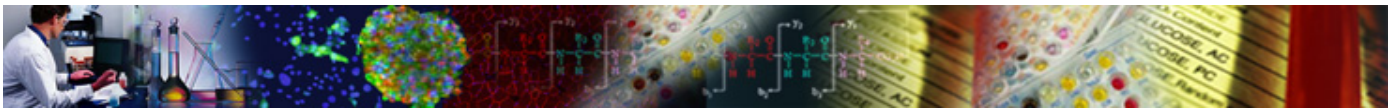
DIABETES RESEARCH CENTERS



2011 Meeting of the Diabetes Centers' Directors

November 9, 2011

Hyatt Regency Bethesda

Bethesda, MD

DIABETES RESEARCH CENTERS

2011 Meeting of the Diabetes Centers' Directors

Table of Contents

19. Diabetes Research Center Featured Publications 2010-2011
    a. Albert Einstein College of Medicine
    b. Baltimore Area (JHU/UMD)
    c. Baylor College of Medicine
    d. Boston Area
    e. Columbia University
    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

Agenda
2011 Diabetes Center Directors' Meeting
November 9, 2011

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

7:30 – 8:00 am	Registration and Light Refreshments
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)
9:00 – 9:15 am	Report from the Diabetes Centers Executive Committee (J. Schaffer)
9:15 – 9:45 am	NIDDK Summer Medical Student Program: report (A. Powers)
9:45 - 10:00 am	Break
10:00 – 10:30 am	Fogarty International Center: global diabetes (Y. Njage, FIC)
10:30 – 11:15 am	Global Diabetes Initiatives at Diabetes Centers (W. Herman, L. Chan, M. Hawkins)
11:15 – 11:30 am	Institutional Diabetes Center Websites – commonalities & diversities (P. Cohen)
11:30 – 12:00 pm	NIH/NIDDK Resources: MMPC update (M. Laughlin)
12:00 – 12:30 pm	NIDDK R24 Collaborative, Interdisciplinary Team Science (P. Smith)
12:30 – 1:30 pm	Lunch (on your own)
1:30 – 2:00 pm	Update: Centers for Diabetes Translation Research (C. Hunter)
2:00 – 2:15 pm	T1D Special Funding: Behavioral Scientists & Bioengineers (C. Hunter & A. Castle)
2:15 – 2:30 pm	Common Fund RFAs: Metabolomics (A. Castle)
2:30 – 2:45 pm	Diabetes Research Centers website: updates/changes (J. Hyde)
2:45 – 3:00 pm	Annual Diabetes Research Center Progress Reports (J. Hyde)
3:00 – 3:15 pm	Diabetes Research Centers: up-coming RFA (J. Hyde)
3:15 – 3:30 pm	Wrap-up, final comments & adjourn
3:30 pm	Transportation to Airport

DIABETES RESEARCH CENTERS

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DIABETES RESEARCH CENTERS

UPCOMING NIH/NIDDK MEETINGS & WORKSHOPS

Human Tissue for Diabetes Complications Research — December 12-13, 2011
Bethesda, MD
http://www2.niddk.nih.gov/News/Calendar/HumanTissues2011.htm

Rare Syndromic Body Fat Disorders: What Can They Teach Us? — March 1-2, 2012
Bethesda, MD
http://www2.niddk.nih.gov/News/Calendar/BodyFatDisorders2012.htm

Isotope Tracers in Metabolic Research — March 12-16, 2012
Little Rock, AR
http://www.mmpc.org/shared/tracers.aspx

NIDDK Network of Minority Research Investigators — April 19-20, 2012
Bethesda, MD
http://nmri.niddk.nih.gov/workshops/

Bile Acid-Mediated Integration of Metabolism and Liver Disease: Translational Science and Therapeutic Engagement — June 8, 2012
Bethesda, MD
http://www2.niddk.nih.gov/News/Calendar/BileAcid2012.htm

DIABETES RESEARCH CENTERS

Requests for Applications (RFAs)

RFA-DK-11-015: Diabetes Research Centers (P30)
Application Receipt Date: February 29, 2012

RFA-DK-11-019: Function of Type 1 Diabetes Genes (DP3)
Application Receipt Date: February 29, 2012

RFA-DK-11-027 Diabetes Research Training for Behavioral Scientists (T32)
Application Receipt Date(s): March 02, 2012

RFA-DK-11-028 Career Development Programs in Diabetes Research for Behavioral Scientists (K12)
Application Receipt Date(s): March 02, 2012

RFA-DK-11-029 Improving Adherence in Pre-teens, Adolescents & Young Adults with T1D (DP3)
Application Receipt Date(s): March 02, 2012

RFA-DK-11-031: Type 1 Diabetes Mouse Resource (UC4)
Application Receipt Date: March 14, 2012

RFA-DK-11-010: Clinical Trial Planning Grants in Type 1 Diabetes (R34)
Application Receipt Date: March 15, 2012

RFA-DK-11-030: NIDDK Interconnectivity Network Coordinating Unit (U24)
Application Receipt Date: March 15, 2012

RFA-DK-11-025: Causes and Consequences of Cystic Fibrosis Related Diabetes (R01)
Application Receipt Date: March 20, 2012

Ongoing Program Announcements

PAR-11-349: Research Using Subjects From The Type 1 Diabetes TrialNet Natural History Study (Living Biobank) (DP3)

PAR-11-350: Research Using Biosamples From Selected Type 1 Diabetes Clinical Studies (DP3)

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

PAR-09-247: Ancillary Studies to Major Ongoing Clinical Research Studies to Advance Areas of Scientific Interest within the Mission of the NIDDK (R01)

PAR-09-223: NIDDK Small Grants for Clinical Scientists to Promote Diversity in Health-Related Research (R03)

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

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

PAR-09-177: Planning Grants for Translational Research for the Prevention and Control of Diabetes and Obesity (R34)

PAR-09-176: Translational Research for the Prevention and Control of Diabetes and Obesity (R18)

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

PAR-11-043: NIDDK Program Project Applications (P01)

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

DIABETES RESEARCH CENTERS

2011 Diabetes Center Directors' Meeting

Bethesda, MD

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

2011 Diabetes Centers Executive Committee

- **Jean Schaffer, Washington University, Chair**
- Bill Herman, University of Michigan
- Jerry Palmer, U Washington (Council liaison)
- Hassy Cohen, University of California, Los Angeles
- Mimmo Accili, Columbia University
- Larry Chan, Baylor College of Medicine
- Gordon Weir, Joslin Diabetes Center, *Ex officio*

DIABETES RESEARCH CENTERS

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

2011 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

DIABETES RESEARCH CENTERS

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

2011 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)
  - Diabetes Centers Research Cores (2011)
  - Additional materials for presentations at the meeting

DIABETES RESEARCH CENTERS

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

2011 Diabetes Center Directors' Meeting

- Brief Overview of Agenda:
  - 8:00 – 9:45: presentations
  - 9:45 – 10:00: break
  - 10:00 – noon: presentations
  - Noon – 1:00: Lunch (on your own; map in book)
  - 1:00 – 3:30: presentations (light refreshments will be available later in the afternoon)

  - Transportation: see Angela at the registration desk to arrange for cab to airport

DIABETES RESEARCH CENTERS

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Perspectives and Opportunities for Diabetes Research

Judith Fradkin, M.D.
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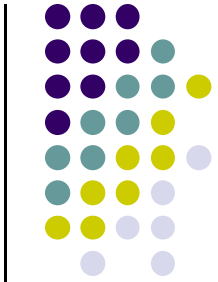
November 9, 2011

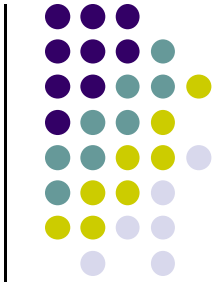
NIDDK | NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Landmark Clinical Studies



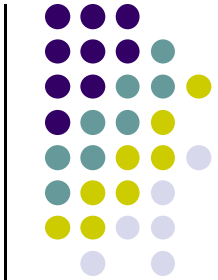
- Diabetes Prevention Program
  - Cost economic analysis
  - Translation through YMCAs
  - Genetics
- DCCT/EDIC
- Discovery of the CF gene yields new therapy

Causes and Consequences of Cystic Fibrosis Related Diabetes (CFRD)



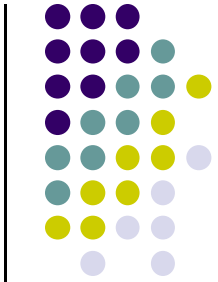
- Improved life expectancy in CF
- Increased incidence of diabetes with aging
- CFRD a distinct form of diabetes
- CFRD associated with poorer outcomes
- Unique diagnostic and therapeutic challenges
- RFA to address gaps in knowledge identified in a May 2011 Workshop (R01)

Clinical Trials



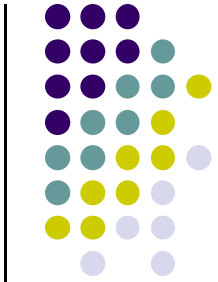
- Recently completed
  - TODAY (treatment options for type 2 diabetes in youth)
  - TINSAL (targeting inflammation using salsalate for glycemic control in type 2 diabetes)
- Mechanism for multicenter trials
  - U34
  - U01
- Planning grants awarded
  - GRADE
  - D2D

Recent Clinical Research Initiatives



- Type 2 diabetes genes in multi-ethnic populations
- Preventing gestational diabetes
- Preserving beta cell function early in the course of type 2 diabetes

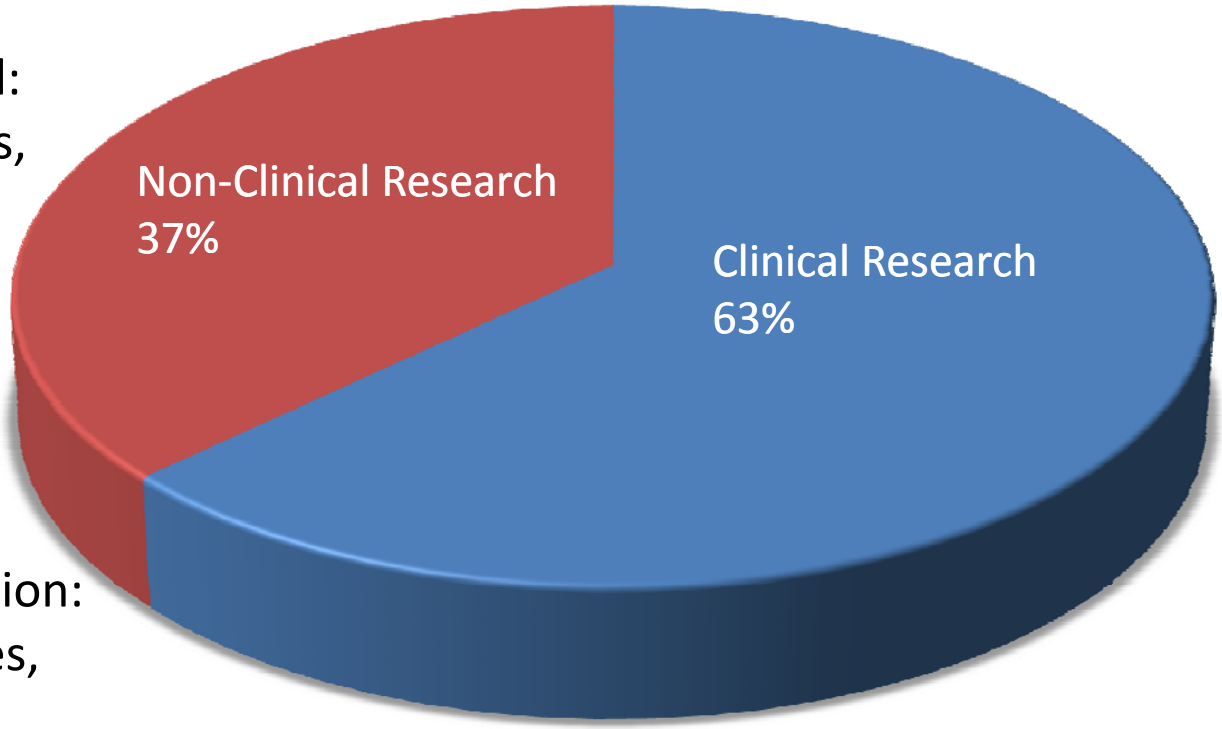
Prioritizing Limited Resources



- Large clinical trials and consortia
- Investigator initiated R01s
- Centers and resources
- New investigators

Special Diabetes Program
Past Allocations

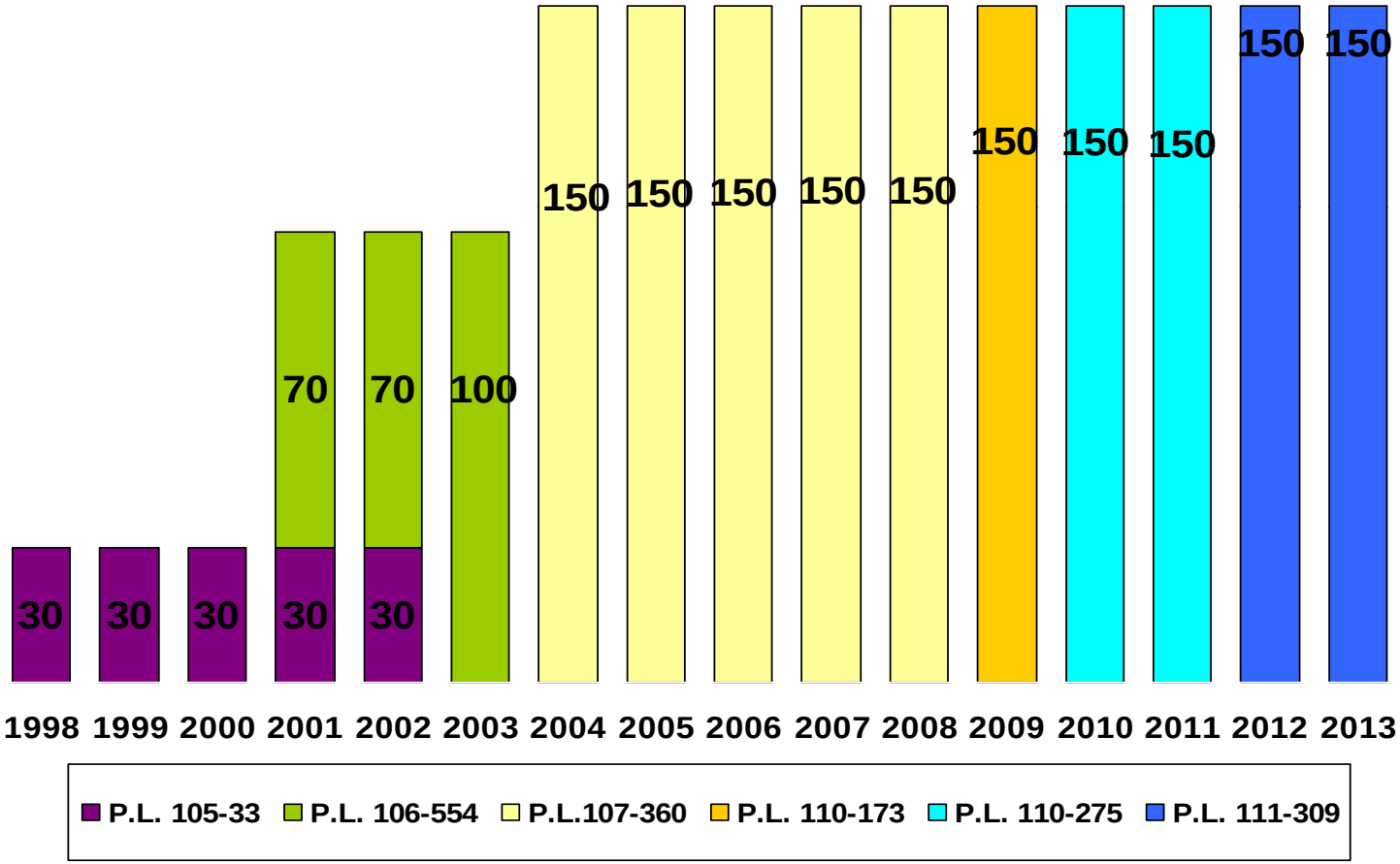
- Translational: Animal Models, BCBC, RAID
- Resources: Islets for basic research
- Standardization: A1C, antibodies, C-peptide



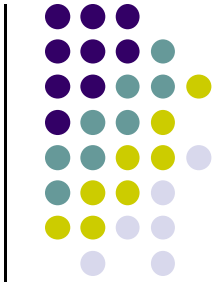
- Genetics
- TEDDY
- SEARCH
- TrialNet
- TRIGR
- CIT
- AP
- DirecNet
- DRCR.net

Since 1998, Special Diabetes Program Funded $1.9 Billion (over 16 years) in Type 1 Diabetes Research

Two year renewal enacted 12/10 extends program through 9/13



Non-traditional Grant Funding Mechanisms



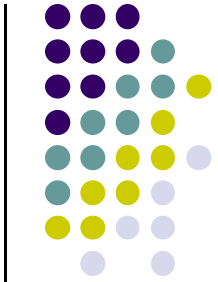
Type 1 Diabetes Targeted Research Award (DP3)

- 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)

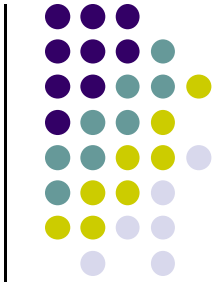
- supports cooperative agreement research projects;
- up to 5 years of research costs are paid in the first FY

FY2011 Initiatives



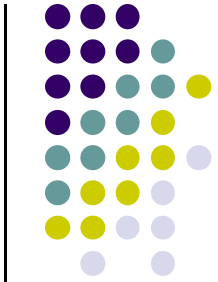
- T1D Impact Award (DP3)
  - $46.7M (9 awards)
- Childhood diabetes research career development (K12)
  - $1.9M (5 awards)
- Feasibility studies for clinical trials (R03)
  - $0.5M (7 awards)

Planning for Use of the Special Funds



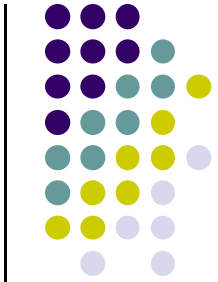
- Collaborative process with other Institutes and Centers at NIH, the CDC, other federal agencies, JDRF, ADA
- Coordinated by the statutory Diabetes Mellitus Interagency Coordinating Committee
- Scientific input from external experts

Process for Initiatives with New Funds



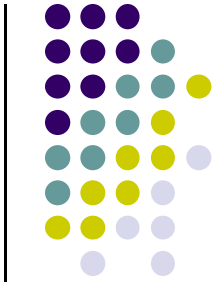
- Solicited proposals from across NIH and CDC
- Workshop May 5-6, 2011To obtain input from scientific and lay experts on future opportunities for research supported in FY12-13 by the *Special Diabetes Program*
- Panel members asked to comment on proposed initiatives and to suggest other ideas for new and emerging opportunities in T1D research that could be pursued with the *Special Funds*.

