NCT00004992 (DPP), clinicaltrials.gov.
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc11-1468/-/DC1.
A slide set summarizing this article is available online.
*A complete list of the members of the Diabetes Prevention Program Research Group, centers, and staff can be found in the Supplementary Data online, and members of the writing group are listed in the APPENDIX.
The opinions expressed in this article are those of the investigators and do not necessarily reflect the views of the funding agencies.

See accompanying articles, pp. 663, 717, and 731.

nature medicine

NF-κB–inducing kinase (NIK) promotes hyperglycemia and glucose intolerance in obesity by augmenting glucagon action

Liang Sheng[1], Yingjiang Zhou[1], Zheng Chen[1], Decheng Ren[1], Kae Won Cho[1], Lin Jiang[1], Hong Shen[1], Yoshiteru Sasaki[2] & Liangyou Rui[1]

The canonical inhibitor of nuclear factor κB kinase subunit β (IKK-β)–nuclear factor of κ light polypeptide gene enhancer in B cells 1 (NF-κB1) pathway has been well documented to promote insulin resistance; however, the noncanonical NF-κB–inducing kinase (NIK)–NF-κB2 pathway is not well understood in obesity. Additionally, the contribution of counter-regulatory hormones, particularly glucagon, to hyperglycemia in obesity is unclear. Here we show that NIK promotes glucagon responses in obesity. Hepatic NIK was abnormally activated in mice with dietary or genetic obesity. Systemic deletion of *Map3k14*, encoding NIK, resulted in reduced glucagon responses and hepatic glucose production (HGP). Obesity is associated with high glucagon responses, and liver-specific inhibition of NIK led to lower glucagon responses and HGP and protected against hyperglycemia and glucose intolerance in obese mice. Conversely, hepatocyte-specific overexpression of NIK resulted in higher glucagon responses and HGP. In isolated mouse livers and primary hepatocytes, NIK also promoted glucagon action and glucose production, at least in part by increasing cAMP response element-binding (CREB) stability. Therefore, overactivation of liver NIK in obesity promotes hyperglycemia and glucose intolerance by increasing the hyperglycemic response to glucagon and other factors that activate CREB.

Obesity is associated with chronic inflammation that is believed to contribute to the pathogenesis of type 2 diabetes[1,2]. Proinflammatory cytokines activate the canonical IKK-β–NF-κB1 and the Jun N-terminal kinase (JNK) pathways, which is believed to impair glucose metabolism by increasing insulin resistance[3–5]. Blood glucose concentrations are determined by a balance between insulin and counter-regulatory hormones (for example, glucagon, catecholamines, glucocorticoids or growth hormone)[6]. In the fasting state, glucagon is secreted by pancreatic α cells and increases HGP by stimulating hepatic glycogenolysis and gluconeogenesis[6]. Glucagon stimulates the phosphorylation and activation of CREB through the cAMP–protein kinase A pathway, and CREB in turn activates the transcription of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase)[7]. In rodents with type 1 diabetes, glucagon action is high and contributes to hyperglycemia and glucose intolerance[8–10]. Blood glucagon concentrations are elevated in rodents with insulin deficiency, and leptin treatments normalize hyperglucagonemia and hyperglycemia[8,9]. Deletion of glucagon receptors prevents streptozotocin (STZ)-induced hyperglycemia and glucose intolerance[10]. In a type 2 diabetes model, leptin treatments also attenuate hyperglucagonemia, leading to improvement in hyperglycemia and glucose intolerance[11]. Insulin suppresses glucagon secretion from α cells[12]; therefore, α cell insulin resistance may lead to hyperglucagonemia, which contributes to hyperglycemia in type 2 diabetes.

A subset of cytokines also stimulates the noncanonical NIK–NF-κB2 pathway; however, the metabolic function of this pathway has not been examined. It is also unclear whether inflammation alters glucagon responses. NIK, also called MAP3K14 (NM_003954), is an essential upstream serine/threonine kinase of the noncanonical NIK–NF-κB2 pathway[13]. NIK protein concentrations are extremely low in quiescent cells as a result of rapid degradation, and cytokines and oxidative stress increase NIK protein stability, leading to NIK activation[14]. NIK has been reported to regulate B and T cell development in mice[15,16]. In this study we show that liver NIK is aberrantly activated in obesity and increases HGP by promoting glucagon action in mice, thus contributing to hyperglycemia and glucose intolerance. NIK promotes glucagon action, at least in part, by increasing CREB stability.

RESULTS

NIK is abnormally activated in the livers of obese mice

We measured NIK activity in two commonly used mouse models of obesity: dietary obesity and genetic obesity with leptin deficiency (*ob/ob*). We immunopurified NIK protein from the livers of mice from these two models and subjected it to *in vitro* kinase assays using the glutathione-S transferase (GST)–IKK-α (amino acids 108–368) fusion protein as a substrate. GST–IKK-α contains an NIK phosphorylation site (IKK-α Ser176) and lacks catalytic activity[17]. NIK activity in the

[1]Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan, USA. [2]Laboratory for Stem Cell Biology, Riken Center for Developmental Biology, Kobe, Japan. Correspondence should be addressed to L.R. (ruily@umich.edu).

Received 31 January; accepted 29 March; published online 13 May 2012; doi:10.1038/nm.2756

nature medicine

Insulin regulates liver metabolism *in vivo* in the absence of hepatic Akt and Foxo1

Mingjian Lu[1], Min Wan[1], Karla F Leavens[1], Qingwei Chu[1], Bobby R Monks[1], Sully Fernandez[1], Rexford S Ahima[1], Kohjiro Ueki[2], C Ronald Kahn[3] & Morris J Birnbaum[1]

Considerable data support the idea that forkhead box O1 (Foxo1) drives the liver transcriptional program during fasting and is then inhibited by thymoma viral proto-oncogene 1 (Akt) after feeding. Here we show that mice with hepatic deletion of *Akt1* and *Akt2* were glucose intolerant, insulin resistant and defective in their transcriptional response to feeding in the liver. These defects were normalized with concomitant liver-specific deletion of *Foxo1*. Notably, in the absence of both Akt and Foxo1, mice adapted appropriately to both the fasted and fed state, and insulin suppressed hepatic glucose production normally. A gene expression analysis revealed that deletion of *Akt* in liver led to the constitutive activation of Foxo1-dependent gene expression, but again, concomitant ablation of *Foxo1* restored postprandial regulation, preventing the inhibition of the metabolic response to nutrient intake caused by deletion of *Akt*. These results are inconsistent with the canonical model of hepatic metabolism in which Akt is an obligate intermediate for proper insulin signaling. Rather, they show that a major role of hepatic Akt is to restrain the activity of Foxo1 and that in the absence of Foxo1, Akt is largely dispensable for insulin- and nutrient-mediated hepatic metabolic regulation *in vivo*.

Eukaryotic organisms have developed mechanisms of varying complexity to deal with periods of starvation and nutritional abundance. In mammals, the obligate requirement for at least some simple carbohydrate at all times is fulfilled during fasting by the liver, which initially breaks down glycogen before transitioning to gluconeogenesis as a means of releasing glucose into the circulation. Just as phylogenetically conserved adaptations to fasting are crucial for survival, a complex interplay of hormonal, neural and nutritional signals drive the coordinated response to feeding. Ultimately, the ability of an organism to survive dietary deprivation depends on the effectiveness of its nutrient storage during the postprandial state, as well as the mobilization of those nutrients during periods of energy deficits.

Almost since its discovery, researchers have recognized the importance of insulin to the metabolic transition that accompanies feeding[1]. In recent years, there has emerged a clear picture of the insulin signaling pathway, beginning with insulin's interaction with its receptor, which phosphorylates the scaffolding protein family insulin-receptor substrate (Irs). This initiates a linear signaling cascade that culminates in the phosphorylation of Akt protein kinases[1–10]. Once activated, Akt kinases use several distinct downstream pathways to modulate metabolism. One pathway branch involves phosphorylation and inactivation of the tuberous sclerosis 1 (Tsc1)-Tsc2 complex, leading to activation of the mammalian target of rapamycin complex 1 (mTorc1) and its immediate downstream target, p70 S6 kinase, which promotes protein translation and cell growth[11,12]. Activation of mTorc1 correlates with and is probably necessary for insulin-induced accumulation of the sterol regulatory element binding transcription factor 1 (Srebf1, also known as Srebp1c), which drives the lipogenic program[12–17]. On another pathway branch, Akt phosphorylates and inactivates glycogen synthase kinase 3 (Gsk3α and Gsk3β), resulting in glycogen synthase activation and glycogen accumulation, as well as reduced phosphorylation and degradation of Srebf1 (ref. 18). On the third pathway branch, Akt phosphorylates and inactivates the Foxo family of transcription factors, which is responsible for the decrease during insulin signaling in the transcription of genes encoding gluconeogenic enzymes[19–22].

In liver, Foxo1 collaborates with the co-activators peroxisome proliferator-activated receptor γ co-activator 1α (Ppargc1a) and Creb-regulated transcription co-activator 2 (Crtc2) to coordinately increase the expression of the genes *G6pc* (glucose-6-phosphatase, catalytic subunit) and *Pck1* (cytosolic phosphoenolpyruvate carboxykinase 1)[23–25]. It is generally accepted that Foxo1 is active during fasting and inactivated by Akt-mediated phosphorylation after feeding and that the antagonism of Foxo1 by Akt is the predominant mechanism by which insulin suppresses hepatic glucose output after a meal[1,25–28]. Data contributed by a number of laboratories in support of this model include the following observations: (i) insulin induces the phosphorylation and activation of Akt after feeding; (ii) activated Akt phosphorylates Foxo1 at Thr24, Ser253 and Ser316 of the mouse protein; (iii) phosphorylated Foxo1 translocates out of the nucleus; and (iv) transcription of Foxo1-dependent genes, such as *G6pc*, *Pck1*, *Igfbp1* (insulin-like growth factor binding protein 1) and *Irs2*, is reduced[19–21,24–26,29]. Nonetheless, there are several gaps and apparent contradictions in the current knowledge about Foxo1.

[1]The Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [2]Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan. [3]Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts, USA. Correspondence should be addressed to M.J.B. (birnbaum@mail.med.upenn.edu).

Received 21 October 2011; accepted 23 January 2012; published online 19 February 2012; doi:10.1038/nm.2686

link function (Proc Genmod, SAS 9.1, SAS Institute Incorporated, Cary, North Carolina). When the infection assays were run over two time blocks, the model also included a block effect and a time-by-block interaction.

The infection assays showed a significant evolutionary response of hosts to epidemics in six of seven lake populations. In three lakes (Island, Midland, and Scott Lakes), host populations became significantly more resistant during epidemics (Fig. 1). However, in three other populations (Canvasback, Downing, and Hale Lakes), hosts became significantly more susceptible to infection. The hosts in the seventh lake, Beaver Dam, did not show a significant change in susceptibility but trended toward increased resistance.

As anticipated by theory (SOM), these evolutionary trajectories correlated with ecologically driven variation in epidemic size. Among the six lake populations showing a significant evolutionary response, change in mean susceptibility correlated strongly with epidemic size (Pearson correlation: $r = 0.86$, $P = 0.030$, $n = 6$; Fig. 2A). Further, in those six lakes, epidemics were larger at lower predation intensity (larger size of hosts; Pearson correlation: $r = 0.86$, $P = 0.029$, $n = 6$; Fig. 2B) and where total nitrogen was higher (Pearson correlation: $r = 0.83$, $P = 0.040$, $n = 6$; Fig. 2C); the trend was similarly directed, but not significant, for total phosphorus (Pearson correlation: $r = 0.50$, $P = 0.3$, $n = 6$; Fig. 2D). Overall, hosts became more susceptible to the yeast in lower productivity lakes with higher vertebrate predation but evolved toward decreased susceptibility in more productive lakes with lower vertebrate predation (Fig. 2, E and F; t tests for differences between two groups; results for body size, $t_4 = 3.19$ and $P = 0.033$; nitrogen, $t_4 = 3.18$ and $P = 0.034$; phosphorus, $t_4 = 0.88$ and $P = 0.43$). Thus, ecological gradients, through their effects on epidemic size, influenced evolutionary outcomes of hosts during outbreaks of a virulent parasite. These qualitative predictions also arose from a general, trait-based epidemiological model built for similar epidemiology and parameterized for our particular system (SOM).