New Type 1 Diabetes Initiatives—Behavioral Science



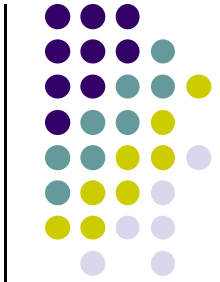
- Improving Adherence in Pre-teens, Adolescents and Young Adults with T1D (DP3)
- Diabetes Research Training for Behavioral Scientists (T32)
- Career Development Programs in Diabetes Research for Behavioral Scientists (K12)

New Type 1 Diabetes Initiatives-- Etiology and Pathogenesis



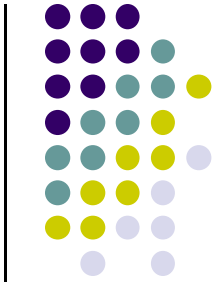
- Research Using Subjects from the Type 1 Diabetes TrialNet Natural History Study (Living Biobank) (DP3)
- Research Using Biosamples from Selected Type 1 Diabetes Clinical Studies (DP3)
- Function of Type 1 Diabetes Genes (DP3)

New Type 1 Diabetes Initiatives- Practical Clinical Trials



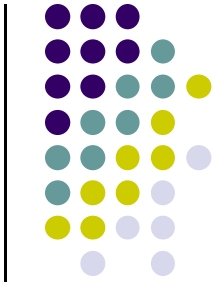
- Planning grant (U34)
- Clinical trial (UC4)

New Type 1 Diabetes Initiatives- Resources



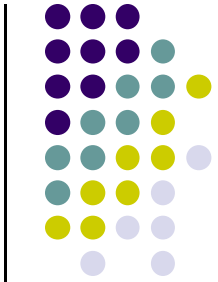
- Type 1 Diabetes Mouse Resource (UC4)
- Human Islets for Research (UC4)

New Type 1 Diabetes Initiatives- Bioengineering and Technology



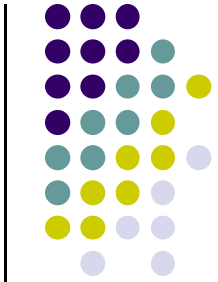
- SBIR
  - New methods and technologies to identify individuals at risk of developing T1D
  - New therapeutics and monitoring technologies for T1D: Toward an Artificial Pancreas
- Training and career development in diabetes research for bioengineers

NIDDK Exploratory Research Grant (R21) Program



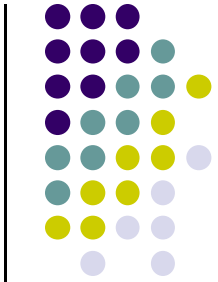
- Per NOT-DK-12-001, NIDDK will no longer co-sponsor the NIH Exploratory/Developmental Research Grant Program (Parent R21) and will not accept any applications (new or resubmission) in response to the Parent R21 Funding Opportunity Announcement
- NIDDK will continue to accept some R21 applications for pilot projects for clinical studies/trials and secondary data analyses

Practical Information



- Electronic submission of administrative supplements
- ARRA awards

Broadening Our Global Health Vision



- One of NIH Director's 5 themes
- United Nations high-level meeting on non-communicable disease prevention and control
- Challenging in a time of limited resources
- What are the unique opportunities for global research in diabetes that can benefit U.S. and global health?

DIABETES RESEARCH CENTERS

Diabetes Centers EXECUTIVE COMMITTEE

2011 Roster

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

Bill Herman, University of Michigan

Hassy Cohen, University of California, Los Angeles

Mimmo Accili, Columbia University

Larry Chan, Baylor College of Medicine

Gordon Weir, Joslin Diabetes Center,
Ex Officio

Jerry Palmer, University of Washington
NIDDK Advisory Council liaison

During 2011 the Diabetes Center Executive Committee included the following Center directors:

Center Director	Program	Lines of communication
Gordon Weir	Joslin	U Mass, BADERC
Bill Herman	Michigan	Chicago, Vanderbilt
Hassy Cohen	UCSD/UCLA	Colorado, UCSF
Jerry Palmer	U Washington	National Council
Jean Schaffer (chair)	Washington U	UAB, Penn
Mimmo Accili	Columbia	Einstein, Yale
Larry Chan	Baylor	JHU/UMD

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

1. Review of Directors' meeting 2010: The meeting was considered successful. A goal for future meetings was to provide all materials electronically to attendees.
2. Agenda for Directors' meeting 2011: A number of potential topics were discussed.
3. Site visits to Universities with multiple NIDDK-funded Centers: The purpose and plans for these visits was discussed.
4. DRC Websites: In an effort led by Hassy Cohen, the Committee solicited information on website design and content from each of the centers and prepared a "best practices" report that will be discussed today.
5. DRC RFA: The most recent RFA was discussed and Center Directors provided feedback regarding changes in requirements for documentation of the research bases.
6. CDTRs: The Directors were kept abreast of developments with the review and selection of the new CDTRs.
7. Annual DRC Progress Report Format: The Directors discussed the new format and agreed with efforts to streamline and create documentation that could be compiled for ease of preparing competitive renewal documents.
8. New Diabetes Centers Program Website: The Directors were alerted to plans for the new site and had an opportunity to view planned changes prior to its launch.

As in the past, the committee will continue to be comprised of those Directors with an interest in participating. The following Directors have agreed to serve on the Executive Committee in 2012:

Center Director	Program
Martin Myers	Michigan
Jeff Pessin	Einstein
Hassy Cohen	UCSD/UCLA
Jerry Palmer	U Washington
Jean Schaffer (chair)	Washington U
Mimmo Acceli	Columbia
Larry Chan	Baylor

Other Directors wishing to volunteer are welcome (contact Jim Hyde or Jean Schaffer).

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 2011

Applications: Year 2011 - 681 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

2011 Program			
Center	# participants	# participants from medical schools associated with a DERC/DRTC	# applicants listing Center as #1, 2, 3 choice
AECOM	4	4	84
Baylor	4	0	58
Boston Area	4	1	51
Columbia	4	3	147
JHopkins/Univ MD	4	0	86
Joslin	6	1	74
UAB	5	5	32
UCLA/UCSD	4	2	109
UCSF	4	1	118
Univ Chicago	4	0	112
Univ Colorado	4	1	57
Univ Michigan	6	1	55
Univ Pennsylvania	4	1	94
Univ Washington	5	0	61
Vanderbilt - NIDDK	6	0	82
Washington Univ	4	0	47
Yale	4	0	57

Vanderbilt - T35 grant	25	6	-

Student Demographics	
Race	
African American	9
American Indian	0
Asian	28
Caucasian	31
Hispanic	6
Other/No answer	2
Gender	
Female	38
Male	38
Citizenship	
US Citizens	74
Permanent Res.	2

Medical Schools of Students Participating in the
2011 NIDDK Medical Student Research Program in Diabetes

Albert Einstein College of Medicine
Boston University
Brown University
Case Western Reserve University
Chicago Med Sch, Rosalind Franklin Univ
Columbia University
Eastern Virginia Medical School
Florida State University
Georgetown University
Howard University
Indiana University
Medical College of Georgia
Medical College of Wisconsin
Meharry Medical College
Michigan State University
New York Medical College
Northeastern Ohio Universities
Pennsylvania State University
Rush Medical College
St. Louis University
Stanford University
SUNY Downstate
Texas Tech Univ Health Sci Ctr, Lubbock
Texas Tech Univ Health Sci Ctr, El Paso
UMDNJ – New Jersey Medical School
UMDNJ – RW Johnson Medical School
University of Alabama, Birmingham
University of Arizona
University of Arkansas
University of California, San Diego
University of Cincinnati
University of Colorado
University of Florida
University of Hawaii
University of Illinois, Chicago
University of Illinois, Urbana-Champaign
University of Iowa
University of Michigan
University of Pennsylvania
University of Rochester
University of South Alabama
University of South Florida
University of Tennessee Health Sci Center
University of Texas, Houston
University of Toledo
University of Utah
Vanderbilt University
Washington University
Wayne State University

NIDDK Diabetes Center Medical Student Summer Research Program

Jim Hyde, Ph.D.
Program Director, NIDDK

Reminders

- NIDDK has already awarded T32 supplemental funds for ALL summer 2012 medical student slots; awarded to FY2011 T32 budget periods
- Most FY2011 T32 budget periods are July 1, 2011 through June 30, 2012
- All medical student appointments must be made by the end of the FY2011 budget period for the T32 training grant (June 30, 2012 for most grants).
- All appointments must be for at least 2 months; maximum is 3 months. NIDDK awarded funds to support up to 3 months per student.

Reminders

- Funds for stipends may be carried forward (beyond the end date of annual budget period) without NIDDK approval as long as the initial appointment was made BEFORE the budget period ends.
- Funds for travel and training-related expenses may NOT be carried forward beyond the budget period without prior approval from NIDDK; an e-mail request from an authorized business official to the NIDDK Grants Management Branch is required each year.

Competing Renewal T32 Applications

- Jim will send out an e-mail reminder to each participating T32 PI (cc: Diabetes Center Director) to include a request to add 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?)

Questions?

Fogarty International Center

Global Diabetes

Yvonne Njage, M.D.
Program Officer

Presentation at the 2011 Diabetes Center Directors' Meeting
November 9, 2011

DEPARTMENT OF HEALTH & HUMAN SERVICES USA
NATIONAL INSTITUTES OF HEALTH
FOGARTY INTERNATIONAL CENTER

Overview of presentation

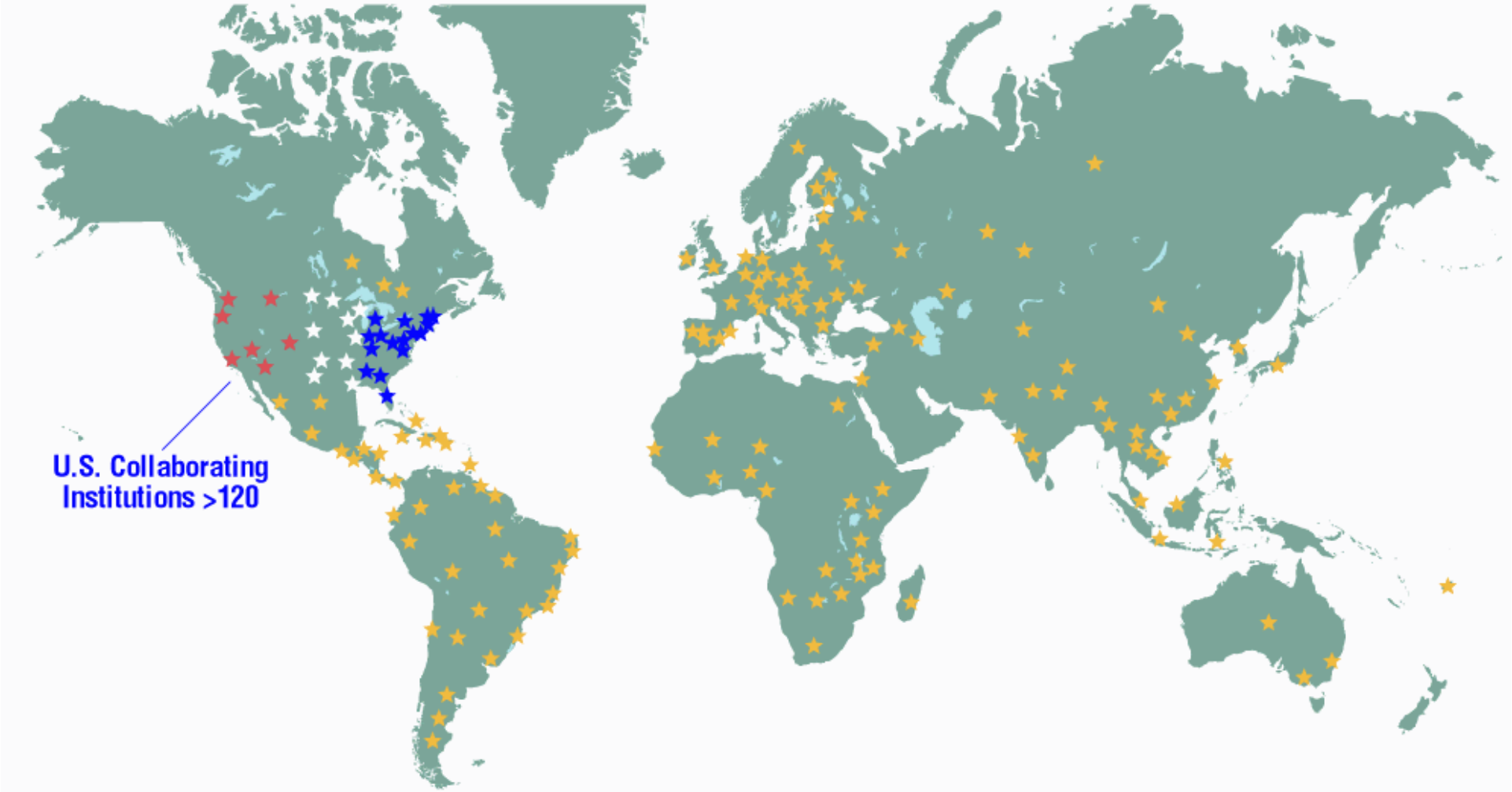
- Introduction to the Fogarty International Center
- Overview of Fogarty Programs
  - Millennium Promise Awards (NCoD)
    NCD-Lifespan program (NCD-L)
  - Framework for Global Health (FRAME)
  - Global Research Initiative Program for New Foreign Investigators (GRIP)
  - Fogarty International Research Collaboration Award (FIRCA)

John E. Fogarty International Center for Advanced Study in the Health Sciences

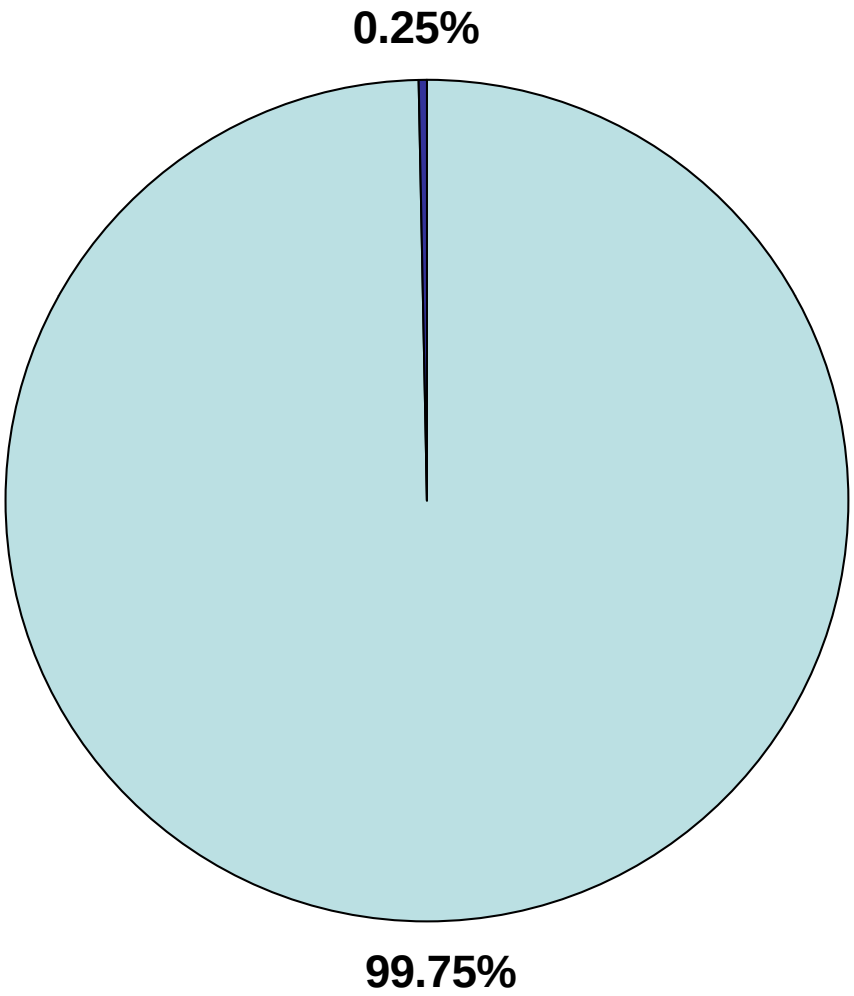
- Established in 1968
- Mission: supporting and facilitating global health research conducted by U.S. and international investigators, building partnerships between institutions, and training the next generation of scientists
- Strategic Plan:
  - Address the growing epidemic of chronic, non-communicable diseases
  - Bridge the implementation research training gap
  - Develop human capital in the developing world
  - Foster a sustainable research environment in low- and middle-income countries
  - Build strategic alliances and funding partnerships

•Fogarty International Center

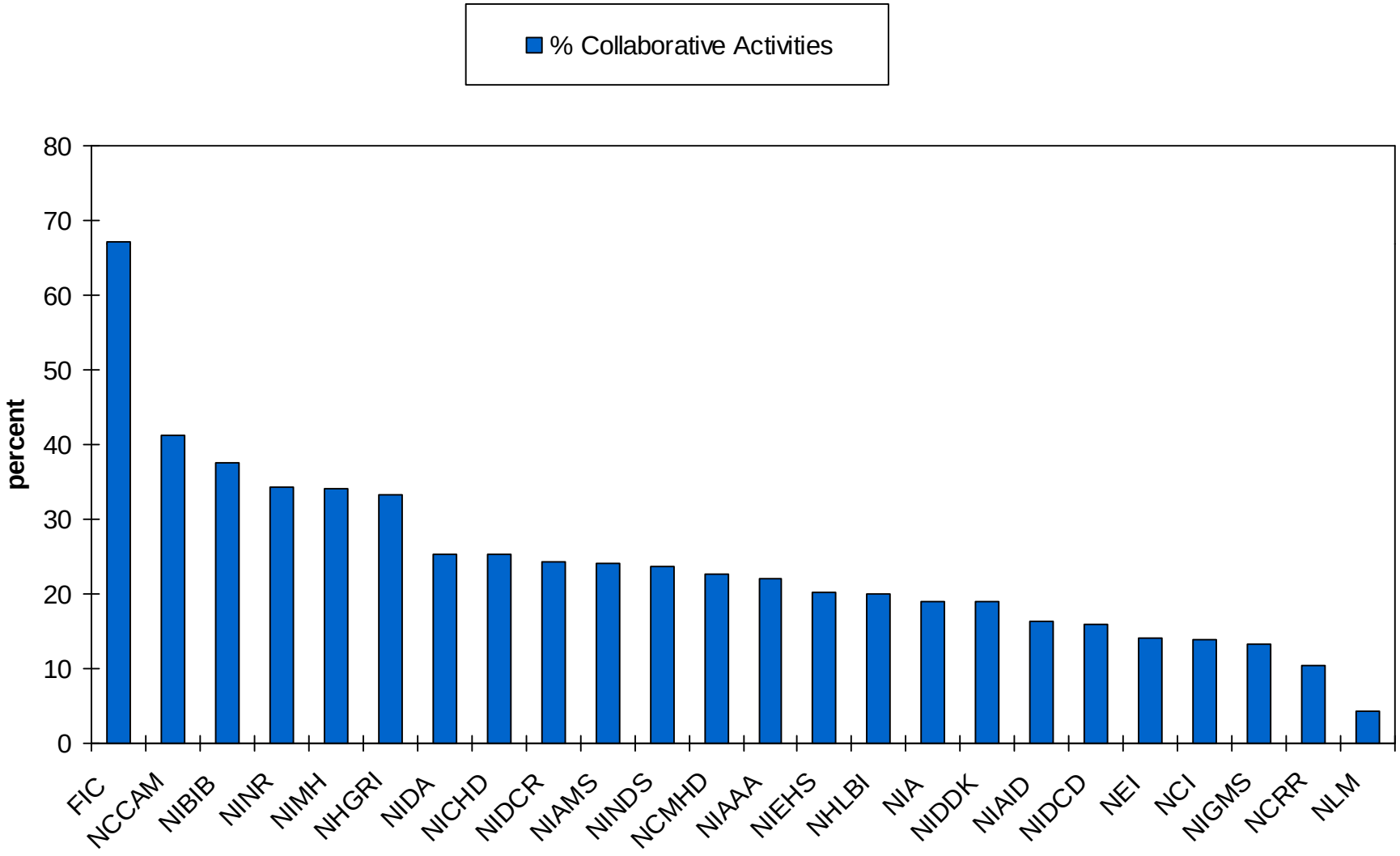
Major Research and Training Sites



Fogarty Intl Center Percent of NIH Budget



NIH Collaborative Activities Report
FY 2009



Guiding Principles of FIC Programs

FOGARTY

- Low- and middle-income countries (LMICs)
- Critical mass of scientists
- Independent & competitive research sites
- Full partnership & collaboration
- Local and national needs
- Long term investment
- Networking
- Leverage
- Funded research base for training

Fogarty Programs

Research Training	Research Programs	Career Development
•NCD-Lifespan	•GRIP	•IRSDA
•AITRP	•FIRCA	•ISGHA
•GID	•Tobacco	•Scholars and Fellows
•Informatics	•Brain disorders	
•Bioethics		
•MEPI		
•Framework		
•Trauma & injury		

For further information, please visit:

http://www.fic.nih.gov/Programs/Pages/default.aspx

Millennium Promise Awards

D43 training grant mechanism

- Goal: build research capacity in LMICs in non-communicable diseases (NCDs)
- Research training program with a goal of training a cadre of experts who can study the multi-disciplinary nature of NCDs and translate research into public health policy and into programs of care
- Focus on chronic, non-communicable diseases
- Has now been incorporated into the NCD-Lifespan program

Millennium promise: Examples

Interdisciplinary research training of NCD epidemiology and prevention in India

- Trainee projects include:
  - Vitamin supplementation and its effects on insulin resistance and beta cell function
  - Optimal treatment of gestational diabetes
  - Factors contributing to childhood obesity and subsequent development of type 2 diabetes
  - Public school policies and practices related to nutrition and physical activity
  - Integration of comprehensive diabetes control in the public health system

Millennium Promise: Examples

Building the Asian NCoD Research Network for Regional Research Capacity

- Trainees from India, Sri Lanka, and Malaysia
- Trainee projects include:
  - Interventions to support physical activity among women
  - Physical activity and peer support effects on glycemic level and medication adherence
  - Lifestyle modification among pre-diabetics
  - Dietary interventions among patients with type 2 diabetes
  - Development of QoL measurement scale for type 2 diabetes

NCD- Lifespan

D43 training grant mechanism

- Aim is to build research capacity in low- and middle-income countries (LMICs) in non-communicable diseases by supporting collaborative research training between institutions in the US and LMICs
- Ultimate goal is to implement evidence-based interventions relevant to the LMIC
- Both U.S. and foreign institutions can apply directly
- Full grants provide up to $250,000 per year for up to 5 years
- Planning grants of $27,000 per year for up to 2 years
- Next receipt date: Sept 21, 2012

NCD-Lifespan: Example

Nutrition-related NCD prevention training in China

- Goal is to train a new generation of scientists in large-scale program and policy research related to the prevention of obesity, diabetes, hypertension, and the related cardio-metabolic problems in China.
- They propose to:
  - Work with the government of China as well as US CDC on the research side of salt-reduction
  - Utilize US experts in nutrition, anthropology, economics, genetics, geography, health economics, mass communication, and urban planning in training related to macro policy research and implementation
  - Enhance current research monitoring dietary changes and the key policy factors impacting them

NCD-Lifespan: Example

Strengthening Indian NCD clinical research and training capacity

- Builds on long-standing ICOHRTA funded program at the Madras Diabetes Research Foundation
- Trainee work has included:
  - Prevalence of DM and obesity among urban school children
  - Prevalence of DM, obesity, and metabolic syndrome among psychiatric patients
  - Determining whether gene variants identified in other populations are assoc with T2D in a South Indian Population
- National impact:
  - Facilitated changes that led to the National Program on Prevention & Control of DM, CVD, and Stroke (NPDCS)
  - Facilitated conduct of India's largest ongoing epidemiologic study of DM prevalence – The Indian Council of Medical Research India Diabetes (ICMR-INDIAB) Study

Framework Programs for Global Health

D43 mechanism – research training grant

- Goal is to build capacity within U.S. and LMIC institutions to develop broadly interdisciplinary, post-doctoral research training programs in global health directed towards encouraging innovation in health-related products, processes, and policies
- Applications must include participation by at least three schools, departments, or entities of distinct disciplines
- Awards are for up to $350,000 total costs per year for up to 5 years
- Receipt date: Dec 15, 2011; Dec 14, 2012

GRIP awards

Global Research Initiative Program for New Foreign Investigators

R01 research grant mechanism

- New, young investigators
  - Trained under a Fogarty D43 program (research training program), other NIH training award, completed within 4 years, and have at least 2 years of research training experience.
- Only foreign institutions can apply
- Up to $50,000 per year for up to 5 years, Plus facilities and administrative (F&A) costs up to 8% for a foreign institution
- Receipt date:
  - Non-AIDS: Jan 10, 2012; Jan 10, 2013
  - AIDS applications: March 9, 2012; March 8, 2012

GRIP: Grantee example

Weight, Diet, Genes, and CVD Risk Factors (Hypertension and Diabetes)

- Study aims to understand how weight history, dietary patterns, and genetic variants independently and jointly affect blood pressure and fasting glucose among adult Filipino women using an ongoing community-based study of over 2000 women which begun in 1983
- Specifically, the PI will determine the:
  - Effect of weight history on risk of having HTN and/or DM
  - Assoc between dietary patterns and HTN and/or DM
  - Independence and co-occurrence of HTN and DM
  - Effects of genetic variants on HTN and DM

FIRCA

Fogarty International Research Collaboration Award

R03 mechanism – small research grant

- Goal is to support collaboration between a U.S. investigator with NIH research funds and a foreign collaborator from a LMIC to help build research capacity
- Applicants must either be an NIH-funded investigator holding an R01, P01, U01, R21, or R03; or a previous LMIC collaborator on an awarded FIRCA
- Awards up to $50,000 in direct costs for up to 3 years
- Next receipt dates: Jan 10, 2012; Jan 10, 2013

FIRCA: Examples

Social and Neighborhood Predictors of Obesity in Belo Horizonte

- Goal is to investigate the role of socioeconomic and neighborhood factors in shaping the distribution of obesity and the related behaviors of diet and physical activity in a large city in Brazil
- Data used is from a large population-based survey of the city

FIRCA: Example

Substituting brown rice for white rice: effect on diabetes risk factors in India

- Goal is to evaluate the efficacy of substituting brown rice for white rice in Chennai. Specifically:
  - Determine the glycemic index of different local rice varieties (brown, red, and fully polished white)
  - Determine the effects of brown rice substitution on fasting biomarker measurements of glucose metabolism (glucose, insulin, HbA1c), dyslipidemia, and inflammation (CRP)
  - Demonstrate the feasibility and cultural appropriateness of this type of intervention in the local environment

FOGARTY

Questions?

All Fogarty program information can be found at http://www.fic.nih.gov/Programs/Pages/default.aspx

Research training awards

- NCD-Lifespan program: The Chronic, Non-Communicable Diseases and Disorders Across the Lifespan: Fogarty International Research Training Award program will support collaborative research training between institutions in the U.S. and low-and middle-income countries (LMIC), defined by the World Bank classification system. The proposed institutional research training program is expected to sustainably strengthen the research capacity of the LMIC institutions, and to train in-country experts to conduct research on chronic, non-communicable diseases and disorders, with the ultimate goal of implementing evidence-based interventions relevant to their countries.

- Framework for Global Health: Framework Programs for Global Health Innovation (FRAME Innovation) will provide support to institutions in the U.S. and in low- and middle-income countries (LMICs) to build capacity within their institutions to develop broadly interdisciplinary, postdoctoral (or post-terminal degree) research training programs in global health directed towards encouraging innovation in health-related products, processes and policies.

Research grants

- FIRCA: The Fogarty International Research Collaboration Award (FIRCA) program fosters international research partnerships between NIH-supported scientists and their collaborators in low- and middle-income countries (LMIC).

- Brain disorders program: The purpose of the program is to develop collaborative research and capacity building projects on brain disorders throughout life, relevant to low- and middle-income countries.

Career development awards

- IRSDA K01 award: The purpose of the award is to provide junior U.S. scientists with an opportunity pursue careers in research on global health, and to prepare them for independent research careers. This award is similar to other NIH K01 career development awards, but requires grantees to spend 50% of the grant period conducting research in developing countries.

- ISGHA K02 award: The overall objective of the Independent Scientist in Global Health Award is to foster the development of outstanding independent scientists and enable them to expand their potential to make significant impact on the health related research needs of developing countries. This award provides three, four, or five years of salary and some research support. This award is similar to other NIH K02 career development awards, but requires grantees to spend 50% of the grant period conducting research in developing countries.

- Fulbright-Fogarty Fellowships in Public Health: Fulbright-Fogarty Fellowships promote the expansion of research in public health and clinical research in resource-limited settings. The Fellowships inaugurated in July 2011 with four fellows in sub-Saharan Africa (Botswana, Malawi and South Africa), and have been expanded for the 2012-2013 application cycle to include countries in Asia and Latin America.

Participation of BADERC investigators in Global Diabetes-related activities.

David M. Nathan M.D.

1. A1c Derived Average Glucose (ADAG) Research Group. Dr. Nathan serves as PI and chair of this international research group (US, Europe and Africa) that established the relationship between mean glucose levels and A1c. The results of this study serve as the basis for all estimated average glucose calculations world-wide. Ongoing analyses of the study data have resulted in ~ 3 papers published per year. Funded by research grants from the ADA and EASD.

2. MAGIC Consortium. PI of one of the contributing research groups. Explores the genetics of diabetes and glycemia.

3. International Expert Committee on the Diagnosis of Diabetes. This international group of diabetes investigators (North America, Europe, Asia, Africa and Australia), which I chair, has updated the diagnostic criteria for diabetes, adding HbA1c as a diagnostic tool in 2010. The Committee is sponsored by the ADA, EASD and IDF.

4. Consensus Committee on the Treatment of Type 2 Diabetes. This international committee (US, Canada, and Europe), which I have chaired since its inception in 2005, has established the algorithm for the treatment of type 2 diabetes. Funded by ADA and EASD.

David Altshuler MD PhD

1. Dr. Altshuler is a co-PI on an NIH-funded program to study diabetes genetics in multiethnic samples (many of which are from around the world.) It is called "Multiethnic Study of Type 2 Diabetes Genes ", and was funded in response to an NIH RFA.
2. A member of the MAGIC consortium, an international consortium that studies the genetics of continuous glycemic traits.

Jose Florez M.D. Ph.D.

1. An investigator on the UO1 "Multiethnic Study of Type 2 Diabetes Genes."
2. A cofounder of the MAGIC consortium, an international consortium that studies the genetics of continuous glycemic traits.

Anthony Rosenzweig M.D.

Dr. Rosenzweig is currently the American Coordinator for a Leducq Foundation Network of Research Excellence that is investigating mechanisms contributing to the intersection of diabetes/metabolic diseases with heart failure. The consortium includes 11 laboratories spread out between Europe and US.

Lee M. Kaplan M.D. Ph.D

Dr. Kaplan has been involved in an international initiative to the study of bariatric surgery for the treatment of type 2 diabetes. This work has included organizing and

participating in several scientific and clinical meetings (Diabetes Surgery Summit in Rome, 2007; 1st and 2nd World Congresses on Interventional Therapies for Type 2 Diabetes in New York, 2008, 2011) and has contributed to the recently revised International Diabetes Federation recommendations on the study and use of surgery for the treatment of diabetes. These efforts have involved clinicians and investigators from Europe, Australia, Asia, the Middle East and North and South America.

Barbara B. Kahn M.D.

Dr. Kahn is working with is the "European Network on Functional Genomics of Type 2 Diabetes at www.eugene2.com, interacting with Ulf Smith, immediate past-president of the EASD and a long-time collaborator at the Univ of Gothenberg, and with Markku Laakso at Kuopio University, Finland. This work has resulted several published reports and some presently under review. Dr. Kahn also has active collaborations with investigators at the University of Leipzig and the University of Amsterdam involving Diabetes-related clinical research

Center for Globalization Capacity and the Global Diabetes Initiative

Baylor College of Medicine's (BCM) Center for Globalization was launched in March of 2011 with a $5 million gift from BCM Trustee Wallace S. Wilson. Dr. Bobby Kapur is the director of the Center, which serves as an umbrella for BCM's existing international programs, as well as new global initiatives in education, research and patient care. BCM's global efforts began with Dr. Michael DeBakey's pioneering heart surgeries for patients around the world and have continued to expand to include the Baylor International Pediatric AIDS Initiative (BIPAI), which has extended BCM's global reach over the past decade. More recently BCM has increased its global reach through the creation of the Center for Global Child Health, led by internationally renowned pediatric hematologist, Dr. Russell Ware, and the creation of The National School of Tropical Medicine, the first dedicated school of its kind in the United States, led by the renowned vaccine researcher, Dr. Peter Hotez. In addition, Dr. Hotez is the president of the Sabin Vaccine Institute and the Institute's vaccine development program will a collaborative between Texas Children's Hospital (TCH) and BCM.