These results show that hosts can evolve enhanced susceptibility to their virulent parasites during epidemics [also see (*27*) for a similar but unreplicated occurrence]. A combination of observations, experiments, and modeling all suggest causation for this initially counterintuitive finding. When ecological factors promote large epidemics, hosts should evolve to become more resistant to infection. However, resistance-fecundity trade-offs can prompt host populations to evolve increased susceptibility when ecology constrains epidemic size. Overall, we demonstrated that ecological context influences epidemic size, which, in turn, determines evolutionary responses of hosts to epidemics. This suggests that alteration of predation pressure on hosts and productivity of ecosystems may influence the ecology and evolution of host-parasite interactions.

References and Notes

1. M. A. Duffy, S. E. Forde, *J. Anim. Ecol.* **78**, 1106 (2009).
2. R. M. Penczykowski, S. E. Forde, M. A. Duffy, *Freshw. Biol.* **56**, 689 (2011).
3. M. F. Dybdahl, C. M. Lively, *Evolution* **52**, 1057 (1998).
4. J. Jokela, M. F. Dybdahl, C. M. Lively, *Am. Nat.* **174** (suppl. 1), S43 (2009).
5. J. Antonovics, P. H. Thrall, *Proc. Biol. Sci.* **257**, 105 (1994).
6. M. Boots, A. Best, M. R. Miller, A. White, *Philos. Trans. R. Soc. London Ser. B* **364**, 27 (2009).
7. M. Boots, Y. Haraguchi, *Am. Nat.* **153**, 359 (1999).
8. R. G. Bowers, M. Boots, M. Begon, *Proc. Biol. Sci.* **257**, 247 (1994).
9. R. M. Anderson, R. M. May, *Infectious Diseases of Humans: Dynamics and Control* (Oxford Univ. Press, Oxford, 1991).
10. M. J. Keeling, P. Rohani, *Modeling Infectious Diseases in Humans and Animals* (Princeton Univ. Press, Princeton, NJ, 2008).
11. K. D. Lafferty, R. D. Holt, *Ecol. Lett.* **6**, 654 (2003).
12. C. Packer, R. D. Holt, P. J. Hudson, K. D. Lafferty, A. P. Dobson, *Ecol. Lett.* **6**, 797 (2003).
13. S. R. Hall, C. R. Becker, M. A. Duffy, C. E. Cáceres, *Am. Nat.* **176**, 557 (2010).
14. M. A. Duffy, L. Sivars-Becker, *Ecol. Lett.* **10**, 44 (2007).
15. M. A. Duffy, S. R. Hall, *Am. Nat.* **171**, 499 (2008).
16. S. R. Hall, C. R. Becker, M. A. Duffy, C. E. Cáceres, *Oecologia* **166**, 833 (2011).
17. E. P. Overholt *et al.*, *Ecol. Lett.* **15**, 47 (2012).
18. M. A. Duffy *et al.*, *BMC Evol. Biol.* **8**, 80 (2008).
19. M. A. Duffy, S. R. Hall, C. E. Cáceres, A. R. Ives, *Ecology* **90**, 1441 (2009).
20. S. R. Hall *et al.*, *Bioscience* **60**, 363 (2010).
21. M. A. Duffy, S. R. Hall, A. J. Tessier, M. Huebner, *Limnol. Oceanogr.* **50**, 412 (2005).
22. A. E. Greenberg *et al.*, Eds., *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, Washington, DC, ed. 19, 1995).
23. J. J. Elser *et al.*, *Ecol. Lett.* **10**, 1135 (2007).
24. S. J. Guildford, R. E. Hecky, *Limnol. Oceanogr.* **45**, 1213 (2000).
25. J. L. Brooks, S. I. Dodson, *Science* **150**, 28 (1965).
26. J. A. Kitchell, J. F. Kitchell, *Limnol. Oceanogr.* **25**, 389 (1980).
27. M. A. Parker, *Evolution* **45**, 1209 (1991).

Acknowledgments: We thank S. Hernandez, K. Jansen, K. Kenline, A. Reynolds, B. Sarrell, K. van Rensburg, and C. Washington for assistance in the lab; K. Boatman, Z. Brown, A. Bowling, C. White, and P. Forys for their help with the field survey; and S. Auld and two anonymous reviewers for comments on the manuscript. This work was supported by NSF (grants DEB-0841679 to M.A.D., DEB-0841817 to S.R.H., and DEB-0845825 and OCE-0928819 to C.A.K.) and by a grant from the James S. McDonnell Foundation (to C.A.K.). We appreciate cooperation from S. Siscoe at the Indiana Department of Natural Resources's Division of Forestry and R. Ronk at the Division of Fish and Wildlife for the field survey. This is KBS contribution no. 1607. Data are available in the SOM and from the lead author (M.A.D.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6076/1636/DC1
Materials and Methods
Fig. S1
Tables S1 and S2
References (*28*–*32*)

18 October 2011; accepted 28 February 2012
10.1126/science.1215429

Rapamycin-Induced Insulin Resistance Is Mediated by mTORC2 Loss and Uncoupled from Longevity

Dudley W. Lamming,[1,2,3,4,5]† Lan Ye,[6]† Pekka Katajisto,[1,2,3,4,5] Marcus D. Goncalves,[7] Maki Saitoh,[1,2,3,4,5] Deanna M. Stevens,[1,2,3,4,5] James G. Davis,[6] Adam B. Salmon,[8] Arlan Richardson,[8] Rexford S. Ahima,[7] David A. Guertin,[1,2,3,4,5]* David M. Sabatini,[1,2,3,4,5]‡ Joseph A. Baur[6]‡

Rapamycin, an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1), extends the life spans of yeast, flies, and mice. Calorie restriction, which increases life span and insulin sensitivity, is proposed to function by inhibition of mTORC1, yet paradoxically, chronic administration of rapamycin substantially impairs glucose tolerance and insulin action. We demonstrate that rapamycin disrupted a second mTOR complex, mTORC2, in vivo and that mTORC2 was required for the insulin-mediated suppression of hepatic gluconeogenesis. Further, decreased mTORC1 signaling was sufficient to extend life span independently from changes in glucose homeostasis, as female mice heterozygous for both mTOR and mLST8 exhibited decreased mTORC1 activity and extended life span but had normal glucose tolerance and insulin sensitivity. Thus, mTORC2 disruption is an important mediator of the effects of rapamycin in vivo.

Age-related diseases—including cancer, neurodegenerative disorders, cardiovascular disease, type II diabetes, and many others—are the major contributors to morbidity and mortality in Western society. The high frequency of these diseases in the elderly limits the benefit that can be obtained by targeting them individually (*1*). However, targeting the aging process directly may offer a way to delay the incidence of many age-related diseases simultaneously. To date, the only molecule that appears to influence the intrinsic rate of aging in mammals, as evidenced by a robust extension of maximum life span, is rapamycin, an inhibitor of mechanistic (previously referred to as mammalian) target of rapamycin complex 1 (mTORC1) (*2*, *3*).

mTOR is a kinase that integrates inputs from many nutrients and growth factors. mTOR is found in two distinct protein complexes: mTORC1, which regulates numerous cellular processes related to

Cell

Foxa1 and Foxa2 Are Essential for Sexual Dimorphism in Liver Cancer

Zhaoyu Li,[1] Geetu Tuteja,[1] Jonathan Schug,[1] and Klaus H. Kaestner[1,*]
[1]Department of Genetics and Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: kaestner@mail.med.upenn.edu
DOI 10.1016/j.cell.2011.11.026

SUMMARY

Hepatocellular carcinoma (HCC) is sexually dimorphic in both rodents and humans, with significantly higher incidence in males, an effect that is dependent on sex hormones. The molecular mechanisms by which estrogens prevent and androgens promote liver cancer remain unclear. Here, we discover that sexually dimorphic HCC is completely reversed in Foxa1- and Foxa2-deficient mice after diethylnitrosamine-induced hepatocarcinogenesis. Coregulation of target genes by Foxa1/a2 and either the estrogen receptor (ERα) or the androgen receptor (AR) was increased during hepatocarcinogenesis in normal female or male mice, respectively, but was lost in Foxa1/2-deficient mice. Thus, both estrogen-dependent resistance to and androgen-mediated facilitation of HCC depend on Foxa1/2. Strikingly, single nucleotide polymorphisms at FOXA2 binding sites reduce binding of both FOXA2 and ERα to their targets in human liver and correlate with HCC development in women. Thus, Foxa factors and their targets are central for the sexual dimorphism of HCC.

INTRODUCTION

Sexual dimorphism is the biological inequality between females and males, favoring females in a variety of conditions including resistance to nutrient deprivation, prevention of premature aging, and resistance to diseases such as vascular and heart disease, brain disorders, and hepatocellular carcinoma (HCC) (Genazzani et al., 2007; Kalra et al., 2008; Stice et al., 2009). Sex hormones, i.e., estrogens in females and androgens in males, are the drivers of sexual dimorphism.

HCC is the fifth most common cancer and ranks third in annual mortality worldwide (Parkin et al., 2005). Women show significantly lower incidence of HCC than men (Parkin et al., 2005). Female rodents are also resistant to HCC compared to males during chemically induced carcinogenesis (Kalra et al., 2008). Male mice treated with estrogen develop fewer liver tumors than control males, and ovariectomized females develop more liver tumors than normal females during chemically induced carcinogenesis (Naugler et al., 2007; Shimizu et al., 1998; Tsutsui et al., 1992; Yamamoto et al., 1991). In addition, female mice deficient for the estrogen receptor alpha (ERα) lose their resistance to HCC (Naugler et al., 2007), while reduced incidence of HCC was observed in male mice lacking the androgen receptor (AR) (Ma et al., 2008; Wu et al., 2010). These data demonstrate that both protective effects of estrogens and deleterious effects of androgens contribute to the sexual dimorphism in HCC incidence. However, the molecular mechanisms of how this is achieved remain to be determined.

A recent report suggested that estrogens prevent HCC through inhibition of IL-6 expression in Kupffer cells, the resident macrophages in the liver, and that this in turn affects hepatocyte proliferation (Naugler et al., 2007). However, follow-up studies employing IL-6 antagonists or estrogen and its analogs to prevent HCC indicate that other mechanisms must also be involved in estrogen-mediated protection from liver cancer (Di Maio et al., 2006; Kalra et al., 2008; Lawrence et al., 2007). Comparatively little is known about the mechanism by which androgen signaling promotes HCC in males, although a recent study suggests that androgens enhance DNA damage and oxidative stress during hepatocarcinogenesis (Ma et al., 2008).

The vertebrate forkhead box A (*Foxa*) gene family of transcription factors consists of three members, Foxa1, Foxa2, and Foxa3, which are encoded by individual genes in mammals (Kaestner, 2010). Previous gene ablation studies of Foxa factors in mice have shown that Foxa1 and Foxa2 redundantly regulate liver development and metabolism, whereas the role of Foxa3 in the liver is limited (Bochkis et al., 2008; Friedman and Kaestner, 2006; Kaestner, 2005; Kaestner et al., 1998, 1999; Lee et al., 2005; Li et al., 2009; Shen et al., 2001; Sund et al., 2000). Though no liver forms in mice when both Foxa1 and Foxa2 are ablated in foregut endoderm following gastrulation (Lee et al., 2005), ablation of both Foxa1 and Foxa2 after liver specification does not affect hepatocyte development or proliferation (Li et al., 2009).

Genome-wide location analyses have revealed that FOXA1 and ERα or AR frequently bind to adjacent *cis*-regulatory elements in their target genes in human breast or prostate cancer cell lines, respectively, and that the recruitment of ERα or AR to their targets depends on FOXA1 (Carroll et al., 2005; Gao et al., 2003; Lupien et al., 2008; Yu et al., 2005). Hence, FOXA1 plays an essential role in estrogen and androgen signaling in breast and prostate epithelia.

We hypothesized that the effects of estrogens and androgens on HCC development are dependent upon Foxa factors. Using liver-specific gene ablation, we demonstrate that sexually

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 44, pp. 37154–37164, October 26, 2012
Published in the U.S.A.