BCM's extensive array of international projects provides an excellent base for the Center for Globalization to launch from. BCM is located in the Texas Medical Center, the largest medical center in the world. BCM has, at one time or another, collaborated with and drawn resources from virtually all of the 49 member institutions of the TMC.

Internally, one of the most significant international presences BCM has is through BIPAI. Established in 1996 and led by Dr. Mark Kline, chair of pediatrics at BCM and physician-in-chief at Texas Children's Hospital, BIPAI has grown from an idea to help a group of children in Romania to a network of care centers across Africa serving over 76,000 children and families receive care across the BIPAI network; provided 350,000 outpatient clinic visits, HIV counseling and testing to 130,000 people, and trained 12,000 local health professionals in the past year. BIPAI has Centers of Excellence in Botswana, Kenya, Lesotho, Malawi, Romania, Swaziland, Tanzania and Uganda. There are also International Program Offices in Ethiopia, Kenya, Libya, Mozambique, and Tanzania. Each BIPAI location is adjusted to fit each countries unique social, economic, legal and healthcare landscape. Each site also officially partners with each country's Ministry of Health, and operates under a memorandum of agreement that makes each site a BIPAI affiliated NGO that embraces BCM's standard of care. BIPAI also provides essential training and mentoring to local health care providers, and this platform is often the only pediatric HIV/AIDS specific training infrastructure in that country.

BIPAI's success and governmental support and trust in those countries it operates in have developed a vast network and infrastructure that can be leveraged to create new BIPAI-type initiatives for other diseases and conditions. For example, the Center for Global Child Health at BCM and TCH builds upon that infrastructure and is setting up a screening and treatment program for sickle cell disease in Luanda, Angola.

Inspired by this success of the BIPAI platform with sickle cell, the Center for Globalization has set a priority of initiating a diabetes program in Africa in line with the global diabetes initiative of the NIH.

Rapid urbanization combined with overburdened or inadequate health system and educational infrastructure has led to an alarming increase in the incidence and prevalence of diabetes in the African continent. In addition, studies have shown that non-HIV individuals with diabetes have a three-fold increased risk of being infected with tuberculosis. Therefore, a strategy to decrease diabetes in Africa will have an exponential positive impact on burden of disease in the region. In order to provide screening and detection of diabetes and metabolic syndrome, a network of care is required. Building upon the trust and framework of the BIPAI system, the Center for Globalization hopes to set up vertically based and independently functioning clinics in conjunction with local governments that provide screening and detection, treatment, health systems training/development, and health policy advancement. We believe that this structure would be the best method of approaching the impending diabetes epidemic in Africa.

Albert Einstein College of Medicine
Global Diabetes Initiative (GDI)

Director: Meredith Hawkins, MD, MS

Mission: With an estimated worldwide prevalence of ~347 million (*Lancet* 378:31, 2011), diabetes is clearly a global health crisis. The Global Diabetes Initiative incorporates research and education projects developed by Einstein faculty since 1996, with the following mission statement: *To combat the global diabetes epidemic by harnessing Einstein's strengths in research and education, and by building strategic international partnerships*.

RESEARCH:

The Global Diabetes Initiative is fortunate to be able to build upon Einstein's strong research prowess both in global health and diabetes, including particular strengths in patient-oriented metabolic research and in behavioral research methodologies.

Metabolic Research: Einstein is collaborating with the renowned Christian Medical College (CMC) in Vellore, India to characterize forms of diabetes that are unique to the developing world. This includes studying the pathogenesis and treatment of 'Malnutrition-modulated diabetes mellitus' (MMDM), which has been reported in many low-and-middle income countries, yet is virtually unknown in the medical literature. The *short term goal* is to delineate the metabolic defects that contribute to malnutrition diabetes, using state-of-the-art insulin clamp studies to assess hepatic and peripheral insulin sensitivity, and magnetic resonance spectroscopy to quantify tissue triglyceride. The *long term goal* is to apply these physiologic insights to develop targeted therapeutic approaches. Thus, research capacity building at CMC Vellore is an important feature of this program, to ensure the long-term sustainability of this work. *This research partnership represents a unique opportunity: performing sophisticated metabolic studies to study a condition that is not encountered in developed countries.*

Behavioral Research: This program represents a collaboration between Einstein's GDI and Makerere University School of Medicine, Uganda. The program's objective is to develop, implement and evaluate a behavioral counseling training program for physicians and nurses, to better equip them to educate and motivate their patients for diabetes self-management. A participatory research approach is being used to gain insight into Ugandan beliefs and practices in motivating behavior change for diabetes self-care. The main goal is to adapt evidence-based behavioral programs and communication techniques to be feasible, acceptable and successful with Ugandans to prevent and control diabetes. To date, we have completed two collaborative programs and are jointly developing a hypoglycemia prevention and management teaching poster for use by community health workers and medical providers. Einstein faculty will work with Ugandan colleagues to oversee its implementation, dissemination and evaluation.

EDUCATION:

Overall Objective: Einstein's Global Diabetes Initiative is building comprehensive diabetes training programs for doctors and nurses working in resource-poor settings in the developing world. This is a collaborative effort with the Endocrinology department at CMC Vellore, who have developed a World Diabetes Foundation-funded comprehensive diabetes curriculum consisting of a textbook and workbooks, in-person and teleconference lectures and seminars, written examinations, and 'on site' training and evaluations, that has trained over a hundred hospitals around India. Einstein's GDI is partnering with CMC to adapt their program to other countries, and to evaluate its efficacy in improving both provider knowledge and patient health outcomes. Einstein and CMC are now collaborating to introduce this program into institutions in the following countries: Uganda, Kenya, Rwanda, Ethiopia, Thailand, Nepal, Sri Lanka, Jordan and Cambodia.

Makerere University School of Medicine, Uganda: Einstein's GDI is collaborating with Makerere University to develop, implement, and evaluate a diabetes training program for health care providers throughout Uganda. Diabetes mellitus is a major emerging health care problem in sub-Saharan Africa. Mulago Hospital, a 1500 bed University hospital that annually serves 120,000 outpatients and 478,000 inpatients, is Uganda's largest referral, teaching and research healthcare center. Unfortunately, this hospital lacks a formalized diabetes training program, leaving healthcare providers ill-equipped to deal

with the diabetes epidemic. Einstein is collaborating with Ugandan health care providers and national diabetes leaders to develop, implement, and evaluate a culturally-adapted diabetes educational conference series. The *short term goal* is to create a series of educational conferences as a collaborative effort with Ugandan colleagues. The *long term goal* will be an ongoing, Ugandan-led series of educational conferences about diabetes management and supporting self-care. To date, Einstein's GDI has organized three comprehensive diabetes conferences that have trained almost 200 doctors, nurses, and medical officers both at Mulago Hospital and from around Uganda. In February 2012, Einstein faculty will help lead the second **Ugandan Endocrine Society meeting** in Kampala, Uganda (following up on our successful collaborative meeting in February 2010), which will include both clinical and research presentations.

International Continuing Medical Education Meetings: Since 2005, Einstein faculty have been providing lectures and practical teaching about diabetes management at these annual medical education meetings, which alternate between Kenya and Thailand, and educate doctors from over 40 countries around Africa, Asia and the Middle East. Under GDI leadership, the diabetes portion of the curriculum has expanded substantially to comprise both formal didactive and highly interactive learning sessions, and has incorporated a strong focus on diabetes management in resource-poor settings. In turn, involvement with these meetings has greatly broadened Einstein's "reach" in global diabetes education.

Global Diabetes Training at Einstein: Under the supervision of Einstein faculty, five medical students, two residents and two fellows have organized global diabetes rotations at CMC Vellore, five trainees spent rotations at Mulago Hospital in Uganda, and seven medical students led a diabetes-focused global health outreach to Guatemala. The Einstein trainees helped to develop various programs at the partner institutions, including a peer support group for patients with type 1 diabetes at CMC Vellore, a systematic evaluation of the diabetes training program at CMC, and therapeutic guidelines for the Ugandan Endocrine Society. GDI faculty also provide education about global diabetes management to Internal Medicine residents at Einstein/Montefiore.

ADVOCACY:

Einstein's Global Diabetes Initiative presented a **Congressional Briefing on the Global Diabetes Epidemic** on September 27, 2010 in Washington, DC. Einstein faculty were joined by Dr. Nihal Thomas, Professor of Medicine at Christian Medical College (CMC) Vellore, India, as well as Dr. Paul Robertson, Director of the Global Diabetes Alliance. The briefing was sponsored by Representatives Diana DeGette (Colorado) and Michael Castle (Delaware), co-chairs of the Diabetes Caucus. Attendees represented many Congressional committees including Science and Technology, Appropriations, Foreign Affairs, Education and Labor, International Relations, and African Global Health. There was representation from the American Diabetes Association, the National Institutes for Health, Centers for Disease Control, and New York City Health and Hospitals Corporation. The briefing was well received and the audience very engaged by the topic, reflecting growing concern for this health crisis.

On September 18, 2011, Einstein's Global Health Center and the International Diabetes Federation will be sponsoring a symposium titled "**Global Diabetes Symposium: Finding the Way to Global Action"** in midtown Manhattan, immediately preceding the United Nations Summit on Non-Communicable Diseases (http://www.einstein.yu.edu/gds). The goal of the symposium is bring together 'thought leaders' from academic institutions, government (National Institutes of Health, New York State Department of Health), international advocacy organizations (eg. International Diabetes Federation) and industry to highlight issues and discuss solutions to the growing impact of diabetes globally.

Penn Diabetes Research Center (DK19525)
Global Diabetes Research 2011

The Penn Diabetes Research Center (DRC) has four primary focus areas: beta cell physiology and pathology, signaling by insulin and other hormones, obesity, and cardiovascular metabolism and complications. Within each of these areas are a diverse group of scientists performing both wet and dry lab research, some highly basic and some translational. Included in the latter are examples of global diabetes research. Two major programs are highlighted here:

Sarah Tishkoff, Ph.D.
David and Lyn Silfen University Associate Professor
Departments of Genetics and Biology
Perelman School of Medicine
University of Pennsylvania

Dr. Tishkoff is interested in examining levels and patterns of genetic variation at the genome level among modern humans and non-human primates in order to elucidate the evolutionary forces (mutation, gene conversion/recombination, migration, drift, selection) that shape and maintain genetic variation in contemporary populations. These data are being used to reconstruct historical demographic and population differentiation events to test hypotheses of modern human origins. Despite the fact that Africa plays a central role in human evolution, African populations have been greatly underrepresented in the study of human genetic diversity. In the African Diversity Project, blood samples have been collected from 7,000 individuals originating from Nigeria, Cameroon, Chad, CAR, DRC, Ethiopia, Tanzania, Sudan, and Kenya. Analysis includes use of the Functional Genomics Core and Biomarker Core of the Penn Diabetes Research Center. For many of these samples, phenotype data has been collected for genotype/phenotype analyses. Genotypic focus includes mtDNA, Y chromosome, and autosomal variation in these populations, including genome-wide analyses of resequencing data and of microsatellite, in/del, and SNP polymorphism data. The study of African genetic diversity is important for reconstructing modern human origins, as well as for the identification of the genetic basis of diseases prevalent in African and African American populations, especially diabetes. In addition, a study of global patterns of linkage disequilibrium (LD) in the human genome is underway to aid in the identification of genes involved in complex diseases such as diabetes and obesity.

Charlene W. Compher, Ph.D., R.D., F.A.D.A., C.N.S.C., L.D.N.
Associate Professor of Nutrition Science
School of Nursing
University of Pennsylvania

Dr. Compher's research focuses on the impact of nutritional and medical interventions to enhance nutrient absorption and obesity prevention. She has been actively studying the nutrition transition in Botswana, and spent the Spring semester 2010 as a Fulbright Africa Research Scholar, where she worked on adolescent obesity prevention and fostered scholarly productivity among faculty at the University of Botswana. Current work in the context of the partnership between the Government of Botswana, the University of Botswana and the University of Pennsylvania focuses on obesity prevention as part of a program to prevent noncommunicable diseases, such as DM.

University of Alabama at Birmingham DRTC

1) Formalized collaboration between Jubilant Organosys (India), University of Alabama at Birmingham, and the Southern Research Institute joint venture for drug discovery in oncology, metabolic diseases and diabetes, and infectious diseases.

2) Fogarty International Center D43 TW005816. Contact PI: Lewis, CE. Title: Clinical Research Training for NCD Studies in India

This is a collaboration between UAB and the Madras Diabetes Research Foundation (MDRF) in Chennai, Tamil Nadu, India, led by Dr. Mohan. The groups undertaking this program have created and operated a training activity that is both broad and deep. Broad in terms of program content, which has been expanded to include most major non-communicable diseases, and in terms of the types of training offered, which includes short-term training in the US, a national seminar involving trainees from throughout India, intensive training sessions for highly selected trainees and workshops, video conferences and special courses for MDRF faculty and staff. Deep in the sense of a focus on diabetes and on long-term training for selected MDRF staff, which is done in India rather than in the US. Together these activities have facilitated the training of numerous research scientist and development of their research programs. Importantly, we created the Indian Non-Communicable Disease (NCD) Network (INN) as a formal entity to facilitate the overall attainment of our goals. Trainees involved to date have completed PhDs or are working toward completing them in their own country and some have assumed key leadership positions in MDRF. We propose to continue and expand these activities, and continue to draw on the extensive training expertise and resources of both UAB and MDRF, an outstanding research organization in Chennai. Training content will include research methods, background on NCDs, and methods to enhance research infrastructure, such as data management, sample handling systems and study operations. For the most part, we plan to conduct the training in India where it can be done in the context of the research to which the training applies and allows for a smooth transition into research programs, with immediate impacts on their public health system. We plan to continue the annual national seminars for trainees from throughout India using Indian and US faculty, special workshops for MDRF faculty and staff, intensive training sessions for selected trainees. We expect also to enhance the activities of the INN so that it can help facilitate the broader establishment of training opportunities and activities. We will expand our innovative approach to using video conferences for research seminars and research planning so that it includes two-four other sites in India and to otherwise further enhance MDRF as a center for training. We also propose to train the trainers to help develop faculty that can conduct workshops for other institutes in India. Our overall goal is to improve, and create where necessary, the clinical research training required to conduct research and prevention programs to help ward off the impending epidemic of diabetes and other NCDs in India.

3) Fogarty International Center D43 TW05750. Contact PI: Sathiakumar, N. Title: International Training and Research in Environmental and Occupational Health

The University of Alabama at Birmingham (DAB) ITREOH, a collaborative research training effort with the Aga Khan University (AKU) of Karachi, Pakistan, seeks to expand its training and research collaboration in South Asia. South Asian countries, including Pakistan, are transitioning from an agrarian to an industrialized economy with a myriad of new environmental and occupational health (EOH) concerns. There are no university-based training programs in this region specifically designed to train the necessary professionals to take on these challenges. The UAB-AKU partnership has resulted in the establishment of a core group of researchers/trainers at AKU who have developed skills in environmental epidemiology, who provide input at the nation level, and have implemented an EOH certificate program at AKU. Our new proposal includes two other principal partner institutions that were identified during our regional training efforts, the Manipal Academy of Higher Education (MAHE) in India and the Ministry of Health (MOH) in Sri Lanka. The proposed training program will focus on prevention and intervention research as applied to the mitigation of environmental and occupational hazards. A major highlight of the collaboration will be the development of an EOH graduate degree program at AKU. The specific aims of this research training program are: (1) Training - To establish and sustain EOH training infrastructure at partner institutions; (2) Research - To further enhance research skills and expand research capacity; (3) Resources - To develop additional resources to support ongoing and future training and research; and (4) Trainee Tracking - To track and document the long-term impact of research training. Training components will include short-term regional training that will consist of principle- and problem-based short courses; intermediate training at UAB or selected sites to enhance research skills, provide mentorship and funds for pilot research. A masters and doctoral degree will be offered as an option to exceptional candidates. Our goal is to develop a core group of trained, independent researchers who are recognized as having expertise in EOH issues and provide input into EOH policies at the national level. Further developing AKU's independence to provide regional leadership and expanding EOH research and training in South Asia that has the potential to translate into practice should be an excellent long-term investment of Fogarty resources.

4) NIH R01DA024875. Title: Network for Tobacco Control among Women in Parana, Brazil
PI: Scarinci, I. Project Period: 09/30/2007 08/31/2013

This proposal develops a Network for Tobacco Control among Women in Paraná, Brazil in order to establish community and institutional capacity to promote gender-relevant tobacco control efforts among Brazilian women through community-based participatory research and training. The goals of the ³Network² are to reduce tobacco use and exposure to environmental tobacco smoke among Brazilian women, and to develop a cadre of well-trained researchers in tobacco control.

5) NIH D43CA153784. Title: Zambian cervical cancer research capacity initiative. PI: Parham G. 07/01/10 – 06/30/13

Tim Nagy, PhD, Director o the DRTC Animal Physiology Core is co-investigator on this training grant. The central focus is to train MDs in Zambia to conduct cancer research. However, there is an emphasis on the Metabolic Syndrome and, recently, an interest in the interaction between diabetes and tuberculosis that is the subject of an application for a DRTC pilot grant during the current cycle.

Summary of On-Going Global Diabetes Initiatives at Yale

Kasia Lipska, MD a junior faculty member in the Section of Endocrinology and Metabolism received a Fulbright Fellowship in 2011 to assess the prevalence of undiagnosed diabetes, prediabetes, and insulin resistance among patients with a recent transient ischemic attack (TIA) or ischemic stroke in India. The prevalence of cardiovascular disease, including ischemic stroke, is dramatically increasing in India, now surpasses that seen in Western countries, and is more likely to lead to fatality. Among patients who experience a transient ischemic attack (TIA) or ischemic stroke, many patients, within a short time will have a recurrent stroke or a myocardial infarction (MI). Therefore, secondary prevention efforts are particularly important in India. Reduction of risk factors (i.e. hypertension, diabetes, dyslipidemia, and smoking) for ischemic stroke is at the cornerstone for secondary prevention. However, despite the use of antiplatelet agents and other proven therapies, recurrent vascular events frequently occur after ischemic stroke. In the U.S., there has been a growing interest in insulin resistance and impaired glucose tolerance (IGT) as additional and potentially modifiable risk factors for secondary prevention of stroke. Although both stroke and glucose disorders are dramatically increasing in India, the association of stroke and TIA with abnormalities of glucose metabolism and insulin resistance has not been previously evaluated in India, despite the high propensity for non-alcoholic fatty liver disease and associated insulin resistance compared to Caucasian counterparts. Because of recent changes in diet and exercise patterns effective lifestyle interventions may be particularly relevant and effective in India.

The study being conducted by Dr. Lipska hopes to help define the prevalence of undiagnosed diabetes and IGT, as well as document levels of insulin resistance among individuals with a recent stroke or TIA and to test the hypothesis that undiagnosed diabetes, IGT, and elevated levels of insulin resistance are highly prevalent among individuals with recent stroke or TIA to in the future design and test potential therapeutic interventions for secondary prevention. This is a 2-site project involving the All India Institute of Medical Sciences (AIIMS) and the Sree Chitra Tirunal Institute for Medical Science and Technology (SCTIMST) that will assess diabetes, prediabetes, and insulin resistance following ischemic stroke or TIA. Men and women older than 30 years with no known history of diabetes who are seen in the stroke clinics at the SCIMST or AIIMS for TIA for an ischemic stroke will be included. One hundred participants will be assessed with an oral glucose tolerance test (with calculation of their insulin sensitivity index) at each site, at least 2 weeks and no longer than 3 months following the acute episode. Blood samples will be obtained in the fasting state for glucose, insulin, and lipoprotein profile. Dietary assessment will be done using previously validated methods.Data analysis will be conducted at the Achutha Menon Centre for Health Science in Trivandrum. De-identified data will be compared with data from the Insulin Resistance in Stroke (IRIS) trial in the U.S.

Marcella Nunez-Smith, MD who is an assistant professor of general internal medicine, assistant director of the Robert Wood Johnson Foundation Clinical Scholars Program,

and a member of Yale's Global Health Leadership Institute has been awarded a 5.3 million dollar grant from the National Institute on Minority Health and Health Disparities to study the risk factors and prevalence of heart disease, cancer and diabetes in the eastern Caribbean. The project aims to form a research collaborative across the eastern Caribbean islands of Puerto Rico, the U.S. Virgin Islands, Barbados and Trinidad & Tobago. The goal is to help improve health outcomes across the region by establishing a cross-island surveillance partnership called "The Eastern Caribbean Health Outcomes Research Network (ECHORN)". ECHORN has two aims: (1) to form a research collaborative across academic partner institutions in the Eastern Caribbean islands of Puerto Rico, the U.S. Virgin Islands, Barbados, and Trinidad & Tobago to recruit and follow a community-dwelling adult cohort to estimate the prevalence of known and potential risk factors associated with the development of heart disease, cancer, and diabetes and (2) to enhance health outcomes research leadership capacity in the region through a series of dedicated activities locally and abroad. ECHORN will expand clinical research with racial/ethnic minority populations in a transitioning part of the globe now threatened with an epidemic of non-communicable chronic diseases (NCD). ECHORN's findings will have direct implications for the health disparities research and policy agenda on the mainland United States. In the long term, the links ECHORN will facilitate with local health policy delegations and global strategic organizational partners will promote the translation of research to improve health outcomes across the region. The collection and storage of biological specimens will also contribute to national bio-monitoring projects and has the potential to identify unique risk and protective factors in the development of NCD.

Kelly Brownell, PhD, Directs the Rudd Center, which was founded at Yale in 2005. The goal of the Center is to marshal the talent of a diverse group of global scientific experts on obesity to assess, critique, and strive to improve practices and policies related to nutrition and obesity so as to inform and empower the public, to promote objective, science-based approaches to policy, and to maximize the impact on public health.
The Rudd Center has a number of global initiatives. It has a close working relationship with the World Health Organization and is currently applying to become an official WHO Collaborating Centre. The Rudd Center is connected with both the world headquarters in Geneva, but also PAHO (The Pan American Health Organization).
In addition, it is closely connected with the International Obesity Task Force, and have worked with officials in a variety of countries with respect to obesity policy (England, Australia, Chile, Argentina, and others).

Global Health Initiatives at the University of Michigan

William H. Herman, MD, MPH

Center for Global Health at the University of Michigan

Generates novel partnerships and approaches and supports research, training, and service to build capacity, improve health, and redress health inequalities.

Areas of Focus

- Noncommunicable diseases and mental health
- Social determinants of health
- Global environment change and health
- Health system strengthening and human resources for health

Collaboration Platforms

- Ghana
- Brazil
- China

Diabetes and Endocrine Initiatives in Brazil

- Improving Diabetes Care in Primary Care Centers in the low-income western region of Sao Paulo, Brazil.
- Michele Heisler and Ken Resnicow and USP Professor Alexandra Brentani
- Developing and testing tailored, interactive, web-based diabetes decision aids for community health workers and patients using iPads
- Developing training program for community health workers in diabetes self-management support
- Funded by Sao Paulo State Foundation

Studies of Congenital Adrenal Hyperplasia due to 17-hydroxylase deficiency (Rich Auchus and Federal University of Sao Paulo Prof. Claudio Kater) and adrenal cortical carcinoma (Gary Hammer and University of Sao Paulo Professors Berenice Mendoca, Ana Latronico, and Claudimara Lofti)

Joint Institute for Translational and Clinical Research

- Partnership between U of M Health System and Peking University Health Sciences Center in China
- Launched October 2010 with combined commitment of $14 million
- 19 U of M Medical School Departments collaborate with 31 Chinese Universities
- Focus on cardiovascular, liver, and pulmonary disease

Obesity, metabolic syndrome, and nonalcoholic fatty liver disease in China

- Liz Speliotes and Linong Ji from Peking University
- 32 genetic variants associated with BMI and 5 associated with NAFLD in individuals of European ancestry
- Assessing environmental and genetic factors associated with NAFLD among urban poor undergoing rapid economic development
- Funded by UM – Peking U Joint Institute for Translational and Clinical Research

Improving Diabetes Care in Mexico with El Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE)

- Bob Anderson and Marti Funnel are providing technical assistance and training to ISSSTE to redesign their health system and implement the patient empowerment approach for diabetes management
- ISSSTE
  - Provides care for 11 million Mexican government workers and their families
  - Integrated health system
  - 13.4% prevalence of diabetes
- Measuring population change in HbA1c
- Funded by ISSSTE

Telehealth Support for Diabetes Self-Management in Honduras and Mexico

- John Piette and collaborators
- Developed, implemented, and assessed feasibility of cell-phone based interactive voice response system for health status monitoring, self-care education, and adherence support in rural Honduras
- HbA1c decreased from 10.0% to 8.9% over 6 weeks
- Now conducting randomized trial of automated telephone monitoring and behavior change telephone calls in Monterrey Mexico

Summary

- Multi-directional collaborations
- Platform-based and investigator initiated
- Leverage strengths and expertise of the partners
- Promote innovative, interdisciplinary research
- Build capacity and provide training for new scientists
- Produce new knowledge to guide action

BCM

Baylor College of Medicine

Diabetes Globalization Initiative: Africa

November 9, 2011

BCM
Baylor College of Medicine

BIPAI Baylor International Pediatric AIDS Initiative at Texas Children's Hospital

History

- Established in 1996
- Founded and Led by Dr. Mark Kline, chair of pediatrics at BCM and Physician-in-Chief at Texas Children's Hospital
- Network of care centers across Africa
- Serving over 76,000 children and families
- Provided 350,000 outpatient clinic visits
- HIV counseling and testing to 130,000 people
- Trained 12,000 local health professionals in the past year

Source: BIPAI.org

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BIPAI Baylor International Pediatric AIDS Initiative at Texas Children's Hospital

Network

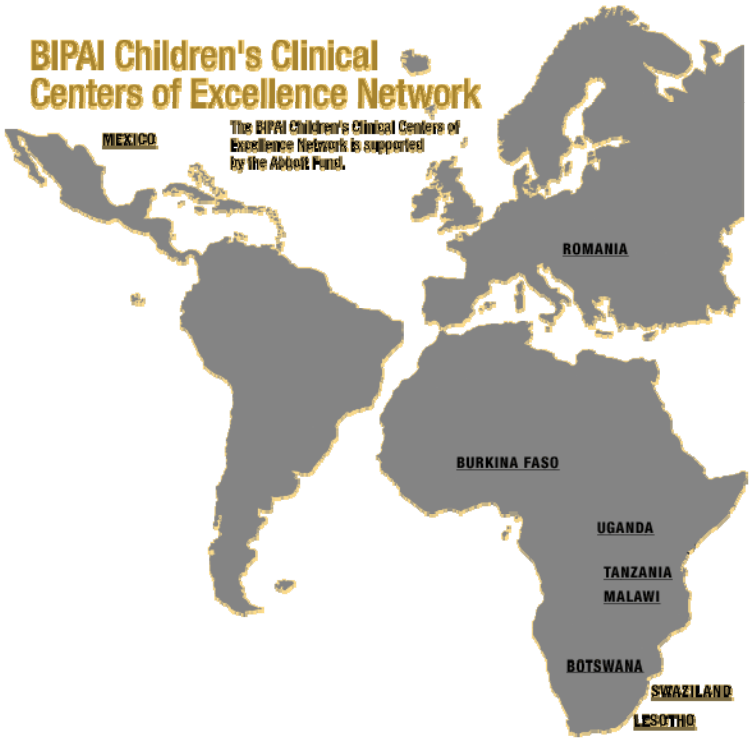
- Centers of Excellence in Botswana, Kenya, Lesotho, Malawi, Romania, Swaziland, Tanzania and Uganda
- International Program Offices in Ethiopia, Kenya, Libya, Mozambique, and Tanzania
- Each location is adjusted to fit each country's unique social, economic, legal and healthcare landscape
- Each site also officially partners with each country's Ministry of Health, and operates under a memorandum of agreement that makes each site a BIPAI affiliated NGO

Source: BIPAI.org

BCM
Baylor College of Medicine

BIPAI Baylor International Pediatric AIDS Initiative at Texas Children's Hospital

Locations



http://www.cyto.purdue.edu
http://pediatrician-in-swaziland.blogspot.com/2007_07_01_archive.html

Baylor College of Medicine

BIPAI Baylor International Pediatric AIDS Initiative at Texas Children's Hospital

Success

BIPAI's success and governmental support and trust in those countries it operates in have developed a vast network and infrastructure that can be leveraged to create new BIPAI-type initiatives for other diseases and conditions

- Screening and treatment program for sickle cell disease in Luanda, Angola launched in 2011

Source: BIPAI.org

Globalization Diabetes Strategy

Inspired by this success of the BIPAI platform, the BCM Center for Globalization has set a priority of initiating a diabetes framework program in Africa in line with the global diabetes initiative of the NIH

Source: BIPAI.org

Diabetes in Africa

WHO Diabetes Prevalence and Projections

Country	2000	2030
Botswana	25,000	45,000
Ethiopia	796,000	1,820,000
Kenya	183,000	488,000
Lesotho	31,000	42,000
Malawi	55,000	118,000
Mozambique	133,000	273,000
Swaziland	13,000	21,000
Tanzania	201,000	605,000
Uganda	98,000	328,000

Source: WHO

Diabetes in Africa

The potential severity of diabetes is such that some epidemiologists predict that its economic impact and death toll will surpass the ravages of HIV and AIDS in the near future.

- Approximately, 7.1 million Africans were suffering from diabetes at the end of 2000, a figure that was expected to rise to 18.6 million by 2030

Source: Azevedo M, Alla S. Diabetes in sub-Saharan Africa: Kenya, Mali, Mozambique, Nigeria, South Africa and Zambia. International Journal of Diabetes in Developing Countries 2008;28:101–8.

Diabetes and HIV

- Recent Swiss study shows that HIV patients over 50 have a five-fold increased risk of Diabetes than a similar aged person without HIV
- In Africa, the enormous HIV burden will lead to a new “double burden” of HIV and Diabetes for this population

Source: *Clinical Infectious Diseases*. Vol 53 Issue 11: pages 1130-1139.

Program Implementation

- Begin at pilot sites where known Diabetes prevalence is significant
- Target parents bringing children into BIPAI sites for Diabetes screening and detection
- Leverage global resources to provide medications and self-monitoring supplies for newly diagnosed patients
- Launch Diabetes clinics adjacent to BIPAI network facilities for ongoing monitoring and treatment

Prevention and Sustainability Measures

- Work with local health authorities and community health clinics to implement prevention measures
- Provide Health Systems Training and Development programs to conduct screening, to staff clinics, and to serve as public health educators

Future Considerations

- Rotations for clinical fellows, residents and medical students
- Clinical Trials

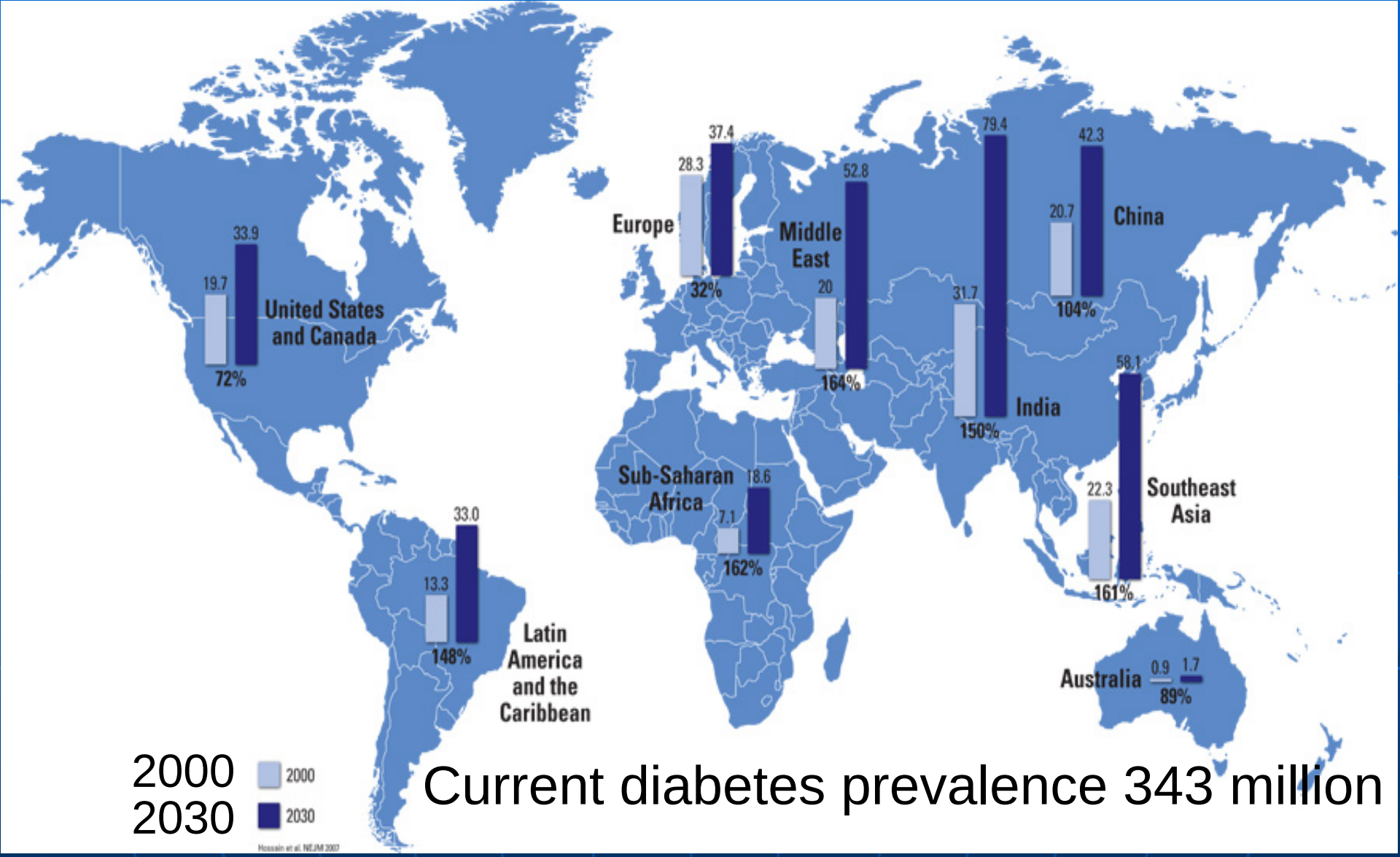
BCM
Baylor College of Medicine

- Thank you!