Overall Sulfation of Heparan Sulfate from Pancreatic Islet β-TC3 Cells Increases Maximal Fibril Formation but Does Not Determine Binding to the Amyloidogenic Peptide Islet Amyloid Polypeptide*

Received for publication, August 10, 2012 Published, JBC Papers in Press, August 30, 2012, DOI 10.1074/jbc.M112.409847

Rebecca L. Hull[‡§1], Michael J. Peters[‡], Susan Potter Perigo[¶], Christina K. Chan[¶], Thomas N. Wight[¶‖], and Michael G. Kinsella[¶]

From the [‡]Veterans Affairs Puget Sound Health Care System, Seattle, Washington 98108, the Departments of [§]Medicine and [‖]Pathology, University of Washington, Seattle, Washington 98195, and the [¶]Hope Heart Matrix Biology Program, Benaroya Research Institute at Virginia Mason, Seattle, Washington 98101

Background: Stimulation of amyloid fibril formation by heparan sulfate is likely mediated by the extent of sulfation.
Results: Islet β-cell heparan sulfate is poorly sulfated but still binds islet amyloid polypeptide (IAPP) and accelerates fibril formation.
Conclusion: The degree of sulfation does not determine all aspects of heparan sulfate-mediated amyloid fibril formation.
Significance: This information will be important for designing amyloid inhibitors targeting peptide-heparan sulfate interactions.

Islet amyloid, a pathologic feature of type 2 diabetes, contains the islet β-cell peptide islet amyloid polypeptide (IAPP) as its unique amyloidogenic component. Islet amyloid also contains heparan sulfate proteoglycans (HSPGs) that may contribute to amyloid formation by binding IAPP via their heparan sulfate (HS) chains. We hypothesized that β-cells produce HS that bind IAPP via regions of highly sulfated disaccharides. Unexpectedly, HS from the β-cell line β-TC3 contained fewer regions of highly sulfated disaccharides compared with control normal murine mammary gland (NMuMG) cells. The proportion of HS that bound IAPP was similar in both cell lines (~65%). The sulfation pattern of IAPP-bound *versus* non-bound HS from β-TC3 cells was similar. In contrast, IAPP-bound HS from NMuMG cells contained frequent highly sulfated regions, whereas the non-bound material demonstrated fewer sulfated regions. Fibril formation from IAPP was stimulated equally by IAPP-bound β-TC3 HS, non-bound β-TC3 HS, and non-bound NMuMG HS but was stimulated to a greater extent by the highly sulfated IAPP-bound NMuMG HS. Desulfation of HS decreased the ability of both β-TC3 and NMuMG HS to stimulate IAPP maximal fibril formation, but desulfated HS from both cell types still accelerated fibril formation relative to IAPP alone. In summary, neither binding to nor acceleration of fibril formation from the amyloidogenic peptide IAPP is dependent on overall sulfation in HS synthesized by β-TC3 cells. This information will be important in determining approaches to reduce HS-IAPP interactions and ultimately prevent islet amyloid formation and its toxic effects in type 2 diabetes.

Islet amyloid deposition is a pathological hallmark of the pancreatic islet in type 2 diabetes (1). Aggregation of islet amyloid polypeptide (IAPP),[2] a normal peptide product of the islet β-cell, underlies the deposition of islet amyloid, a process that contributes to the decreased β-cell volume that characterizes type 2 diabetes (2–4). The mechanism(s) that govern the aggregation of this normally soluble polypeptide are poorly understood. In addition to amyloidogenic IAPP, several other components of islet amyloid have been identified, including apolipoprotein E (5, 6), serum amyloid P component (7), and the heparan sulfate proteoglycan perlecan (6, 8).

Heparan sulfate proteoglycans (HSPGs) are a heterogeneous population of proteoglycans involved in a diverse range of cellular processes ranging from vascular development to cell signaling (9). HSPGs are components of amyloid deposits that form in a number of diseases, including type 2 diabetes (6, 8) and Alzheimer disease (10). HSPGs may play a role in stimulating amyloid deposition via a direct interaction with amyloidogenic peptides. We and others have shown that HSPGs are capable of binding the amyloidogenic peptide IAPP (11, 12), its precursor pro-IAPP (13) and other amyloidogenic peptides, including amyloid-β (14), and serum amyloid A (15), the unique amyloidogenic peptides from Alzheimer disease-related amyloid and inflammation-associated AA amyloidosis, respectively. Upon binding amyloidogenic peptides, HSPGs or their oligosaccharide heparan sulfate (HS) glycosaminoglycan

* This work was supported by the Department of Veterans Affairs, Veterans Affairs Puget Sound Health Care System (Seattle, WA) and National Institutes of Health Grants DK74404 (to R. L. H.), HL18645 and HL92969 (to T. N. W.), and DK17047 (to the University of Washington Diabetes Research Center).

[1] To whom correspondence should be addressed: Veterans Affairs Puget Sound Health Care System, 1660 S. Columbian Way, Seattle, WA 98108. Tel.: 206-764-2897; Fax: 206-764-2164; E-mail: rhull@u.washington.edu.

[2] The abbreviations used are: IAPP, islet amyloid polypeptide; GlcN, glucosamine; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; NMuMG, normal murine mammary gland; V_t, total permeation volume; TBS, Tris-buffered saline; UA, uronic acid.

Diabetes promotes an inflammatory macrophage phenotype and atherosclerosis through acyl-CoA synthetase 1

Jenny E. Kanter[a], Farah Kramer[a], Shelley Barnhart[a], Michelle M. Averill[a], Anuradha Vivekanandan-Giri[b], Thad Vickery[c], Lei O. Li[d], Lev Becker[e], Wei Yuan[e,1], Alan Chait[e], Kathleen R. Braun[f], Susan Potter-Perigo[f], Srinath Sanda[g], Thomas N. Wight[f], Subramaniam Pennathur[b], Charles N. Serhan[c], Jay W. Heinecke[e], Rosalind A. Coleman[d], and Karin E. Bornfeldt[a,2]

[a]Department of Pathology, Diabetes and Obesity Center of Excellence, University of Washington School of Medicine, Seattle, WA 98109; [b]Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109; [c]Center of Experimental Therapeutics, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; [d]Department of Nutrition, University of North Carolina, Chapel Hill, NC 27599; [e]Department of Medicine, Diabetes and Obesity Center of Excellence, University of Washington School of Medicine, Seattle, WA 98109; and [f]Hope Heart Matrix Biology Program and [g]Diabetes Research Program, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101

Edited* by Joseph A. Beavo, University of Washington School of Medicine, Seattle, WA, and approved December 8, 2011 (received for review July 17, 2011)

The mechanisms that promote an inflammatory environment and accelerated atherosclerosis in diabetes are poorly understood. We show that macrophages isolated from two different mouse models of type 1 diabetes exhibit an inflammatory phenotype. This inflammatory phenotype associates with increased expression of long-chain acyl-CoA synthetase 1 (ACSL1), an enzyme that catalyzes the thioesterification of fatty acids. Monocytes from humans and mice with type 1 diabetes also exhibit increased ACSL1. Furthermore, myeloid-selective deletion of ACSL1 protects monocytes and macrophages from the inflammatory effects of diabetes. Strikingly, myeloid-selective deletion of ACSL1 also prevents accelerated atherosclerosis in diabetic mice without affecting lesions in nondiabetic mice. Our observations indicate that ACSL1 plays a critical role by promoting the inflammatory phenotype of macrophages associated with type 1 diabetes; they also raise the possibilities that diabetic atherosclerosis has an etiology that is, at least in part, distinct from the etiology of nondiabetic vascular disease and that this difference is because of increased monocyte and macrophage ACSL1 expression.

It is becoming increasingly clear that monocytes isolated from humans with type 1 diabetes display an inflammatory phenotype and secrete higher levels of proinflammatory cytokines, such as IL-6 and IL-1β, than do monocytes from subjects without diabetes (1–5). Furthermore, inflammatory monocytes found in the setting of type 1 diabetes have recently been shown to result in Th17 cell activation (2, 6), suggesting that the proinflammatory effect of diabetes on monocytes/macrophages may result in wide-ranging effects on the immune system. Monocytes and macrophages are centrally important in atherogenesis and may play critical roles in diabetes-accelerated cardiovascular disease (7) and other complications of diabetes. Atherosclerotic vascular disease is the leading cause of death among people with types 1 and 2 diabetes, but this greatly increased incidence cannot be completely explained by the traditional cardiovascular risk factors. Systemic factors, such as dyslipidemia, hyperglycemia, hypertension, microalbuminuria, and low-grade inflammation, are often viewed as culprits in diabetic vascular disease. For example, clinical studies suggest that suboptimal glycemic control early in the cardiovascular disease progression promotes cardiovascular events later in life in subjects with type 1 diabetes (8). This hypothesis is supported by studies on animal models of the disease (9–11).

One viewpoint that has gained increased traction is that direct effects of diabetes on atherosclerotic lesion cells, such as macrophages, play an additional important role (12). Thus, the increased macrophage expression of inflammatory mediators associated with diabetes can be mimicked by elevated glucose concentrations in vitro (13). In addition, fatty acids exert inflammatory effects in macrophages, which could contribute to inflammation in the setting of diabetes (14), diabetes-accelerated atherosclerosis, and possibly other complications. After entering the cell, these fatty acids are thioesterified into their acyl-CoA derivatives, a process catalyzed by long-chain acyl-CoA synthetases (ACSLs).

In the current studies, we investigated the potential link between ACSL and diabetes in macrophages and monocytes. We show that ACSL1, an enzyme that hitherto has been implicated only in fatty acid incorporation into cellular lipids and use for β-oxidation, is up-regulated in monocytes and macrophages by type 1 diabetes, concomitant with well-known inflammatory mediators. Furthermore, myeloid-targeted deletion of ACSL1 inhibits the inflammatory activation of macrophages in the setting of diabetes and prevents diabetes-accelerated atherosclerosis in an established mouse model of type 1 diabetes. Our observations show that ACSL1 is centrally and selectively important in mediating the inflammatory phenotype of macrophages associated with diabetes.

Results

Diabetes Results in an Inflammatory Macrophage Phenotype Characterized by Increased ACSL1 Expression and Increased Arachidonoyl-CoA Levels. Monocytes isolated from human subjects with type 1 diabetes exhibit increased secretion of proinflammatory cytokines (1, 2). Using a transgenic mouse model of type 1 diabetes, in which T-cell–mediated destruction of pancreatic β-cells expressing a viral glycoprotein (GP) can be induced at will by lymphocytic choriomeningitis virus (LCMV) injection (10), we investigated if diabetes promotes a proinflammatory state in macrophages. LCMV-injected low-density lipoprotein receptor $(LDLR)^{-/-}$;GP^{+} mice were hyperglycemic (Fig. 1*A*) but showed no significant changes in blood cholesterol (Fig. 1*B*) compared with saline-injected $LDLR^{-/-}$;GP^{+} mice or LCMV-injected $LDLR^{-/-}$ mice without the GP transgene (Fig. S1 *A* and *B*). In both resident and thioglycollate-

Author contributions: J.E.K., L.B., C.N.S., and K.E.B. designed research; J.E.K., F.K., S.B., M.M.A., A.V.-G., T.V., L.O.L., L.B., K.R.B., and S.P.-P. performed research; W.Y., A.C., S.S., T.N.W., J.W.H., and R.A.C. contributed new reagents/analytic tools; J.E.K., L.B., S.S., S.P., and K.E.B. analyzed data; and J.E.K. and K.E.B. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]Present address: Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, MD 20850.

[2]To whom correspondence should be addressed. E-mail: bornf@uw.edu.

See Author Summary on page 4353 (volume 109, number 12).

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1111600109/-/DCSupplemental.

Obesity is associated with hypothalamic injury in rodents and humans

Joshua P. Thaler,[1,2] Chun-Xia Yi,[3] Ellen A. Schur,[2] Stephan J. Guyenet,[1,2] Bang H. Hwang,[1,2,4] Marcelo O. Dietrich,[5] Xiaolin Zhao,[1,2,6] David A. Sarruf,[1,2] Vitaly Izgur,[7] Kenneth R. Maravilla,[7] Hong T. Nguyen,[1,2] Jonathan D. Fischer,[1,2] Miles E. Matsen,[1,2] Brent E. Wisse,[1,2] Gregory J. Morton,[1,2] Tamas L. Horvath,[5,8] Denis G. Baskin,[1,2,4] Matthias H. Tschöp,[3] and Michael W. Schwartz[1,2]

[1]Division of Metabolism, Endocrinology and Nutrition, Diabetes and Obesity Center of Excellence, and [2]Department of Medicine, University of Washington, Seattle, Washington, USA. [3]Metabolic Diseases Institute, Division of Endocrinology, Department of Medicine, University of Cincinnati, Cincinnati, Ohio, USA. [4]Research and Development Service, Department of Veterans Affairs Puget Sound Health Care System, Seattle, Washington, USA. [5]Program in Integrative Cell Signaling and Neurobiology of Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut, USA. [6]Department of Physiology and Pathophysiology, School of Medicine at Xi'an Jiaotong University, Xi'an, China. [7]Department of Radiology, University of Washington, Seattle, Washington, USA. [8]Department of Obstetrics/Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut, USA.

Rodent models of obesity induced by consuming high-fat diet (HFD) are characterized by inflammation both in peripheral tissues and in hypothalamic areas critical for energy homeostasis. Here we report that unlike inflammation in peripheral tissues, which develops as a consequence of obesity, hypothalamic inflammatory signaling was evident in both rats and mice within 1 to 3 days of HFD onset, prior to substantial weight gain. Furthermore, both reactive gliosis and markers suggestive of neuron injury were evident in the hypothalamic arcuate nucleus of rats and mice within the first week of HFD feeding. Although these responses temporarily subsided, suggesting that neuroprotective mechanisms may initially limit the damage, with continued HFD feeding, inflammation and gliosis returned permanently to the mediobasal hypothalamus. Consistent with these data in rodents, we found evidence of increased gliosis in the mediobasal hypothalamus of obese humans, as assessed by MRI. These findings collectively suggest that, in both humans and rodent models, obesity is associated with neuronal injury in a brain area crucial for body weight control.

Introduction

Obesity has emerged as a major health problem in industrialized nations. Despite substantial progress in understanding the neurobiology of energy homeostasis (the biological process through which energy intake and expenditure are matched to one another so as to promote stability in the amount of fuel stored as fat) (1), little is known regarding how brain systems designed to promote weight stability are altered in common forms of obesity (2, 3).

Growing evidence implicates immune cell–mediated tissue inflammation as an important mechanism linking obesity to insulin resistance in metabolically active organs, such as liver, skeletal muscle, and adipose tissue (4–6). In rodent models of diet-induced obesity (DIO), increased inflammatory signaling in the mediobasal hypothalamus (MBH) similarly contributes to leptin resistance and weight gain (7–12), but the cellular interactions underlying this inflammatory response remain uncharacterized. The goal of the current study was to identify the neuroanatomical correlates of obesity-associated hypothalamic inflammation and to determine whether similar responses occur in humans.

We report that unlike inflammation in peripheral tissues, a process that develops over weeks to months of high-fat diet (HFD) feeding in rodent models (13–15), markers of hypothalamic inflammation are elevated within 24 hours of HFD exposure. Within the first week of HFD, markers of neuron injury also become evident in the hypothalamic arcuate nucleus (ARC) and adjacent median eminence (ARC-ME) in association with reactive gliosis involving recruitment of both microglia and astrocytes. Although initially transient, suggesting an effective neuroprotective response, inflammation and gliosis return and become established with continued HFD exposure. Using an established MRI method (16–19), we also report evidence of increased gliosis in the MBH of obese humans. These findings collectively suggest that, in both humans and rodent models, obesity is associated with injury to a key brain area for energy homeostasis.

Results

Time course of HFD-induced hypothalamic inflammatory gene expression. Consistent with a large volume of literature (7–11, 13, 14, 20), we found that expression of several proinflammatory genes increased by approximately 50% in both hypothalamus (Figure 1A) and liver (Figure 1B) of adult male rats subjected to long-term (20 weeks) consumption of a HFD (60% of calories from fat) relative to that in controls fed standard chow. By comparison, whereas inflammation was not detected in either liver or adipose tissue after only 4 weeks of HFD, hypothalamic inflammation was clearly evident at this earlier time point (Figure 1, C–E). Thus, the effect of HFD feeding to induce hypothalamic inflammatory gene expression seems unlikely to arise from a systemic inflammatory process.

Since rats fed the HFD for 4 weeks gained more than 50 g more body weight and more than 10% more fat mass than chow-fed controls over the same time frame (data not shown), it remains possible that hypothalamic inflammation at this time point is a consequence of obesity. To address this question, we analyzed hypothalamic proinflammatory gene expression in rats during the

Authorship note: Chun-Xia Yi and Ellen A. Schur contributed equally to this work.
Conflict of interest: The authors have declared that no conflict of interest exists.
Citation for this article: *J Clin Invest.* 2012;122(1):153–162. doi:10.1172/JCI59660.

Multimodal image coregistration and inducible selective cell ablation to evaluate imaging ligands

John Virostko[a], Joseph Henske[b], Laurent Vinet[c], Smaragda Lamprianou[c], Chunhua Dai[b], Aramandla Radhika[b], Ronald M. Baldwin[a,d], Mohammad S. Ansari[d], Franz Hefti[e], Daniel Skovronsky[e], Hank F. Kung[f], Pedro L. Herrera[c], Todd E. Peterson[a,d], Paolo Meda[c], and Alvin C. Powers[b,g,h,1]

[a]Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN 37232; [b]Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University, Nashville, TN 37232; [c]Department of Cell Physiology and Metabolism, University of Geneva Medical School, 1211 Geneva 4, Switzerland; [d]Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN 37232; [e]Avid Radiopharmaceuticals, Inc., Philadelphia, PA 19104; [f]Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104; [g]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232; and [h]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN 37232

Edited by Michael E. Phelps, University of California, Los Angeles, CA, and approved November 2, 2011 (received for review June 15, 2011)

We combined multimodal imaging (bioluminescence, X-ray computed tomography, and PET), tomographic reconstruction of bioluminescent sources, and two unique, complementary models to evaluate three previously synthesized PET radiotracers thought to target pancreatic beta cells. The three radiotracers {[^{18}F]fluoropropyl-(+)-dihydrotetrabenazine ([^{18}F]FP-DTBZ), [^{18}F](+)-2-oxiranyl-3-isobutyl-9-(3-fluoropropoxy)-10-methoxy-2,3,4,6,7,11b-hexahydro-1H-pyrido[2,1-a]isoquinoline (^{18}F-AV-266), and (2*S*,3*R*,11b*R*)-9-(3-fluoropropoxy)-2-(hydroxymethyl)-3-isobutyl-10-methoxy-2,3,4,6,7,11b-hexahydro-1*H*-pyrido[2,1-*a*]isoquinolin-2-ol (^{18}F-AV-300)} bind vesicular monoamine transporter 2. Tomographic reconstruction of the bioluminescent signal in mice expressing luciferase only in pancreatic beta cells was used to delineate the pancreas and was coregistered with PET and X-ray computed tomography images. This strategy enabled unambiguous identification of the pancreas on PET images, permitting accurate quantification of the pancreatic PET signal. We show here that, after conditional, specific, and rapid mouse beta-cell ablation, beta-cell loss was detected by bioluminescence imaging but not by PET imaging, given that the pancreatic signal provided by three PET radiotracers was not altered. To determine whether these ligands bound human beta cells in vivo, we imaged mice transplanted with luciferase-expressing human islets. The human islets were imaged by bioluminescence but not with the PET ligands, indicating that these vesicular monoamine transporter 2-directed ligands did not specifically bind beta cells. These data demonstrate the utility of coregistered multimodal imaging as a platform for evaluation and validation of candidate ligands for imaging islets.

diabetes | insulin | molecular imaging | pancreatic islet | bioluminescence tomography

The ability to image and quantify rare cell populations would aid diagnosis and treatment of Parkinson disease (1), Alzheimer's disease (2), certain cancers (3), and diabetes mellitus (4). In the case of diabetes, the rare cell population of interest is the pancreatic beta cell, the sole cell type capable of synthesizing and releasing the hormone insulin in vertebrates. Reduced beta-cell mass is a cardinal feature of type 1 diabetes (5) and is now recognized as a critical feature in type 2 diabetes as well (6–8), but we cannot noninvasively quantify beta-cell mass in humans. Despite the efforts of a number of investigators, approaches for noninvasive assessment of beta-cell mass in vivo are presently limited. The small size of pancreatic islets (50–300 μm in diameter), their sparse frequency (1–2% of pancreatic mass), and their scattered distribution throughout the pancreas create a number of scientific and technical challenges for noninvasive imaging and quantification of beta-cell mass. Additionally, the location of the pancreas deep within the abdomen, its proximity to other major organs, and artifacts from respiratory, cardiac, and intestinal motion pose further challenges to imaging beta cells.

The dynamic changes in beta-cell mass accompanying diabetes are neither well understood nor well characterized by functional measurements of glycemic control. Although the amount of insulin/C-peptide secreted after a glucose or meal challenge gives insight into insulin secretory capacity (4, 9), these only indirectly reflect beta-cell mass and do not determine whether this mass is increasing or declining. For example, glucose homeostasis is unaffected until beta-cell mass is reduced to less than half its original value, presumably because of a large functional reserve capacity, and overt diabetes develops only when beta-cell mass is greatly reduced (10–12). Morphometric analysis of histological sections (13) requires removal of the pancreas to quantify beta-cell mass. Because we incompletely understand the time course and development of both forms of diabetes, our ability is limited to effectively test interventions to prevent beta-cell loss. Furthermore, efforts to regenerate beta-cell mass (14, 15) would be aided by the ability to image the beta cells both during preclinical development of regenerative protocols and during clinical monitoring of therapeutic response.

Recently, the vesicular monoamine transporter 2 (VMAT2) has been identified as a possible target for noninvasive positron-emission tomography (PET) imaging of pancreatic beta cells. Gene-expression studies on human tissues indicate that VMAT2 expression is greater in islets than in the exocrine tissue of the pancreas (16), and immunohistochemical studies have shown costaining of VMAT2 and insulin (17, 18). A radiolabeled ligand to VMAT2, dihydrotetrabenazine ([^{11}C]DTBZ) (19), has been extensively used in PET imaging of both human and rodent brains, revealing altered density of VMAT2 in diseased states such as Parkinson disease (20). [^{11}C]DTBZ has also been used for pancreatic islet imaging, showing reduced pancreatic uptake in rodent models of autoimmune and streptozotocin-induced diabetes (21, 22). Imaging in humans has demonstrated lower pancreatic [^{11}C]DTBZ radioactivity in type 1 diabetics (23). However, [^{11}C]DTBZ has high nonspecific binding to the exocrine pancreas, suggesting that it may not be suitable for imaging beta-cell mass (24, 25). An ^{18}F-labeled analog of DTBZ ([^{18}F]DTBZ) has been previously synthesized (26), and the longer half-life of ^{18}F (110 min versus 20 min for ^{11}C) facilitates imaging at longer intervals after radiotracer administration, allowing more

Author contributions: J.V., J.H., R.M.B., M.S.A., F.H., D.S., H.F.K., T.E.P., P.M., and A.C.P. designed research; J.V., J.H., L.V., S.L., C.D., A.R., and R.M.B. performed research; H.F.K. and P.L.H. contributed new reagents/analytic tools; J.V., P.M., and A.C.P. analyzed data; and J.V., T.E.P., P.M., and A.C.P. wrote the paper.

Conflict of interest statement: F.H., D.S., and H.F.K. either are employed at Avid Radiopharmaceuticals or have a financial interest in the company. A.C.P. has received an investigator-initiated research grant from Eli Lilly and Company.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. E-mail: al.powers@vanderbilt.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1109480108/-/DCSupplemental.