Global Diabetes Initiative

Meredith A. Hawkins, MD, MS

Diabetes Centers Directors' Meeting
November 9, 2011
Bethesda, MD



- **80-90% of new cases occur in developing countries**
- **Life expectancy ~ 1yr after Dx in rural Africa**

Global Diabetes Initiative

Strategic global partnerships:

- **research**
- **education**
- **advocacy**

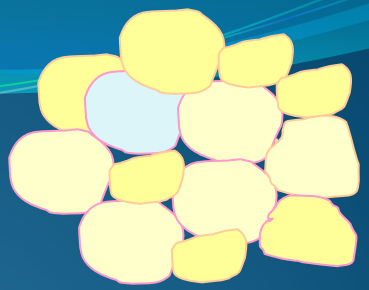
Global Partnerships

Malnutrition-Modulated Diabetes Mellitus

- **Age:** Onset by 10-30 years of age
- **Where:** ~50% of young patients with diabetes in India, sub-Saharan Africa
- **History:** Poor nutrition
- **Body mass Index** 11-16 kg/m2
- **Complications:** severe

Characteristics: reduced insulin secretion, high insulin requirements, not ketosis prone

Decreased insulin secretion



Reduced Fat Mass

Malnutrition Diabetes

Fatty Liver

Infectious Diseases and Inflammation

CMC Vellore - Einstein Research Partnership

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

NOT TO BE MINISTERED UNTO BUT TO MINISTER
CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

Global Diabetes Initiative

Strategic global partnerships:

- **research**
- **education**
- **advocacy**

CLINICAL DIABETES CONFERENCE

Mukono Agricultural Research Center

August 23rd-August 26th, 2009

THE REPUBLIC OF UGANDA

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

WORLD DIABETES FOUNDATION

International Continuing Medical Education Conferences

- Annual meetings for past 30 years
- Attended by hundreds of physicians serving in most African and Asian countries
- Forming liasons with Global Diabetes Initiative to develop culturally-adapted diabetes training

Nairobi, Kenya

Chiang Mai, Thailand

Global Diabetes Initiative

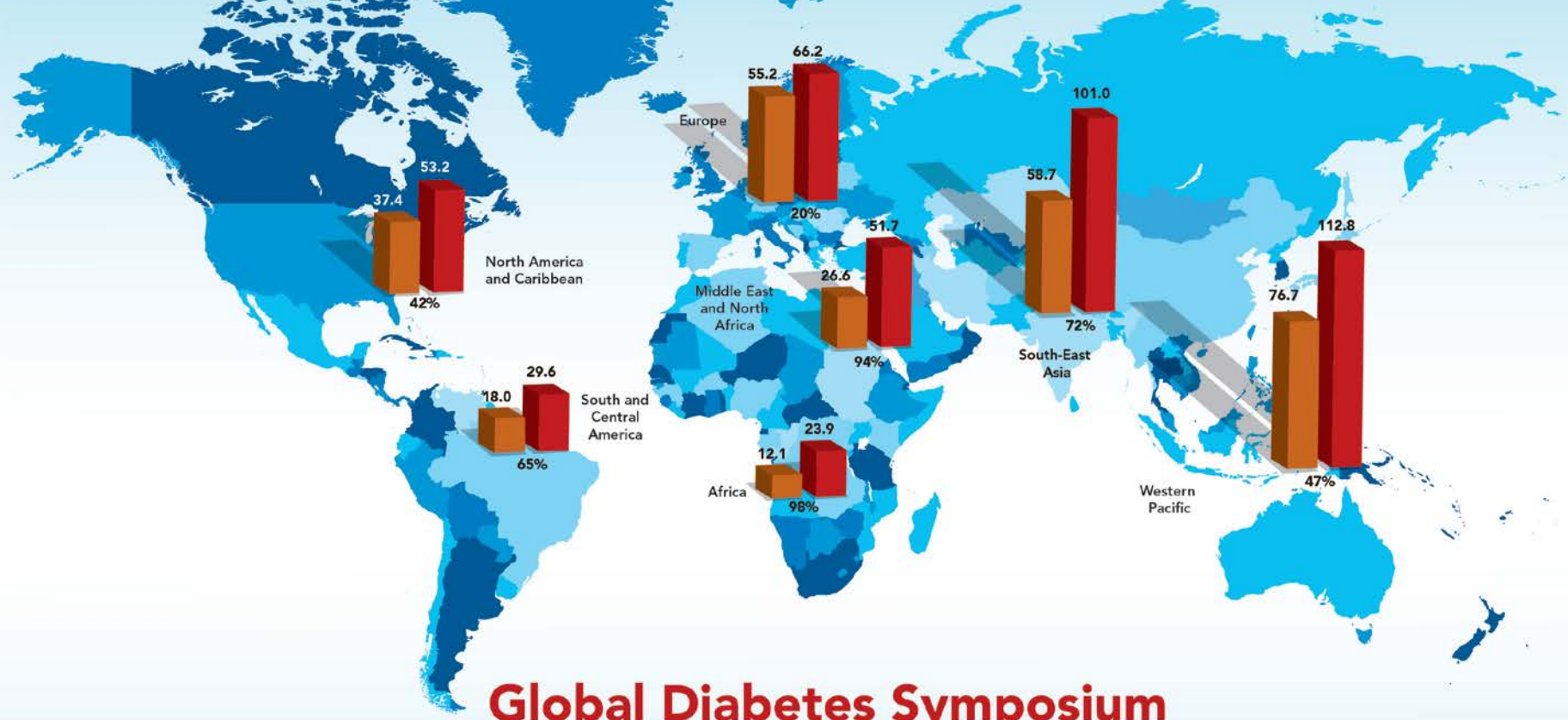
Strategic global partnerships:

- **research**
- **education**
- **advocacy**

Partnerships for Advocacy

Briefing to United States Congressional Staff

September 27, 2010



Global Diabetes Symposium

Finding the Way to Global Action

September 18, 2011
New York, NY

EINSTEIN
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OF YESHIVA UNIVERSITY

International Diabetes Federation


• Clinical research and clinical trials
• Special needs for special populations
• Diabetes complications
• Clinical research to practice
• Resource and infrastructure needs
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Science at the hear of medicine
EINSTEIN
Albert Einstein College of Medicine
International Diabetes Federation
Global Diabetes Symposium
EINSTEIN
Albert Einstein College of Medicine

Faces of Malnutrition Diabetes

Acknowledgements:

Dean Allen Spiegel

Dr. Jason Baker

Prof. Elizabeth Walker

Prof. Nihal Thomas and Endocrinology Dept, CMC Vellore

Drs. Fred Nakwagala, Agatha Nambuya, Silver Bahendeka

Stephanie Lawrence, Jill Raufman, Melissa Gorton

Gary Goldenberg: www.garygoldenberg.com

DIABETES RESEARCH CENTERS

2011 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://diabetescenters.org/center/derc-uclaucsd

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/index.html

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

Washington University in St. Louis: http://drtc.im.wustl.edu/

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

Diabetes Centers Websites Edit

Design Survey Collect Responses Analyze Results

View Summary
Browse Responses
Filter Responses
Crosstab Responses
Download Responses
Share Responses

Default Report + Add Report

Response Summary

Total Started Survey: 16
Total Completed Survey: 16 (100%)

PAGE: 1

1. What type of individual designed your Diabetes Center website? Create Chart Download

	Response Percent	Response Count
Graduate student/post-doc	0.0%	0
Faculty member	0.0%	0
Institutional computer information technologist	**50.0%**	**8**
External computer information technologist	6.3%	1
A team comprised of a combination of above	37.5%	6
Other (please specify) Show Responses	6.3%	1
	answered question	16
	skipped question	0

2. Approximately how much did your Center pay to have your Diabetes Center website designed/created? (If created internally for no 'official' charge/fee, please estimate amount of time and cost). Create Chart Download

	Response Percent	Response Count
<$500	6.3%	1
$500-$2,000	25.0%	4
$2,000-$5000	12.5%	2
>$5,000	**56.3%**	**9**
	answered question	16
	skipped question	0

3. How often is your website updated? Create Chart Download

	Response Percent	Response Count
Weekly	**31.3%**	**5**
Monthly	**31.3%**	**5**
A few times a year	**31.3%**	**5**
Once a year or less	6.3%	1
Updated?	0.0%	0
	answered question	**16**
	skipped question	**0**

4. What website design software are you currently using for your Diabetes Center website? Create Chart Download

	Response Percent	Response Count
Drupal	6.3%	1
Dreamweaver	**50.0%**	**8**
Webstudio	6.3%	1
Microsoft Expression	6.3%	1
An Institutional Content Management System	6.3%	1
Other (please specify) Hide Responses	25.0%	4

Responses (4) | **Text Analysis** | **My Categories** (0)

GOLD FEATURE: Text Analysis allows you to view frequently used words and phrases, categorize responses and turn open-ended text into data you can really use. To use Text Analysis, upgrade to a GOLD or PLATINUM plan. Learn More Upgrade »

Showing **4** text responses No responses selected

Estrada
9/7/2011 6:51 AM View Responses

Tridion content management system
5/10/2011 9:34 AM View Responses

BCM
5/10/2011 6:24 AM View Responses

adobe contribute
5/9/2011 4:29 PM View Responses

answered question	**16**
skipped question	**0**

4. What website design software are you currently using for your Diabetes Center website? Create Chart Download

answered question	**16**
skipped question	**0**

5. Where is your Diabetes Center website hosted? Create Chart Download

	Response Percent	Response Count
Lab server	0.0%	0
Departmental server	12.5%	2
Institutional server	**81.3%**	**13**
Cloud	0.0%	0
Commercial server	6.3%	1
answered question		**16**
skipped question		**0**

6. What is the charge for hosting and maintenance for your site? Create Chart Download

	Response Percent	Response Count
<$200/year	25.0%	4
$200-$500/year	**31.3%**	**5**
$500-$2,000/year	18.8%	3
>$2,000/year	25.0%	4
answered question		**16**
skipped question		**0**

7. What type of individual maintains/curates your website? Create Chart Download

	Response	Response
answered question		**16**
skipped question		**0**

7. What type of individual maintains/curates your website? Create Chart Download

	Percent	Count
Graduate student/post-doc	0.0%	0
Faculty member	6.3%	1
Institutional computer information technologist	6.3%	1
External computer information technologist	0.0%	0
A DERC staff person	**37.5%**	**6**
A team comprised of a combination of above	31.3%	5
Other (please specify) Show Responses	18.8%	3
	answered question	16
	skipped question	0

8. Is there a link to the NIDDK central Diabetes Center website (www.diabetescenters.org) on your website? Create Chart Download

	Response Percent	Response Count
No (but there will be soon)	31.3%	5
Yes, of course	**68.8%**	**11**
	answered question	16
	skipped question	0

9. How many hits does your institutional Diabetes Center website have in an average month? Create Chart Download

	Response Percent	Response Count
<50	0.0%	0
50-200	25.0%	4
200-500	12.5%	2
>500	6.3%	1
Don’t know; we do not have a hit counter	**56.3%**	**9**
	answered question	16
	skipped question	0

10. Does your Diabetes Center website manager communicate with Jodee Allen (central Diabetes Center website manager)?	Create Chart	Download
	Response Percent	Response Count
Yes	25.0%	4
Jodee who? (no)	75.0%	12
	answered question	16
	skipped question	0

commonalities and diversities in Institutional Diabetes Center Websites

Pinchas (Hassy) Cohen
Professor and Vice Chair for Research
Mattel Children's Hospital
Chief, Pediatric Endocrinology UCLA
Co-Director, UCSD/UCLA DERC

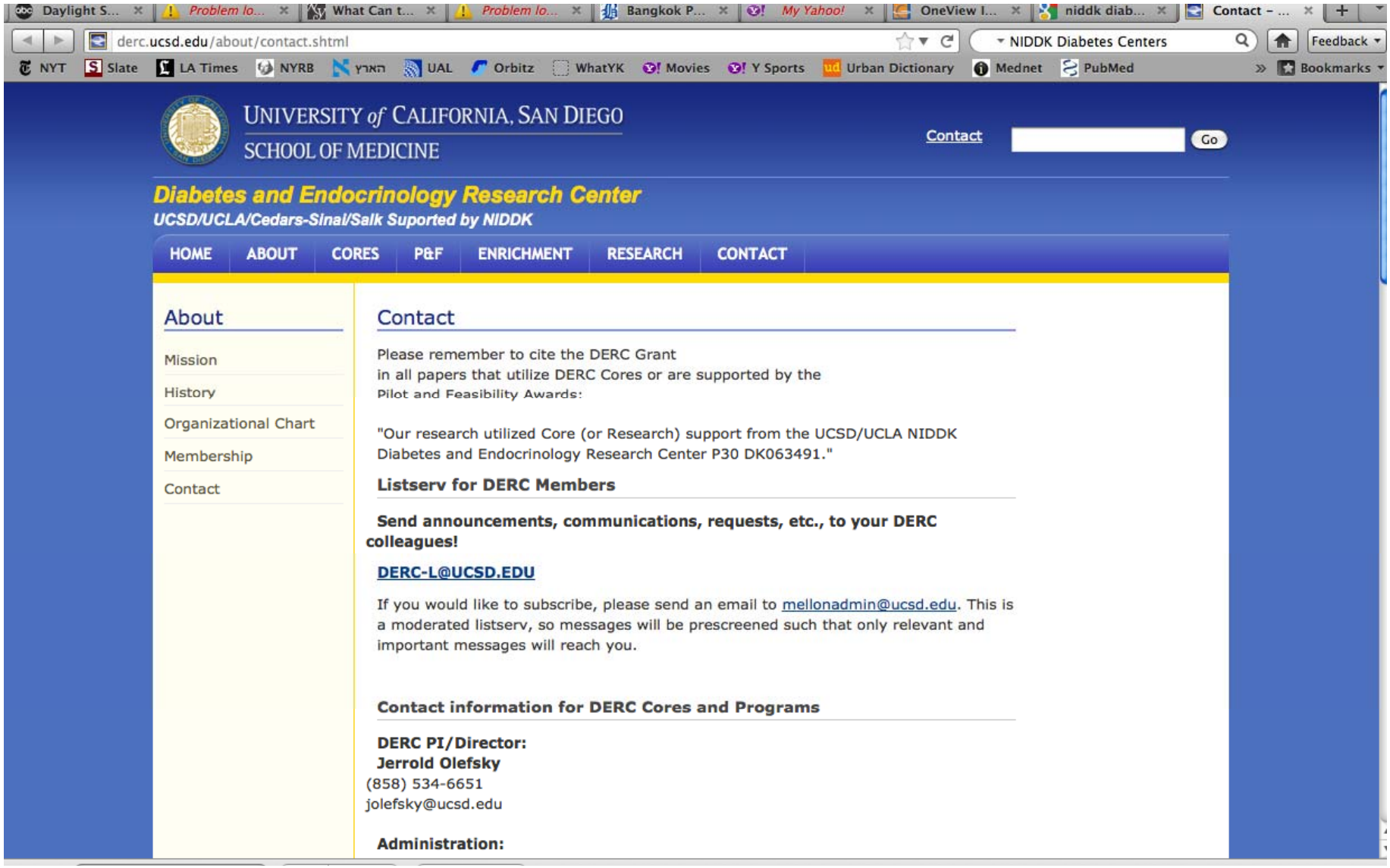
Contributors

- **The Diabetes Centers Executive Committee**

  - Jim Hyde (NIDDK)
  - Jean Schaffer (Wash U)
  - Mimmo Accili (Columbia)
  - Gordon Weir (Harvard)
  - Hassy Cohen (UCLA)
  - Christine Carter-Su (Michigan)
  - Jerry Palmer (UW)
  - Bill Herman (U of Michigan)
  - Larry Chan (Baylor)

The Central NIDDK Diabetes Centers Website

UCSD/UCLA site



BCM site

Daylight Savings Tim... | What Can the DNA of ... | The New York Times – ... | Los Angeles Times – C... | bcm diabetes center –... | My Yahoo!

www.bcm.edu/diabetescenter/

NYT | Slate | LA Times | NYRB | הארץ | UAL | Orbitz | WhatYK | Movies | Y Sports | Urban Dictionary | Mednet | PubMed | UCLA Logon

BCM
Baylor College of Medicine

Diabetes and Endocrinology Research Center

>BCM Home >BCM Centers >BCM Departments >Find a BCM person >Giving

Houston, Texas Search: Go

Home
About the DERC
Pilot/Feasibility
Enrichment Programs
Cores/Fees
Resources
Announcements
BCM Home

DERC

Baylor College of Medicine is the newest member of an integrated program for diabetes and related endocrinology and metabolism research overseen by the National Institute of Diabetes and Digestive and Kidney Diseases. Information on all ongoing NIDDK Diabetes Research Centers can be found at www.diabetescenters.org.