Connective tissue growth factor acts within both endothelial cells and β cells to promote proliferation of developing β cells

Michelle A. Guney[a], Christine P. Petersen[b], Andre Boustani[b], Matthew R. Duncan[c], Uma Gunasekaran[b], Renuka Menon[b], Courtney Warfield[b], Gary R. Grotendorst[c], Anna L. Means[d,e], Aris N. Economides[f], and Maureen Gannon[a,b,d,1]

[a]Department of Molecular Physiology and Biophysics, [b]Department of Medicine, Division of Diabetes, Endocrinology and Metabolism, [d]Department of Cell and Developmental Biology, and [e]Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232; [c]The Lovelace Respiratory Institute, Albuquerque, NM 87108; and [f]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591

Edited* by Roger D. Cone, Vanderbilt University School of Medicine, Nashville, TN, and approved July 28, 2011 (received for review January 3, 2011)

Type 1 and type 2 diabetes result from an absolute or relative reduction in functional β-cell mass. One approach to replacing lost β-cell mass is transplantation of cadaveric islets; however, this approach is limited by lack of adequate donor tissue. Therefore, there is much interest in identifying factors that enhance β-cell differentiation and proliferation in vivo or in vitro. Connective tissue growth factor (CTGF) is a secreted molecule expressed in endothelial cells, pancreatic ducts, and embryonic β cells that we previously showed is required for β-cell proliferation, differentiation, and islet morphogenesis during development. The current study investigated the tissue interactions by which CTGF promotes normal pancreatic islet development. We found that loss of CTGF from either endothelial cells or β cells results in decreased embryonic β-cell proliferation, making CTGF unique as an identified β cell-derived factor that regulates embryonic β-cell proliferation. Endothelial CTGF inactivation was associated with decreased islet vascularity, highlighting the proposed role of endothelial cells in β-cell proliferation. Furthermore, CTGF overexpression in β cells during embryogenesis using an inducible transgenic system increased islet mass at birth by promoting proliferation of immature β cells, in the absence of changes in islet vascularity. Together, these findings demonstrate that CTGF acts in an autocrine manner during pancreas development and suggest that CTGF has the potential to enhance expansion of immature β cells in directed differentiation or regeneration protocols.

Pancreas development initiates at embryonic day (E) 9.5 in the mouse as dorsal and ventral evaginations from the posterior foregut endoderm that undergo branching morphogenesis. Notch/Delta signaling within the ductal epithelium generates a population of endocrine progenitor cells marked by expression of the transcription factor neurogenin3 (Ngn3) (1–4). These progenitors delaminate from the epithelium and differentiate into hormone-positive cells that subsequently proliferate. Islets are complex microorgans responsible for maintaining glucose homeostasis and consist of at least four different endocrine cell types, including insulin-producing β cells and glucagon-producing α cells. Insufficient β-cell mass characterizes both type 1 (autoimmune) and type 2 diabetes. Thus, strategies to generate β cells de novo or increase their number in vivo are a potential approach for the treatment of diabetes and are being widely investigated.

Generation of the correct numbers of the different endocrine cell types requires tight coordination of waves of differentiation and proliferation that are regulated by both paracrine and autocrine signals. The pancreatic vascular endothelium secretes paracrine factors important for pancreas differentiation (5–8). Factors such as retinoic acid, FGFs, and bone morphogenetic proteins (BMPs) regulate the outgrowth of the epithelium, as well as the differentiation of multipotent pancreatic progenitors (9). Signals from the dorsal aorta are necessary for growth of the dorsal pancreas as well as expression of *Ptf1a*, a transcription factor essential for the development of pancreatic progenitors (7, 10). Furthermore, in vitro coculture experiments demonstrated that recombination of prepatterned dorsal endoderm with aorta endothelium is sufficient to induce differentiation of insulin-expressing cells (8). Blood vessel-derived sphingosine-1-phosphate stimulates growth of the pancreas, and endothelial cells secrete additional factors that have a direct role on the endoderm (11). For example, the concomitant formation of blood vessels and islets involves communication between the endothelial cells and endocrine cells. VEGFA is expressed in islet endocrine cells from very early in development through adulthood and is required for proper development of the islet vasculature (12). Overexpression of VEGFA throughout the entire pancreatic epithelium under the direction of the *pancreatic and duodenal homeobox-1* (*Pdx-1*) promoter leads to an increase in pancreatic vascularization and total islet mass at the expense of exocrine tissue (8). The main VEGF receptor, VEGFR2/flk-1, is not expressed in islets, suggesting that the increase in islet mass is secondary to the increase in vasculature rather than a direct consequence of VEGFA overexpression on the endoderm (8, 12, 13). Thus, although endothelial cells have long been understood to have a role in pancreas growth and endocrine development, the specific secreted factors that mediate its effects on the pancreatic epithelium have not yet been identified.

Although endocrine cell neogenesis generates the majority of the endocrine cells early in development, adequate endocrine cell replication during late gestation (E18.5) and in the neonatal period is necessary to generate the correct number of β cells in the adult. Only a few factors, however, have been shown to regulate embryonic β-cell proliferation in vivo. The eIF2α kinase PERK is important for β-cell proliferation in embryos and neonates but not in the adult (14). The transcription factor Pdx-1 is essential for pancreas organogenesis and β-cell proliferation (15–17). Removal of *Pdx-1* specifically in embryonic β cells leads to a significant decrease in β-cell proliferation and a concomitant increase in α-cell proliferation at late gestation (17). Recent evidence has shown that Ngn3-positive proendocrine cells are unipotent; each progenitor cell gives rise to only one endocrine cell type (18). The fact that β- and α-cell proliferation are re-

Author contributions: M.A.G. and M.G. designed research; M.A.G., C.P.P., A.B., M.R.D., U.G., R.M., C.W., A.L.M., and M.G. performed research; G.R.G. and A.N.E. contributed new reagents/analytic tools; M.A.G. and M.G. analyzed data; and M.A.G. and M.G. wrote the paper.

Conflict of interest statement: A.N.E. is a paid employee and shareholder of Regeneron Pharmaceuticals.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: maureen.gannon@vanderbilt.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1100072108/-/DCSupplemental.

Reversal of Type 1 Diabetes in Mice by Brown Adipose Tissue Transplant

Subhadra C. Gunawardana and David W. Piston

Current therapies for type 1 diabetes (T1D) involve insulin replacement or transplantation of insulin-secreting tissue, both of which suffer from numerous limitations and complications. Here, we show that subcutaneous transplants of embryonic brown adipose tissue (BAT) can correct T1D in streptozotocin-treated mice (both immune competent and immune deficient) with severely impaired glucose tolerance and significant loss of adipose tissue. BAT transplants result in euglycemia, normalized glucose tolerance, reduced tissue inflammation, and reversal of clinical diabetes markers such as polyuria, polydipsia, and polyphagia. These effects are independent of insulin but correlate with recovery of the animals' white adipose tissue. BAT transplants lead to significant increases in adiponectin and leptin, but with levels that are static and not responsive to glucose. Pharmacological blockade of the insulin receptor in BAT transplant mice leads to impaired glucose tolerance, similar to what is seen in nondiabetic animals, indicating that insulin receptor activity plays a role in the reversal of diabetes. One possible candidate for activating the insulin receptor is IGF-1, whose levels are also significantly elevated in BAT transplant mice. Thus, we propose that the combined action of multiple adipokines establishes a new equilibrium in the animal that allows for chronic glycemic control without insulin.
Diabetes **61:674–682, 2012**

Type 1 diabetes (T1D) involves autoimmune-mediated destruction of β-cells, which leads to an absolute deficiency of insulin and loss of glycemic control. The ultimate cure for T1D would require permanent renormalization of blood glucose homeostasis. Although insulin is believed to be the major regulator of blood glucose levels, numerous other hormones are also involved (1). In particular, adipose tissue is a versatile endocrine organ secreting a variety of adipokines with both hyperglycemic and hypoglycemic properties (1–3). Two adipokines, adiponectin and leptin, have recently been reported to ameliorate diabetes phenotypes in the absence of exogenous insulin (4–7), indicating that specific adipokines may be able to compensate for a loss of insulin.

In addition to the destruction of pancreatic β-cells, T1D is associated with a loss of adipose tissue. Successful treatment of T1D results in spontaneous recovery of adipose tissue along with the restoration of normal glucose levels and dynamics (8–10). However, a two-way relationship between adipose tissue and blood glucose homeostasis has not yet been documented. Previous data following streptozotocin (STZ)-diabetic mice (a model of T1D) treated by embryonic pancreatic tissue transplants suggest that healthy adipose tissue contributes to the correction of T1D, even when insulin levels are low (11). In some immune-deficient nude mice (NCRNU; Taconic), we observed return to euglycemia and reversal of clinical signs of diabetes despite a lack of any measurable increase in plasma insulin. These animals exhibited a robust recovery of body weight and adipose tissue, as well as a simultaneous increase in plasma adiponectin and visfatin (11,12). Thus, we hypothesized that adipose tissue can compensate for a lack of insulin. Our previous subcutaneous transplants were performed using embryonic pancreatic buds with their surrounding tissue, resulting in remarkable replenishment of recipients' subcutaneous white adipose tissue (WAT) (11,12). To delineate the contributions from different tissue types, we transplanted a panel of embryonic tissues subcutaneously into STZ-treated mice. Pancreatic bud transplants correct T1D as expected. Surprisingly, subcutaneous transplantation of embryonic brown adipose tissue (BAT) into immune-competent C57BL/6J T1D recipients results in marked improvement of glucose homeostasis and reversal of diabetes, accompanied by robust regeneration of the recipients' subcutaneous WAT. None of the other transplanted tissue types produce similar results. The positive changes after the BAT transplant do not require exogenous insulin or immunosuppression, which indicates that healthy adipose tissue is sufficient to maintain glucose homeostasis in the absence of insulin.

RESEARCH DESIGN AND METHODS

Embryonic BAT was transplanted in the subcutaneous space of STZ-diabetic recipients. Recipient mice included C57BL/6J and NCRNU-M-M nude mice. Donor adipose tissue came from E16–E17 C57BL/6J embryos. Weight was recorded and basal-fed blood samples were collected at regular intervals from mice that received BAT transplants as well as normal nondiabetic control mice and untreated diabetic control mice. Intraperitoneal glucose tolerance tests (IPGTTs) were performed before BAT transplants and at monthly intervals after BAT transplants. Metabolic parameters such as blood glucose, insulin response to glucose and arginine, and plasma levels of adiponectin, leptin, glucagon, and IGF-1 were measured from transplant and control groups at regular intervals. Three months after BAT transplant, mice were subjected to an additional glucose tolerance test in the presence of S961, an inhibitor of insulin receptor. One insulin tolerance test was also performed between 2 and 3 months posttransplant. BAT transplant mice were killed at different time points after 3 months and tissues were collected. Pancreata were tested for insulin content by immunohistochemistry and radioimmunoassay, and adipose tissue at and around the transplant site was examined for uncoupling protein-1 (UCP-1), IGF-1, and signs of inflammation by immunohistochemistry.

Animals. Recipients were immune-deficient NCRNU-M-M nude mice (Taconic) and immune-competent C57BL/6J mice (Jackson Laboratories) rendered diabetic with STZ (125 mg/kg dissolved in ice-cold Na^+ citrate buffer at pH 4.5, injected intraperitoneally, and repeated biweekly until diabetes induced). All recipients were 3–6 months of age at the time of transplants. Donor embryonic BAT was obtained from C57BL/6J embryos at gestational age E16.5–E17.5. Parents were purchased from Jackson Laboratories and maintained in the Vanderbilt animal care facility. Animals were fed standard laboratory chow and

From the Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee.
Corresponding author: Subhadra C. Gunawardana, subhadra.gunawardana@vanderbilt.edu.
Received 15 April 2011 and accepted 15 December 2011.
DOI: 10.2337/db11-0510
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db11-0510/-/DC1.

Xenin-25 Amplifies GIP-Mediated Insulin Secretion in Humans With Normal and Impaired Glucose Tolerance but Not Type 2 Diabetes

Burton M. Wice,[1] Dominic N. Reeds,[2] Hung D. Tran,[1] Dan L. Crimmins,[3] Bruce W. Patterson,[2] Judit Dunai,[1] Michael J. Wallendorf,[4] Jack H. Ladenson,[3] Dennis T. Villareal,[5,6] and Kenneth S. Polonsky[1,7]

Glucose-dependent insulinotropic polypeptide (GIP) potentiates glucose-stimulated insulin secretion (GSIS). This response is blunted in type 2 diabetes (T2DM). Xenin-25 is a 25–amino acid neurotensin-related peptide that amplifies GIP-mediated GSIS in hyperglycemic mice. This study determines if xenin-25 amplifies GIP-mediated GSIS in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or T2DM. Each fasting subject received graded glucose infusions to progressively raise plasma glucose concentrations, along with vehicle alone, GIP, xenin-25, or GIP plus xenin-25. Plasma glucose, insulin, C-peptide, and glucagon levels and insulin secretion rates (ISRs) were determined. GIP amplified GSIS in all groups. Initially, this response was rapid, profound, transient, and essentially glucose independent. Thereafter, ISRs increased as a function of plasma glucose. Although magnitudes of insulin secretory responses to GIP were similar in all groups, ISRs were not restored to normal in subjects with IGT and T2DM. Xenin-25 alone had no effect on ISRs or plasma glucagon levels, but the combination of GIP plus xenin-25 transiently increased ISR and plasma glucagon levels in subjects with NGT and IGT but not T2DM. Since xenin-25 signaling to islets is mediated by a cholinergic relay, impaired islet responses in T2DM may reflect defective neuronal, rather than GIP, signaling. ***Diabetes*** **61:1793–1800, 2012**

Peptides secreted from the gastrointestinal tract play an important role in regulating insulin secretion (1,2). To date, attention has focused on two intestinal peptides: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is produced predominantly by L cells in the distal bowel, whereas GIP is produced mainly by K cells in the proximal intestine. Both peptides are released into the blood immediately after eating and potentiate glucose-stimulated insulin secretion (GSIS) (3–5). Unlike GLP-1, which stimulates insulin secretion in type 2 diabetes (T2DM), persons with T2DM are thought to be resistant to the actions of GIP (6–8), and increasing GIP signaling has not been pursued as a therapeutic target for T2DM.

To better understand how GIP regulates the incretin response, we generated and characterized transgenic mice that lack GIP-producing cells by driving expression of an attenuated diphtheria toxin transgene with regulatory elements from the GIP gene (9). These GIP/DT mice demonstrated markedly attenuated insulin secretory responses to oral glucose even though GLP-1 release was normal (9). Moreover, the GIP/DT mice exhibited a blunted insulin secretory response to exogenously administered GIP but not GLP-1 (10). Thus, GIP-producing cells may secrete a hormone(s) in addition to GIP that plays a critical role in the incretin response.

Xenin-25 is a 25–amino acid neurotensin-related peptide reportedly produced by a subset of K cells (11,12) and would also be reduced in the GIP/DT mice. In vivo experiments demonstrated that xenin-25 potentiated the insulin secretory response to GIP but had little effect alone (10). Similar results were observed in hyperglycemic NONcNZO10/Ltj mice (10)—a polygenic model of human T2DM in which diabetes spontaneously develops with age (13,14). These studies raise the possibility that xenin-25 could increase the insulin secretory response to GIP in humans. The purpose of the current study was to determine if xenin-25, either alone or with GIP, could amplify the insulin secretory response to glucose in humans with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and/or T2DM.

RESEARCH DESIGN AND METHODS

Studies in human subjects. All protocols were approved by Washington University's Human Research Protection Office and the Food and Drug Administration (IND 103,374) and are registered with ClinicalTrials.gov (NCT00798915). Studies were performed in the Clinical Research Unit of the Institute of Clinical and Translational Sciences of Washington University after obtaining written informed consent. Male and female subjects with NGT, IGT, and mild T2DM were studied (Table 1). Glucose tolerance was defined by the 2-h plasma glucose level during a 75-g oral glucose tolerance test using diagnostic criteria of the American Diabetes Association (15). Baseline characteristics were determined during a screening visit after a 10-h fast. HbA_{1c} levels were required to be ≤9.0 in all subjects. Subjects treated with insulin were excluded. Subjects treated with oral antidiabetic medications were enrolled if the agent(s) could be safely discontinued for 48 h preceding each study visit. These selection criteria were designed to exclude T2DM subjects with advanced β-cell failure and to identify participants with residual insulin secretion who have the potential to respond to incretin peptides. Women of childbearing potential were required to use birth control. Subjects were excluded if they *1*) had a history of chronic pancreatitis and/or risk factors for chronic pancreatitis, *2*) had a history of gastrointestinal disorders, *3*) were taking nondiabetes medications known to

From the [1]Division of Endocrinology, Metabolism, and Lipid Research, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri; the [2]Division of Nutritional Science, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri; the [3]Division of Laboratory and Genomic Medicine, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri; the [4]Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri; the [5]Division of Geriatrics, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri; the [6]Department of Geriatrics, University of New Mexico School of Medicine and New Mexico Veterans Affairs Health Care System, Albuquerque, New Mexico; and the [7]Division of Biological Sciences and Pritzker School of Medicine, The University of Chicago, Chicago, Illinois.

Corresponding author: Kenneth S. Polonsky, polonsky@bsd.uchicago.edu, or Burton M. Wice, bwice@dom.wustl.edu.

Received 13 October 2011 and accepted 29 February 2012.

DOI: 10.2337/db11-1451. Clinical trial reg. no. NCT00798915, clinicaltrials.gov.

Peroxisome Proliferator-Activated Receptor Pathway Gene Polymorphism Associated With Extent of Coronary Artery Disease in Patients With Type 2 Diabetes in the Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial

Sharon Cresci, MD; Jun Wu, MD, MS; Michael A. Province, PhD; John A. Spertus, MD, MPH; Michael Steffes, MD, PhD; Janet B. McGill, MD; Edwin L. Alderman, MD; Maria Mori Brooks, PhD; Sheryl F. Kelsey, PhD; Robert L. Frye, MD; Richard G. Bach, MD; on behalf of the BARI 2D Study Group

Background—Coronary artery disease (CAD) is the major cause of death in patients with type 2 diabetes mellitus. Although demographic and clinical factors associated with extent of CAD in patients with diabetes mellitus have been described, genetic factors have not. We hypothesized that genetic variation in peroxisome proliferator-activated receptor (PPAR) pathway genes, important in diabetes mellitus and atherosclerosis, would be associated with extent of CAD in patients with diabetes mellitus.

Methods and Results—We genotyped 1043 patients (702 white, 175 blacks) from the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) genetic cohort for 3351 variants in 223 PPAR pathway genes using a custom targeted-genotyping array. Angiographic end points were determined by a core laboratory. In whites, a single variant (rs1503298) in *TLL1* was significantly ($P=5.5\times10^{-6}$) associated with extent of CAD, defined as number of lesions with percent diameter stenosis ≥20%, after stringent Bonferroni correction for all 3351 single nucleotide polymorphisms. This association was validated in the diabetic subgroups of 2 independent cohorts, the Translational Research Investigating Underlying Disparities in Acute Myocardial Infarction Patients' Health Status (TRIUMPH) post–myocardial infarction registry and the prospective Family Heart Study (FHS) of individuals at risk for CAD. *TLL1*rs1503298 was also significantly associated with extent of severe CAD (≥70% diameter stenosis; $P=3.7\times10^{-2}$) and myocardial jeopardy index ($P=8.7\times10^{-4}$). In general linear regression modeling, *TLL1*rs1503298 explained more variance of extent of CAD than the previously determined clinical factors.

Conclusions—We identified a variant in a single PPAR pathway gene, *TLL1,* that is associated with the extent of CAD independently of clinical predictors, specifically in patients with type 2 diabetes mellitus and CAD.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00006305.

(*Circulation*. 2011;124:1426-1434.)

Key Words: coronary artery disease ■ calcium ■ diabetes mellitus, type 2 ■ genetic variation ■ peroxisome proliferator-activated receptors ■ Tolloid-like metalloproteinases

Diabetes mellitus (DM) currently affects >17 million people in the United States, and ≈1.6 million new cases are diagnosed each year.[1] Among persons with DM, coronary atherosclerosis is highly prevalent and accounts for the majority of deaths.[1] In patients with coronary artery disease (CAD), patients with DM have lower 10-year survival than those without DM,[2] and the extent of CAD predicts mortality.[3] Atherosclerosis in patients with DM has an accelerated phenotype, with more diffuse and extensive disease that shows more rapid progression, suggesting a distinctive pathogenesis.[4] The distinct pathogenesis of the accelerated atherosclerosis observed among patients with DM is poorly understood,[5] and the role of genetic factors is unknown.

Clinical Perspective on p 1434

Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) was a multicenter, randomized clinical trial that investigated the effect of different approaches to the

Received July 2, 2010; accepted July 26, 2011.

From the Washington University School of Medicine, Department of Medicine, St. Louis, MO (S.C., J.W., M.A.P., J.B.M., R.G.B.); Saint Luke's Mid America Heart Institute and the University of Missouri–Kansas City (J.A.S.); University of Minnesota, Minneapolis (M.S.); Stanford University, Stanford, CA (E.L.A.); University of Pittsburgh, Pittsburgh, PA (M.M.B., S.F.K.); and Mayo Clinic, Rochester, MN (R.L.F.).

A complete list of the BARI 2D Study Group is provided in the Appendix in the online-only Data Supplement.

Guest Editor for this article was Kari E. North, PhD.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.111.029173/-/DC1.

Correspondence to Sharon Cresci, MD, Cardiovascular Division, Washington University School of Medicine, 660 S Euclid Ave, Campus Box 8086, St. Louis, MO 63110-1093. E-mail scresci@dom.wustl.edu

Cell Metabolism
Article

Inhibiting Adipose Tissue Lipogenesis Reprograms Thermogenesis and PPARγ Activation to Decrease Diet-Induced Obesity

Irfan J. Lodhi,[1] Li Yin,[1] Anne P.L. Jensen-Urstad,[1] Katsuhiko Funai,[1] Trey Coleman,[1] John H. Baird,[1] Meral K. El Ramahi,[1] Babak Razani,[1,2] Haowei Song,[1] Fong Fu-Hsu,[1] John Turk,[1] and Clay F. Semenkovich[1,3,*]
[1]Division of Endocrinology, Metabolism & Lipid Research
[2]Cardiovascular Division
[3]Department of Cell Biology and Physiology
Washington University School of Medicine, St. Louis, MO 63110, USA
*Correspondence: csemenko@wustl.edu
http://dx.doi.org/10.1016/j.cmet.2012.06.013

SUMMARY

De novo lipogenesis in adipocytes, especially with high fat feeding, is poorly understood. We demonstrate that an adipocyte lipogenic pathway encompassing fatty acid synthase (FAS) and PexRAP (*per*oxisomal *r*eductase *a*ctivating *P*PARγ) modulates endogenous PPARγ activation and adiposity. Mice lacking FAS in adult adipose tissue manifested increased energy expenditure, increased brown fat-like adipocytes in subcutaneous adipose tissue, and resistance to diet-induced obesity. FAS knockdown in embryonic fibroblasts decreased PPARγ transcriptional activity and adipogenesis. FAS-dependent alkyl ether phosphatidylcholine species were associated with PPARγ and treatment of 3T3-L1 cells with one such ether lipid increased PPARγ transcriptional activity. PexRAP, a protein required for alkyl ether lipid synthesis, was associated with peroxisomes and induced during adipogenesis. PexRAP knockdown in cells decreased PPARγ transcriptional activity and adipogenesis. PexRAP knockdown in mice decreased expression of PPARγ-dependent genes and reduced diet-induced adiposity. These findings suggest that inhibiting PexRAP or related lipogenic enzymes could treat obesity and diabetes.

INTRODUCTION

A relentless increase in mean global body weight since 1980 has resulted in an estimated 1.5 billion overweight people worldwide, of which a half billion are obese (Finucane et al., 2011). Obesity leads to diabetes, which is associated with premature death from many causes (Seshasai et al., 2011). Obesity is caused by positive energy balance leading to expansion of adipocyte mass. However, adipocytes possess functional pathways that might be targeted to complement therapies altering energy balance. De novo lipogenesis, an adipocyte function that requires the multifunctional enzyme fatty acid synthase (FAS) (Semenkovich, 1997), is one such potential target since adipose tissue FAS has been implicated in obesity and insulin resistance in humans (Moreno-Navarrete et al., 2009; Roberts et al., 2009; Schleinitz et al., 2010).