Housed in the Margaret M. Alkek Building for Biomedical Research, the BCM Diabetes and Endocrinology Research Center:

- Serves a biomedical research base of 57 researchers in 8 different departments of the college
- Provides support via research core laboratories
- Presents enrichment opportunities via meetings and seminars
- Offers a Pilot and Feasibility Program

Advancing diabetes research

Receiving its designation in January 2008, the Houston-based DERC is the only NIH diabetes center in Texas and one of 17 nationwide. Hear comments from Center Director Dr. Lawrence Chan (RealPlayer required).

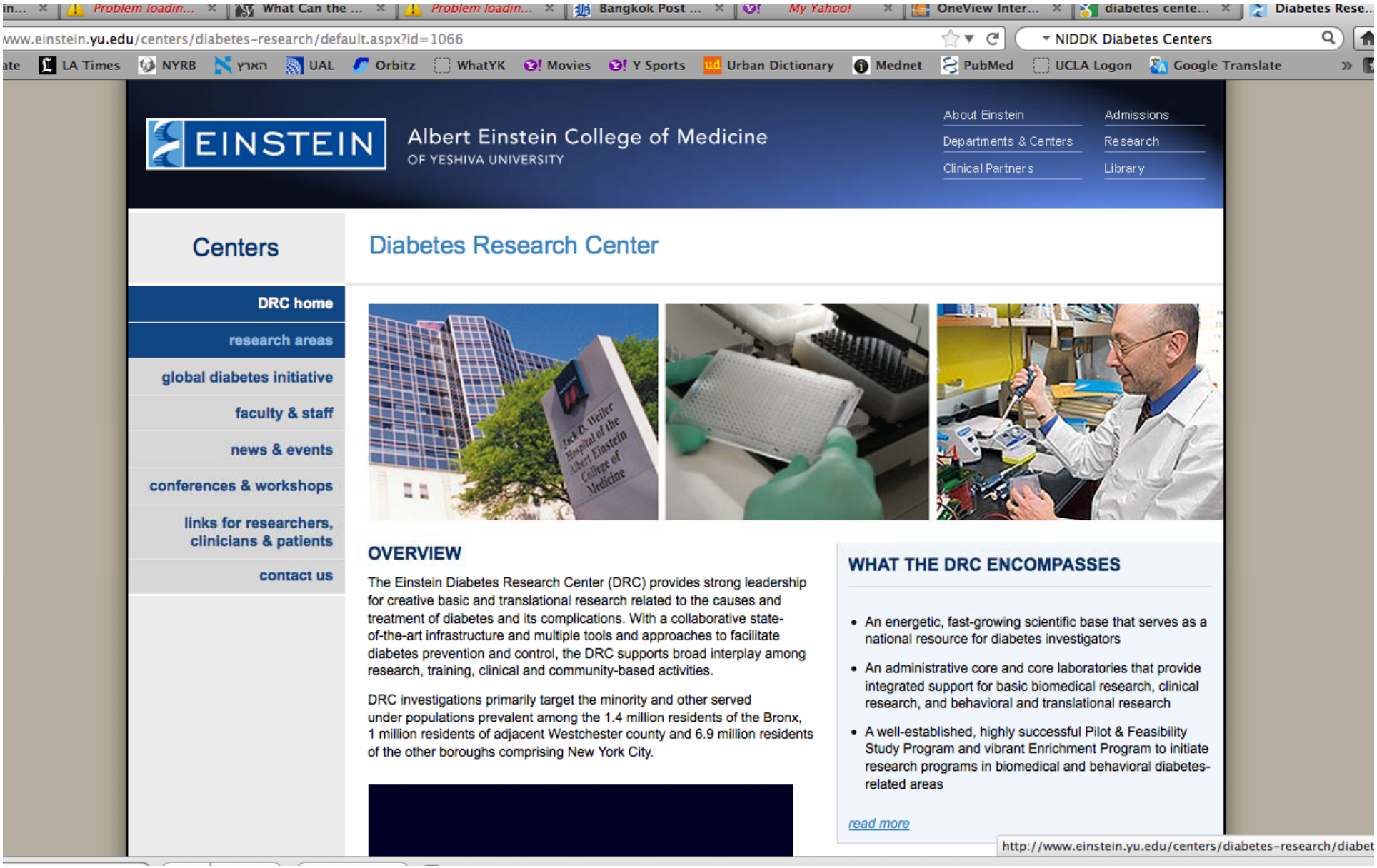
Research Areas

The BCM DERC covers four major research areas:

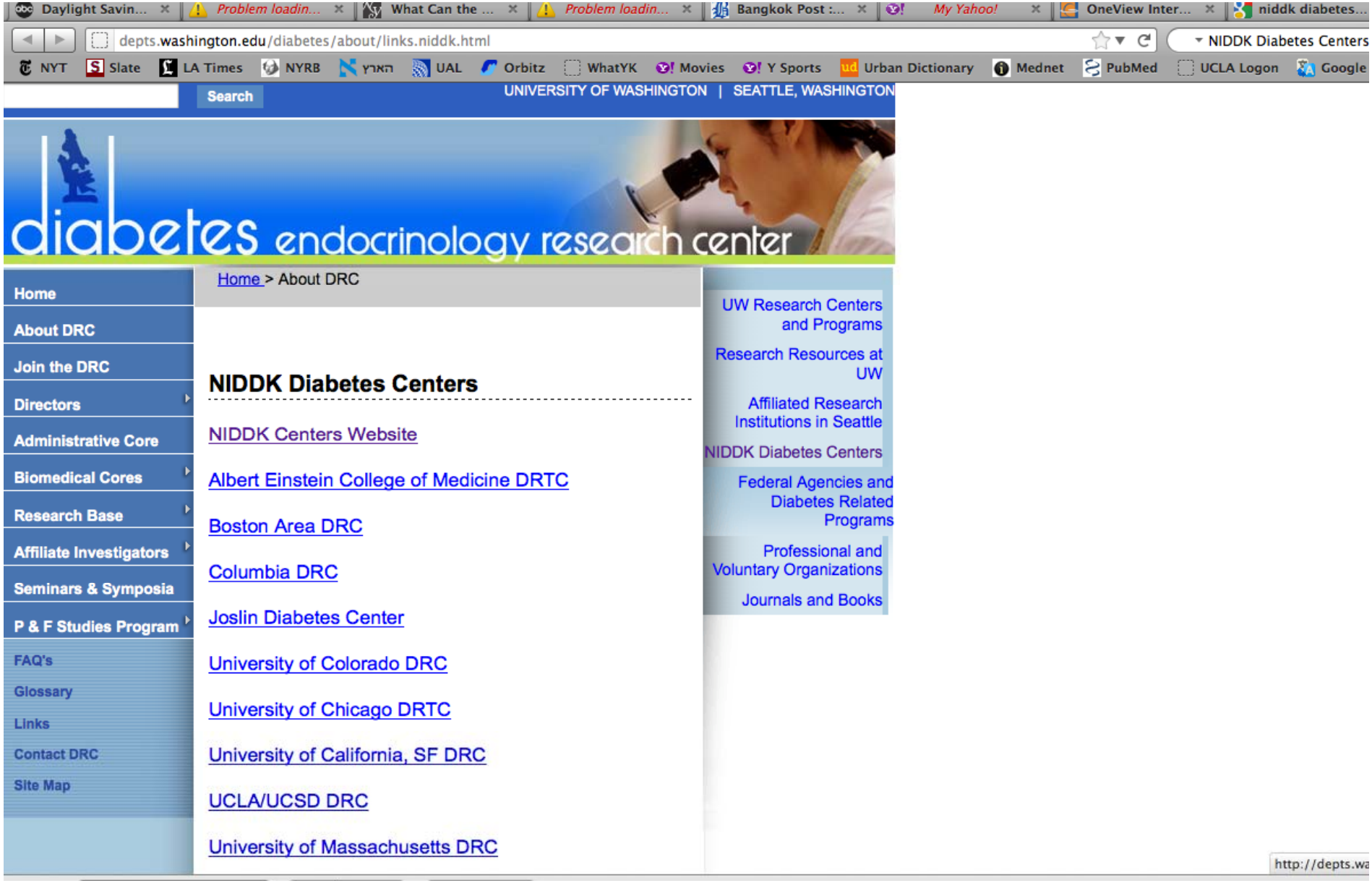
- Clinical diabetes, metabolism and nutrition

Find: nep | Next | Previous | Highlight all | Match case

AECOM site


www.einstein.yu.edu/centers/diabetes-research/default.aspx?id=1066
NIDDK Diabetes Centers
LA Times
NYRB
UAL
Orbitz
WhatYK
Movies
Y Sports
Urban Dictionary
Mednet
PubMed
UCLA Logon
Google Translate
EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY
About Einstein
Admissions
Departments & Centers
Research
Clinical Partners
Library
Centers
DRC home
research areas
global diabetes initiative
faculty & staff
news & events
conferences & workshops
links for researchers, clinicians & patients
contact us
Diabetes Research Center
OVERVIEW
The Einstein Diabetes Research Center (DRC) provides strong leadership for creative basic and translational research related to the causes and treatment of diabetes and its complications. With a collaborative state-of-the-art infrastructure and multiple tools and approaches to facilitate diabetes prevention and control, the DRC supports broad interplay among research, training, clinical and community-based activities.
DRC investigations primarily target the minority and other served under populations prevalent among the 1.4 million residents of the Bronx, 1 million residents of adjacent Westchester county and 6.9 million residents of the other boroughs comprising New York City.
WHAT THE DRC ENCOMPASSES
An energetic, fast-growing scientific base that serves as a national resource for diabetes investigators
An administrative core and core laboratories that provide integrated support for basic biomedical research, clinical research, and behavioral and translational research
A well-established, highly successful Pilot & Feasibility Study Program and vibrant Enrichment Program to initiate research programs in biomedical and behavioral diabetes-related areas
read more
http://www.einstein.yu.edu/centers/diabetes-research/diabet

UW Site


depts.washington.edu/diabetes/about/links.niddk.html
NIDDK Diabetes Centers
Search
UNIVERSITY OF WASHINGTON | SEATTLE, WASHINGTON
diabetes endocrinology research center
Home
About DRC
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Directors
Administrative Core
Biomedical Cores
Research Base
Affiliate Investigators
Seminars & Symposia
P & F Studies Program
FAQ's
Glossary
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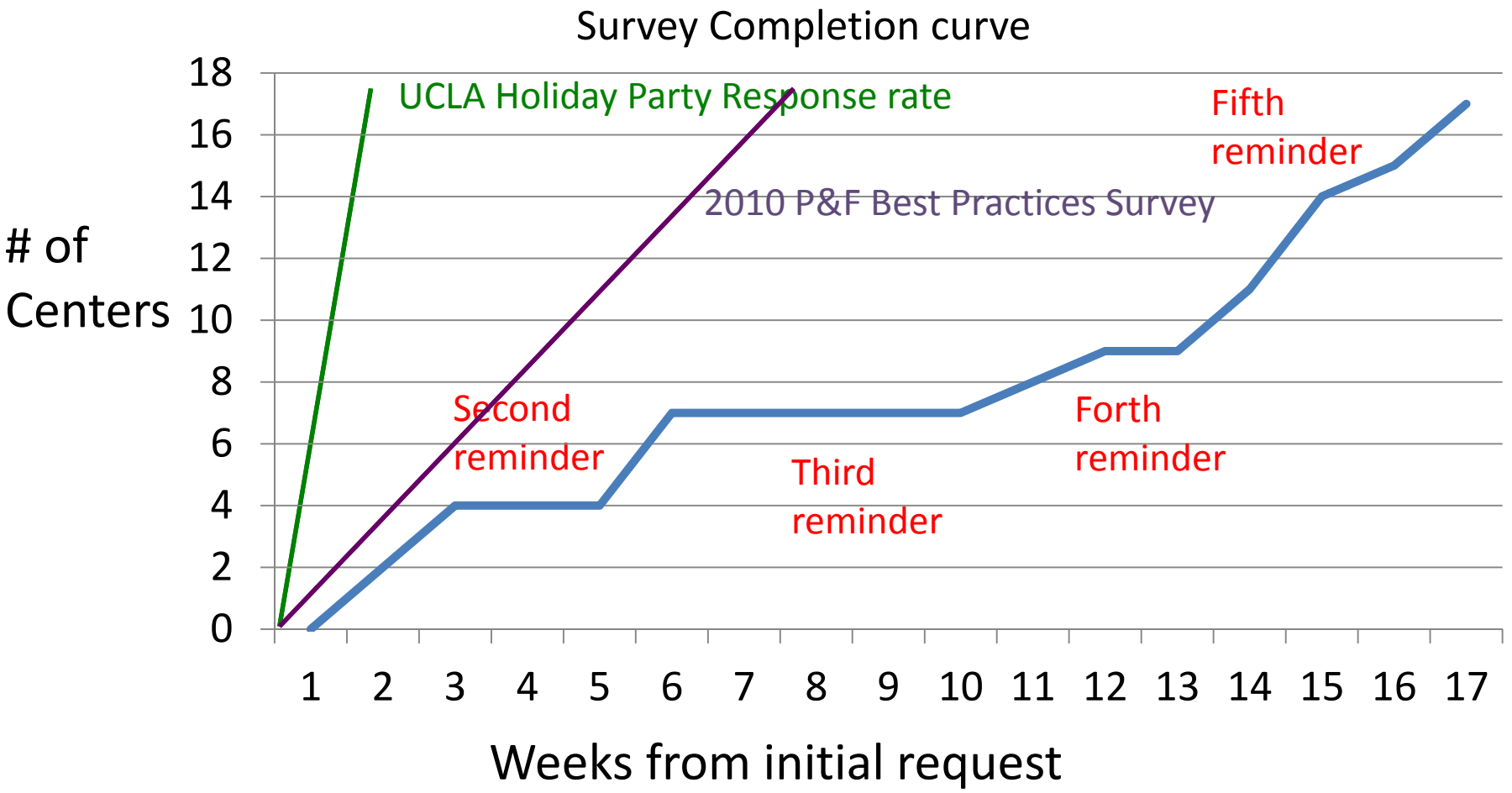
Site Map
Home > About DRC
NIDDK Diabetes Centers
NIDDK Centers Website
Albert Einstein College of Medicine DRTC
Boston Area DRC
Columbia DRC
Joslin Diabetes Center
University of Colorado DRC
University of Chicago DRTC
University of California, SF DRC
UCLA/UCSD DRC
University of Massachusetts DRC
UW Research Centers and Programs
Research Resources at UW
Affiliated Research Institutions in Seattle
NIDDK Diabetes Centers
Federal Agencies and Diabetes Related Programs
Professional and Voluntary Organizations
Journals and Books

Background

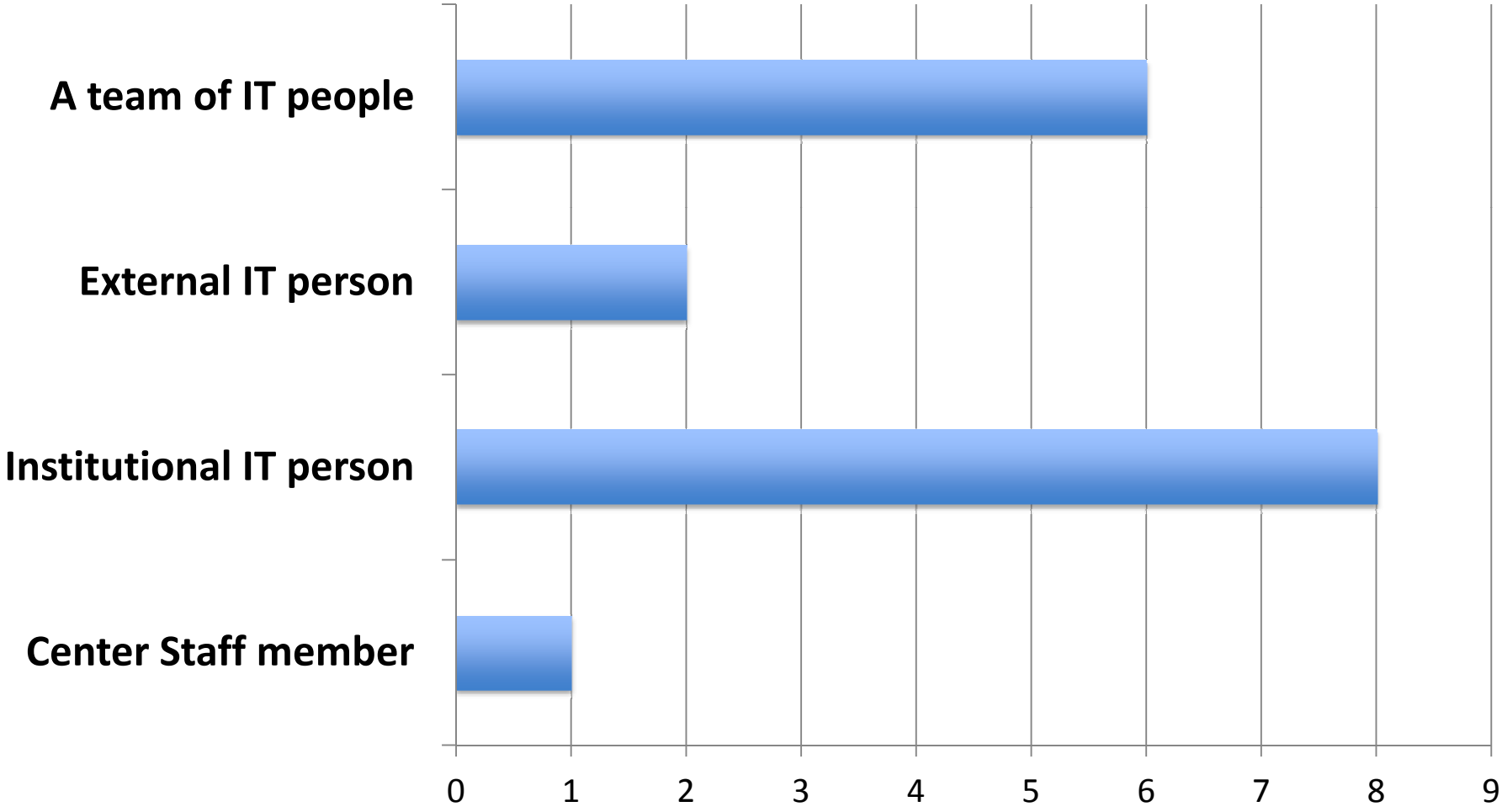
- Diabetes Centers are expected to manage individual Websites.
- These Websites are an important part of the mission of the Centers.
- The Websites are managed locally with no NIDDK oversight.
- Actual Website management, design, and content are quite variable.
- There is no apparent interconnectivity among sites at the current time.
- Information about the management of these Websites, the cost involved, the personnel committed, and the tools employed was unavailable.
- The Diabetes Centers Executive Committee commissioned a survey…