Fatty acid synthase catalyzes the first committed step in de novo lipogenesis. The magnitude of de novo lipogenesis is different in rodents and people. Lipogenesis is thought to be a relatively minor contributor to whole body lipid stores in a present-day human consuming a typical high fat diet (Aarsland et al., 1996; Letexier et al., 2003; McDevitt et al., 2001). However, pharmacologic or genetic manipulation of enzymes in the lipogenic pathway can have profound metabolic consequences (Postic and Girard, 2008), suggesting that de novo lipogenesis might serve a signaling function independent of the generation of lipid stores (Lodhi et al., 2011). Consistent with this concept, FAS in liver is part of a lipogenic pathway involved in the generation of a ligand for peroxisome proliferator-activated receptor α (PPARα) (Chakravarthy et al., 2009), a key transcriptional regulator of fatty acid oxidation.

PPARs, consisting of PPARα, PPARδ and PPARγ, are ligand activated transcription factors that form obligate heterodimers with the retinoid X receptor (RXR) and regulate metabolism (Wang, 2010). Ligand binding results in a conformational change in the receptor, promoting dissociation of repressors, recruitment of coactivators, and subsequent activation of target gene expression. This nuclear receptor family was identified and named based on activation by chemicals that promote proliferation of peroxisomes (Dreyer et al., 1992; Issemann and Green, 1990).

Peroxisomes participate in the oxidation of certain fatty acids as well as the synthesis of bile acids and ether lipids (Wanders and Waterham, 2006). These single membrane-enclosed organelles are present in virtually all eukaryotic cells. In adipocytes they tend to be small and were referred to as microperoxisomes by Novikoff and colleagues, who documented a large increase in peroxisome number during the differentiation of 3T3-L1 adipocytes (Novikoff and Novikoff, 1982; Novikoff et al., 1980).

We sought to evaluate the role of de novo lipogenesis in adipocyte function and metabolism. Here we show that a lipogenic pathway encompassing FAS and PexRAP (*per*oxisomal *r*eductase *a*ctivating *P*PARγ), an enzyme localized to peroxisomes

Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease

Naoki Kumashiro[a,b], Derek M. Erion[a,b,c], Dongyan Zhang[a], Mario Kahn[b], Sara A. Beddow[b], Xin Chu[d], Christopher D. Still[d], Glenn S. Gerhard[d], Xianlin Han[e], James Dziura[b], Kitt Falk Petersen[b], Varman T. Samuel[b,f,1], and Gerald I. Shulman[a,b,c,1]

[a]Howard Hughes Medical Institute and Departments of [b]Internal Medicine and [c]Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510; [d]Weis Center for Research, Geisinger Clinic, Danville, PA 17822; [e]Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, Orlando, FL 32827; and [f]Veterans Affairs Medical Center, West Haven, CT 06516

Contributed by Gerald I. Shulman, August 24, 2011 (sent for review July 22, 2011)

Insulin resistance is associated with nonalcoholic fatty liver disease (NAFLD) and is a major factor in the pathogenesis of type 2 diabetes. The development of hepatic insulin resistance has been ascribed to multiple causes, including inflammation, endoplasmic reticulum (ER) stress, and accumulation of hepatocellular lipids in animal models of NAFLD. However, it is unknown whether these same cellular mechanisms link insulin resistance to hepatic steatosis in humans. To examine the cellular mechanisms that link hepatic steatosis to insulin resistance, we comprehensively assessed each of these pathways by using flash-frozen liver biopsies obtained from 37 obese, nondiabetic individuals and correlating key hepatic and plasma markers of inflammation, ER stress, and lipids with the homeostatic model assessment of insulin resistance index. We found that hepatic diacylglycerol (DAG) content in cytoplasmic lipid droplets was the best predictor of insulin resistance ($R = 0.80$, $P < 0.001$), and it was responsible for 64% of the variability in insulin sensitivity. Hepatic DAG content was also strongly correlated with activation of hepatic PKCε ($R = 0.67$, $P < 0.001$), which impairs insulin signaling. In contrast, there was no significant association between insulin resistance and other putative lipid metabolites or plasma or hepatic markers of inflammation. ER stress markers were only partly correlated with insulin resistance. In conclusion, these data show that hepatic DAG content in lipid droplets is the best predictor of insulin resistance in humans, and they support the hypothesis that NAFLD-associated hepatic insulin resistance is caused by an increase in hepatic DAG content, which results in activation of PKCε.

Hepatic insulin resistance is associated with nonalcoholic fatty liver disease (NAFLD) and is a major factor in the pathogenesis of type 2 diabetes (T2D) and the metabolic syndrome (1–3). Although there is general consensus that insulin resistance is caused by defects in intracellular insulin signaling, multiple causes have been proposed to explain how these insulin signaling defects arise in NAFLD. Inflammation, activation of endoplasmic reticulum (ER) stress pathways, and accumulation of hepatocellular lipids have all been suggested to cause insulin resistance in animal models of NAFLD (Fig. S1) (4–7). First, intracellular diacylglycerols (DAGs) can inhibit insulin signaling by activation of novel PKC isoforms (6, 8, 9), which in turn, block insulin receptor kinase phosphorylation of insulin receptor substrates 1 and 2. Intracellular ceramides are thought to prevent Akt2 activation (10–12) (Fig. S1). Second, adipocytokines (e.g., TNF-α, IL-1β, and IL-6) interfere with insulin signaling through activation of the JNK or inhibitor of IκB kinase-β pathways (13–15). Finally, the unfolded protein response, or ER stress pathways are also implicated in the pathogenesis of insulin resistance. This response is initiated with the disassociation of immunoglobulin heavy-chain binding protein (BiP) from key mediators of a coordinated ER stress pathway, dsRNA-activated kinase-like ER kinase (PERK), activating transcription factor (ATF) 6, and inositol requiring ER to nucleus signal kinase (IRE) 1α. The latter has been reported to impair insulin signaling by activation of JNK (14, 16). Although animal studies have supported each of these hypotheses, few studies have examined these potential mechanisms in a comprehensive fashion in humans. Therefore, whether these same mechanisms translate to humans with NAFLD is unknown.

To determine whether any of these putative mechanisms for insulin resistance translate to humans, we assessed these potential pathways in liver tissue obtained from nondiabetic obese individuals undergoing bariatric surgery. Under these conditions, fresh liver biopsies could safely be obtained in sufficient quantities to determine the potential hepatic cellular and molecular changes that relate to insulin resistance in humans.

Results

Participant Characteristics. We studied 37 obese, nondiabetic (hemoglobin $A_{1C} < 6.5\%$) subjects (Table 1). As an aggregate, these subjects were insulin-resistant, which was assessed by the homeostatic model assessment of insulin resistance index (HOMA-IR; 4.6 ± 2.2 mg/dL × μU/mL; normal < 2.0 mg/dL × μU/mL) (17). However, the individuals within this cohort had a large range of values (1.4–9.3 mg/dL × μU/mL), showing that some remain insulin-sensitive despite being morbidly obese. The analyses that we performed sought to understand what factors best predicted the variation of the insulin resistance in these individuals.

Hepatic DAG Content and Insulin Resistance. As in subjects with lesser degrees of obesity, there was a positive but relatively weak association ($R = 0.39$) between body mass index (BMI) and HOMA-IR (Fig. 1*A*) (18). Using flash-frozen liver specimens, we comprehensively assessed whether changes in lipid species, activation of the unfolded protein response, or systemic or tissue inflammation could better account for insulin resistance in this cohort. The intrahepatic concentrations of long-chain fatty acyl-CoAs (LCCoAs) or ceramides did not relate to HOMA-IR (Fig. 1 *E* and *F*). In contrast, hepatic DAG content was found to be strongly and positively correlated with HOMA-IR ($R = 0.73$, $P < 0.001$) (Fig. 1*B*). DAGs are present as constituent lipids within either the plasma membrane or cytosolic lipid droplets (19). Liver samples were separated into membrane and cytosolic lipid droplet compartments, and DAG was quantified again. Surprisingly, the DAG content in lipid droplets correlated with HOMA-IR strongly ($R = 0.80$, $P < 0.001$) (Fig. 1*C*), and the correlation was stronger than either membrane DAG or total DAG content (Fig. 1 *B* and *D*). The relationship between DAG content in lipid

Author contributions: N.K., K.F.P., V.T.S., and G.I.S. designed research; N.K., D.M.E., D.Z., M.K., S.A.B., X.C., C.D.S., G.S.G., and X.H. performed research; X.C., C.D.S., G.S.G., X.H., and J.D. contributed new reagents/analytic tools; N.K., D.M.E., D.Z., M.K., S.A.B., C.D.S., G.S.G., X.H., J.D., K.F.P., V.T.S., and G.I.S. analyzed data; and N.K., G.S.G., K.F.P., V.T.S., and G.I.S. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

[1]To whom correspondence may be addressed. E-mail: varman.samuel@yale.edu or gerald.shulman@yale.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1113359108/-/DCSupplemental.

ARTICLE

doi:10.1038/nature10809

Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity

Jorge Henao-Mejia[1]*, Eran Elinav[1]*, Chengcheng Jin[1,2]*, Liming Hao[3], Wajahat Z. Mehal[4], Till Strowig[1], Christoph A. Thaiss[1], Andrew L. Kau[5,6], Stephanie C. Eisenbarth[7], Michael J. Jurczak[4], Joao-Paulo Camporez[4], Gerald I. Shulman[4,8], Jeffrey I. Gordon[5], Hal M. Hoffman[9] & Richard A. Flavell[1,8]

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome and the leading cause of chronic liver disease in the Western world. Twenty per cent of NAFLD individuals develop chronic hepatic inflammation (non-alcoholic steatohepatitis, NASH) associated with cirrhosis, portal hypertension and hepatocellular carcinoma, yet the causes of progression from NAFLD to NASH remain obscure. Here, we show that the NLRP6 and NLRP3 inflammasomes and the effector protein IL-18 negatively regulate NAFLD/NASH progression, as well as multiple aspects of metabolic syndrome via modulation of the gut microbiota. Different mouse models reveal that inflammasome-deficiency-associated changes in the configuration of the gut microbiota are associated with exacerbated hepatic steatosis and inflammation through influx of TLR4 and TLR9 agonists into the portal circulation, leading to enhanced hepatic tumour-necrosis factor (TNF)-α expression that drives NASH progression. Furthermore, co-housing of inflammasome-deficient mice with wild-type mice results in exacerbation of hepatic steatosis and obesity. Thus, altered interactions between the gut microbiota and the host, produced by defective NLRP3 and NLRP6 inflammasome sensing, may govern the rate of progression of multiple metabolic syndrome-associated abnormalities, highlighting the central role of the microbiota in the pathogenesis of heretofore seemingly unrelated systemic auto-inflammatory and metabolic disorders.

The prevalence of non-alcoholic fatty liver disease (NAFLD) ranges from 20–30% in the general population and up to 75–100% in obese individuals[1,2]. NAFLD is considered one of the manifestations of metabolic syndrome[3]. Whereas most patients with NAFLD remain asymptomatic, 20% progress to develop chronic hepatic inflammation (non-alcoholic steatohepatitis, NASH), which in turn can lead to cirrhosis, portal hypertension, hepatocellular carcinoma and increased mortality[4–6]. Despite its high prevalence, factors leading to progression from NAFLD to NASH remain poorly understood and no treatment has proven effective[7,8].

A "two hit" mechanism is proposed to drive NAFLD/NASH pathogenesis[9]. The first hit, hepatic steatosis, is closely associated with lipotoxicity-induced mitochondrial abnormalities that sensitize the liver to additional pro-inflammatory insults. These second hits include enhanced lipid peroxidation and increased generation of reactive oxygen species (ROS)[10]. Inflammasomes are cytoplasmic multi-protein complexes composed of one of several NLR and PYHIN proteins, including NLRP1, NLRP3, NLRC4 and AIM2. Inflammasomes are sensors of endogenous or exogenous pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)[11] that govern cleavage of effector pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18 (refs 12, 13). Most DAMPs trigger the generation of ROS, which are known to activate the NLRP3 inflammasome[14]. Therefore, we propose that inflammasome-dependent processing of IL-1β and IL-18 may have an important role in the progression of NAFLD.

Results

Feeding adult mice a methionine-choline-deficient diet (MCDD) for 4 weeks beginning at 8 weeks of age induces several features of human NASH, including hepatic steatosis, inflammatory cell infiltration and ultimately fibrosis[15]. To investigate the role of inflammasomes in NASH progression, we fed MCDD to C57Bl/6 wild type (NCI), apoptosis-associated speck-like protein containing a CARD ($Asc^{-/-}$, also known as *Pycard*) and caspase 1 ($Casp1^{-/-}$) mutant mice to induce early liver damage in the absence of fibrosis (Fig. 1a–d and Supplementary Fig. 1c). Compared to wild-type animals, age- and gender-matched $Asc^{-/-}$ and $Casp1^{-/-}$ mice that were fed MCDD were characterized by significantly higher serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, by enhanced microvesicular and macrovesicular hepatic steatosis, and by accumulation of multiple immune subsets in the liver from the innate and adaptive arms of the immune system (as defined by pathological examination and flow cytometry; $n = 7$–11 mice per group; Fig. 1a–d and Supplementary Figs 1c, 2a). Remarkably, the hepatic accumulation of T and B cells seems to be dispensable for this phenotype because $Asc^{-/-}$ mice lacking adaptive immune cells ($Asc^{-/-}$; $Rag^{-/-}$) also showed more severe NASH compared to wild-type animals, and comparable degrees of pathology to $Asc^{-/-}$ animals (Supplementary Fig. 2b–d).

To test whether the increased NASH observed in *Asc*- and *Casp1*-deficient mice was mediated by IL-1β or IL-18, we performed similar experiments using mice deficient in either the IL-1 receptor ($Il1r^{-/-}$)

[1]Department of Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520, USA. [2]Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06520, USA. [3]Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06520, USA. [4]Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA. [5]Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St Louis, Missouri 63108, USA. [6]Division of Allergy and Immunology, Department of Internal Medicine, Washington University School of Medicine, St Louis, Missouri 63108, USA. [7]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA. [8]Howard Hughes Medical Institute, Chevy Chase, Maryland 20815, USA. [9]Department of Pediatrics, Rady Children's Hospital San Diego, University of California at San Diego, La Jolla, California 92093, USA.
*These authors contributed equally to this work.

Effect of Pramlintide on Prandial Glycemic Excursions During Closed-Loop Control in Adolescents and Young Adults With Type 1 Diabetes

Stuart A. Weinzimer, MD[1]
Jennifer L. Sherr, MD[1]
Eda Cengiz, MD[1]
Grace Kim, MD[1]
Jessica L. Ruiz[1]
Lori Carria, BS[1]
Gayane Voskanyan, PHD[2]
Anirban Roy, PHD[2]
William V. Tamborlane, MD[1]

OBJECTIVE—Even under closed-loop (CL) conditions, meal-related blood glucose (BG) excursions frequently exceed target levels as a result of delays in absorption of insulin from the subcutaneous site of infusion. We hypothesized that delaying gastric emptying with preprandial injections of pramlintide would improve postprandial glycemia by allowing a better match between carbohydrate and insulin absorptions.

RESEARCH DESIGN AND METHODS—Eight subjects (4 female; age, 15–28 years; A1C, 7.5 ± 0.7%) were studied for 48 h on a CL insulin-delivery system with a proportional integral derivative algorithm with insulin feedback: 24 h on CL control alone (CL) and 24 h on CL control plus 30-μg premeal injections of pramlintide (CLP). Target glucose was set at 120 mg/dL; timing and contents of meals were identical on both study days. No premeal manual boluses were given. Differences in reference BG excursions, defined as the incremental glucose rise from premeal to peak, were compared between conditions for each meal.

RESULTS—CLP was associated with overall delayed time to peak BG (2.5 ± 0.9 vs. 1.5 ± 0.5 h; $P < 0.0001$) and reduced magnitude of glycemic excursion (88 ± 42 vs. 113 ± 32 mg/dL; $P = 0.006$) compared with CL alone. Pramlintide effects on glycemic excursions were particularly evident at lunch and dinner, in association with higher premeal insulin concentrations at those mealtimes.

CONCLUSIONS—Pramlintide delayed the time to peak postprandial BG and reduced the magnitude of prandial BG excursions. Beneficial effects of pramlintide on CL may in part be related to higher premeal insulin levels at lunch and dinner compared with breakfast.

Diabetes Care 35:1994–1999, 2012

External closed-loop (CL) artificial pancreas systems, consisting of insulin infusion pumps, transcutaneous continuous glucose sensors, and controller algorithms to regulate the rate of insulin delivery automatically, have emerged as one of the most promising technologies for the care of people with type 1 diabetes. Short-term, inpatient studies have demonstrated the general feasibility of the CL approach, especially in achieving safe and effective night-time glucose control (1–7). Performance of CL systems for mealtime control has proved more difficult, however, in great part because of the limitations imposed by use of the subcutaneous route of insulin delivery. Systems that respond to meals only when sensor glucose levels begin to rise are inevitably associated with delays in insulin absorption and action and result in exaggerated increases in glucose levels immediately after meals, as well as a tendency toward hypoglycemia in the late postprandial period. Approaches that attempt to anticipate the increased demand for mealtime insulin by giving premeal priming doses of insulin may succeed in reducing prandial glucose excursions (2,5) but require manual inputs, thereby detracting from the goal of a fully autonomous system.

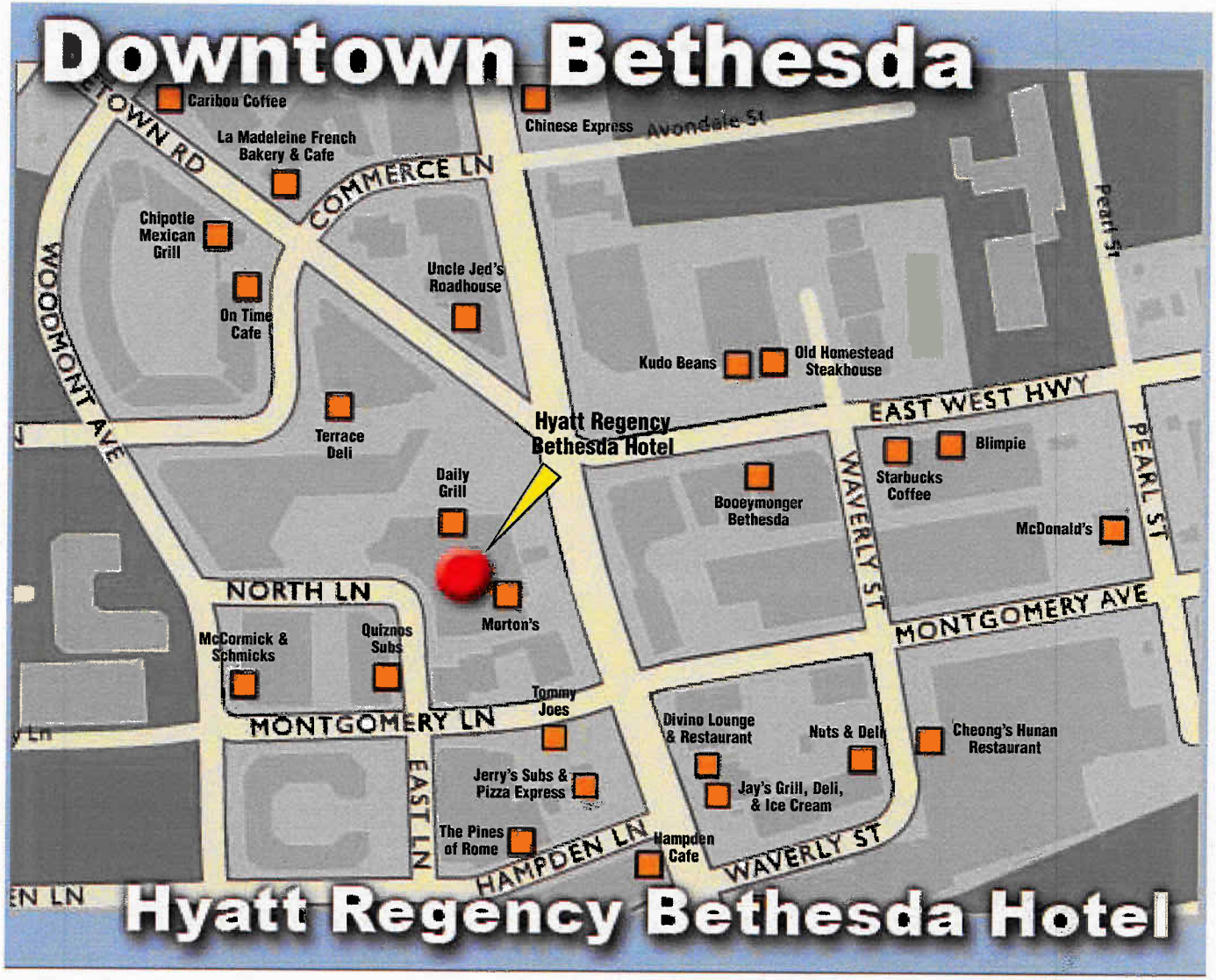
To improve the performance of a CL system around mealtimes, a potential alternative to accelerating insulin appearance would be to delay carbohydrate appearance. This strategy would theoretically slow the gastrointestinal carbohydrate absorption and allow the system to deliver insulin, with its slower absorption characteristics, with a more optimal timing. Pramlintide, an analog of the naturally occurring β-cell peptide amylin, has been introduced as an adjunct to insulin in patients with type 1 diabetes. It has been shown to be effective in reducing postprandial glucose excursions and A1C levels in patients with type 1 and type 2 diabetes (8–15), presumably through the mechanism of delaying gastric emptying and slowing carbohydrate appearance (16,17). An additional mechanism may involve lowering of plasma glucagon levels (18–20). Preliminary studies have suggested similar mechanisms (21–24) and efficacy (25) in adolescents with type 1 diabetes. We hypothesized that the ability of pramlintide to delay gastric emptying and slow carbohydrate appearance would improve the performance of an external CL system in controlling meal-stimulated glucose excursions by enabling a better match between carbohydrate and insulin absorption.

RESEARCH DESIGN AND METHODS

Study subjects and enrollment

Eleven subjects meeting the following enrollment criteria were recruited from the Yale Type 1 Diabetes Program and local advertising: age, 15–30 years; clinical diagnosis of type 1 diabetes of at least 1 year's duration; current use of insulin pump

From the [1]Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut; and [2]Medtronic Diabetes, Northridge, California.
Corresponding author: Stuart A. Weinzimer, stuart.weinzimer@yale.edu.
Received 15 February 2012 and accepted 23 April 2012.
DOI: 10.2337/dc12-0330
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc12-0330/-/DC1.


Downtown Bethesda
Caribou Coffee
La Madeleine French Bakery & Cafe
Chinese Express
Avondale St
COMMERCE LN
WOODMONT AVE
Chipotle Mexican Grill
On Time Cafe
Uncle Jed's Roadhouse
Kudo Beans
Old Homestead Steakhouse
Pearl St
EAST WEST HWY
Terrace Deli
Hyatt Regency Bethesda Hotel
Daily Grill
Booeymonger Bethesda
Blimpie
Starbucks Coffee
McDonald's
WAVERLY ST
PEARL ST
NORTH LN
Morton's
McCormick & Schmicks
Quiznos Subs
MONTGOMERY AVE
Tommy Joes
MONTGOMERY LN
Divino Lounge & Restaurant
Nats & Deli
Cheong's Hunan Restaurant
Jerry's Subs & Pizza Express
Jay's Grill, Deli, & Ice Cream
EAST LN
The Pines of Rome
HAMPDEN LN
Hampden Cafe
WAVERLY ST
Hyatt Regency Bethesda Hotel