Survey-Monkey to assess diversity

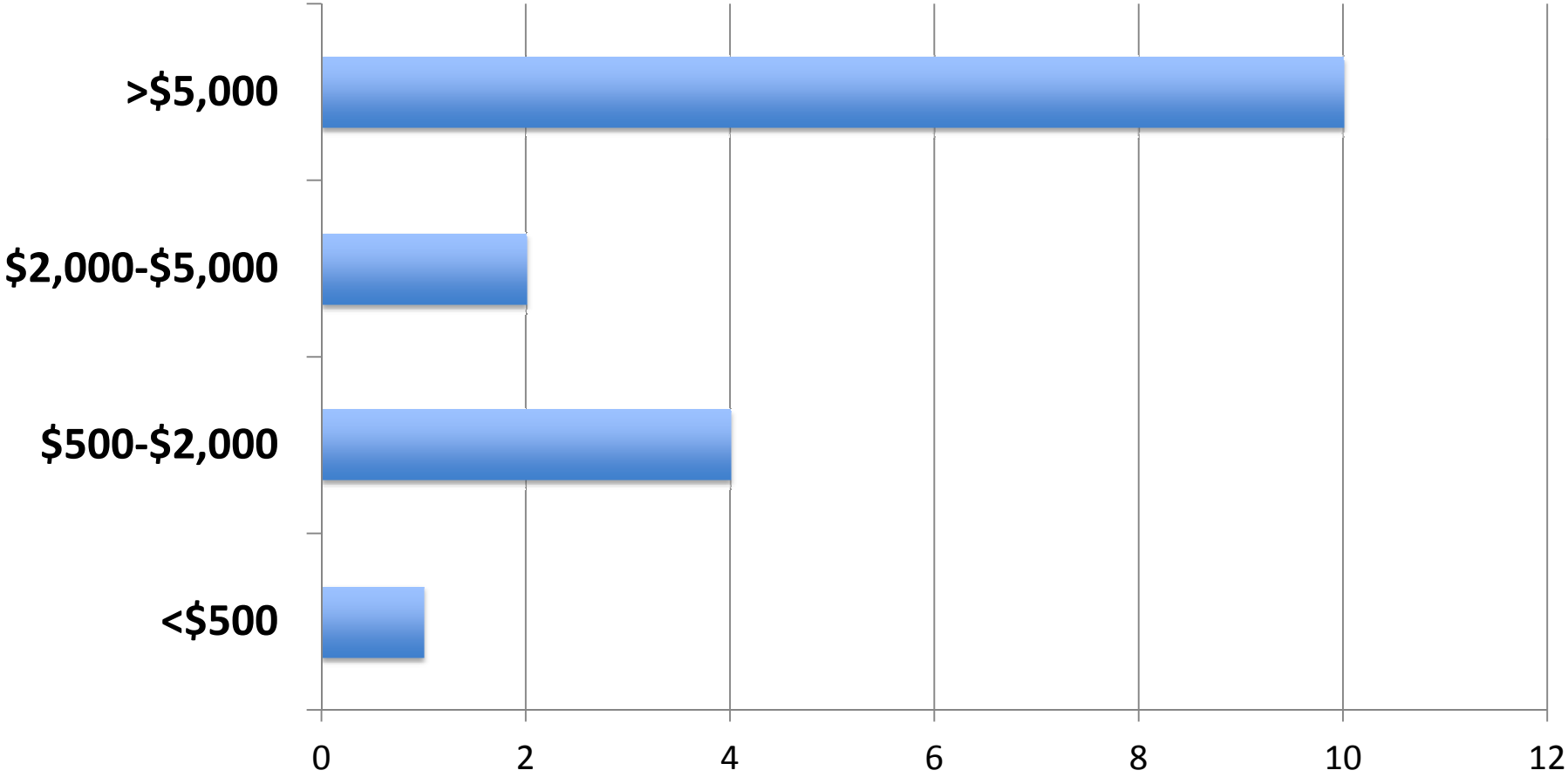
(17 Center Directors Contacted April-Sept 2011)



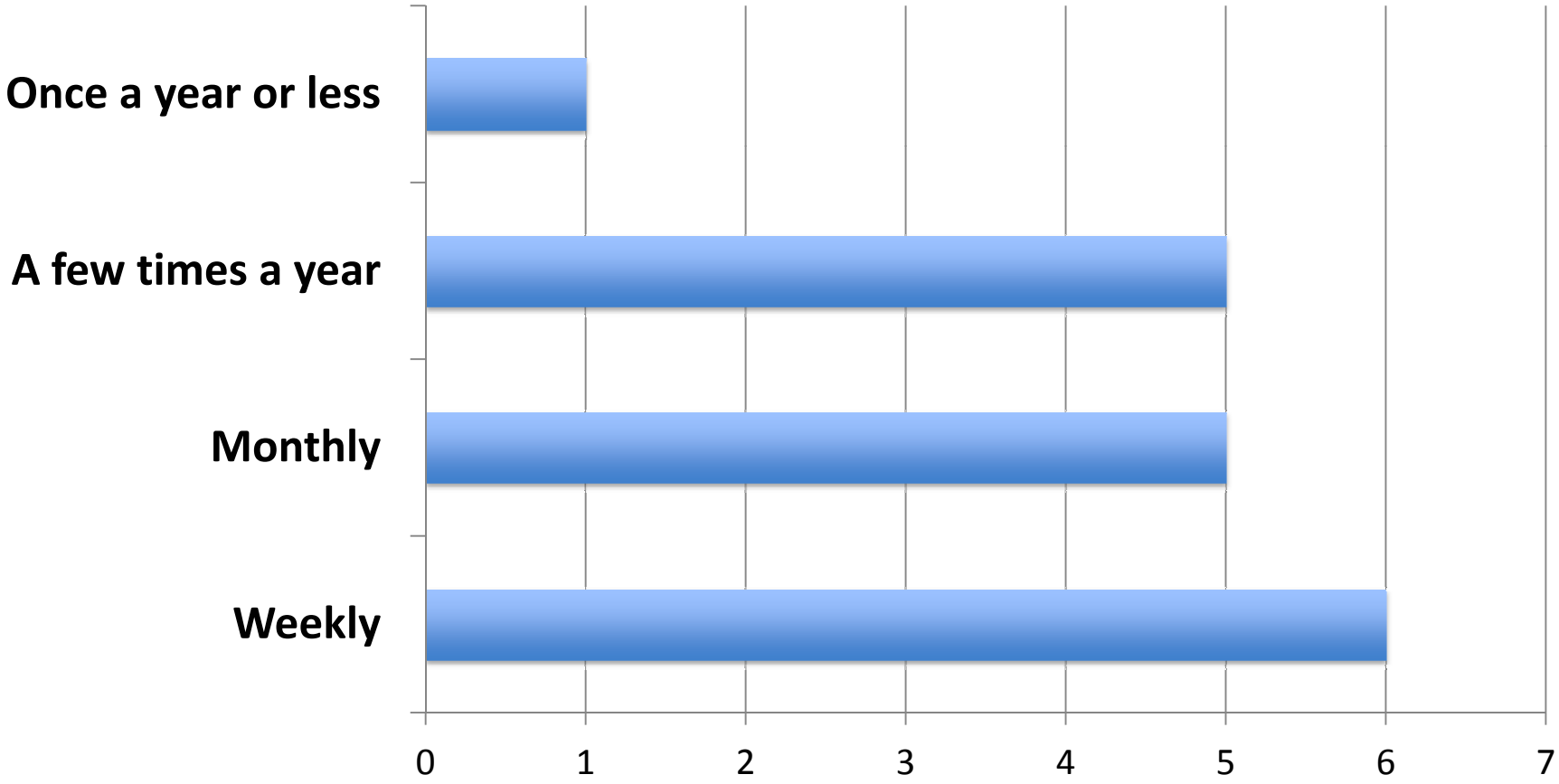
What type of individual designed your Diabetes Center website?



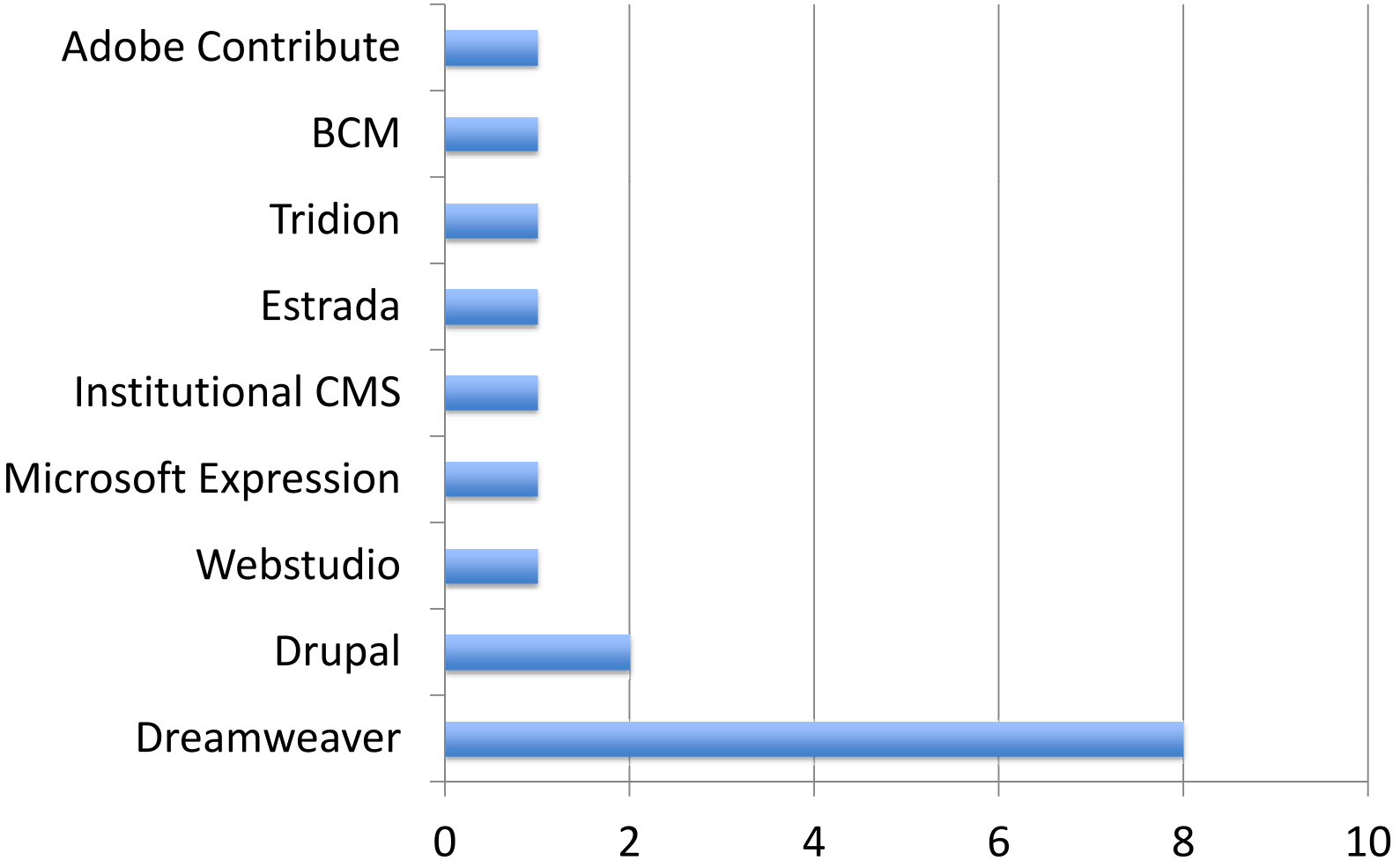
Approximately how much did your Center pay to have your Diabetes Center website designed/created?



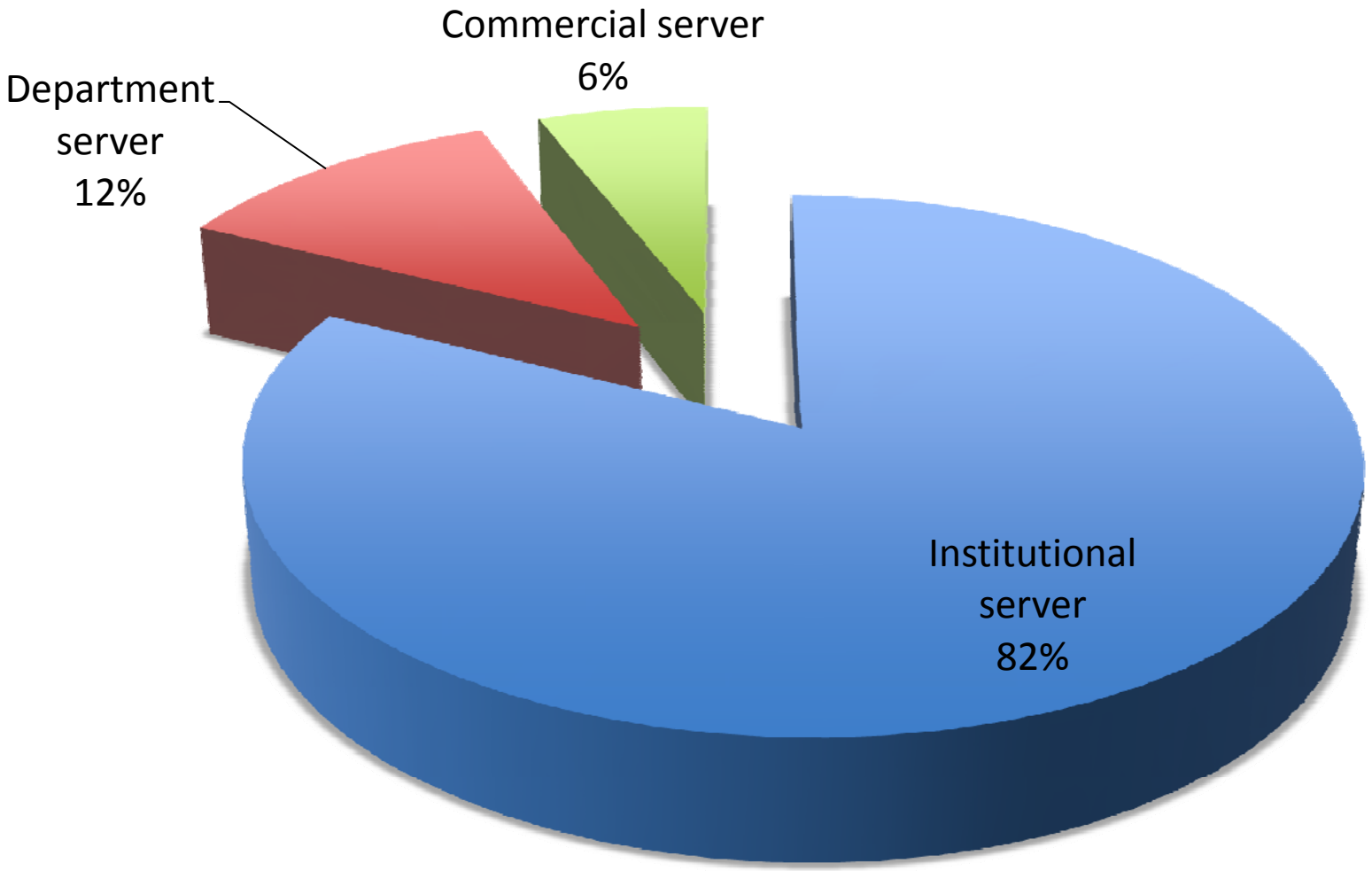
How often is your website updated?



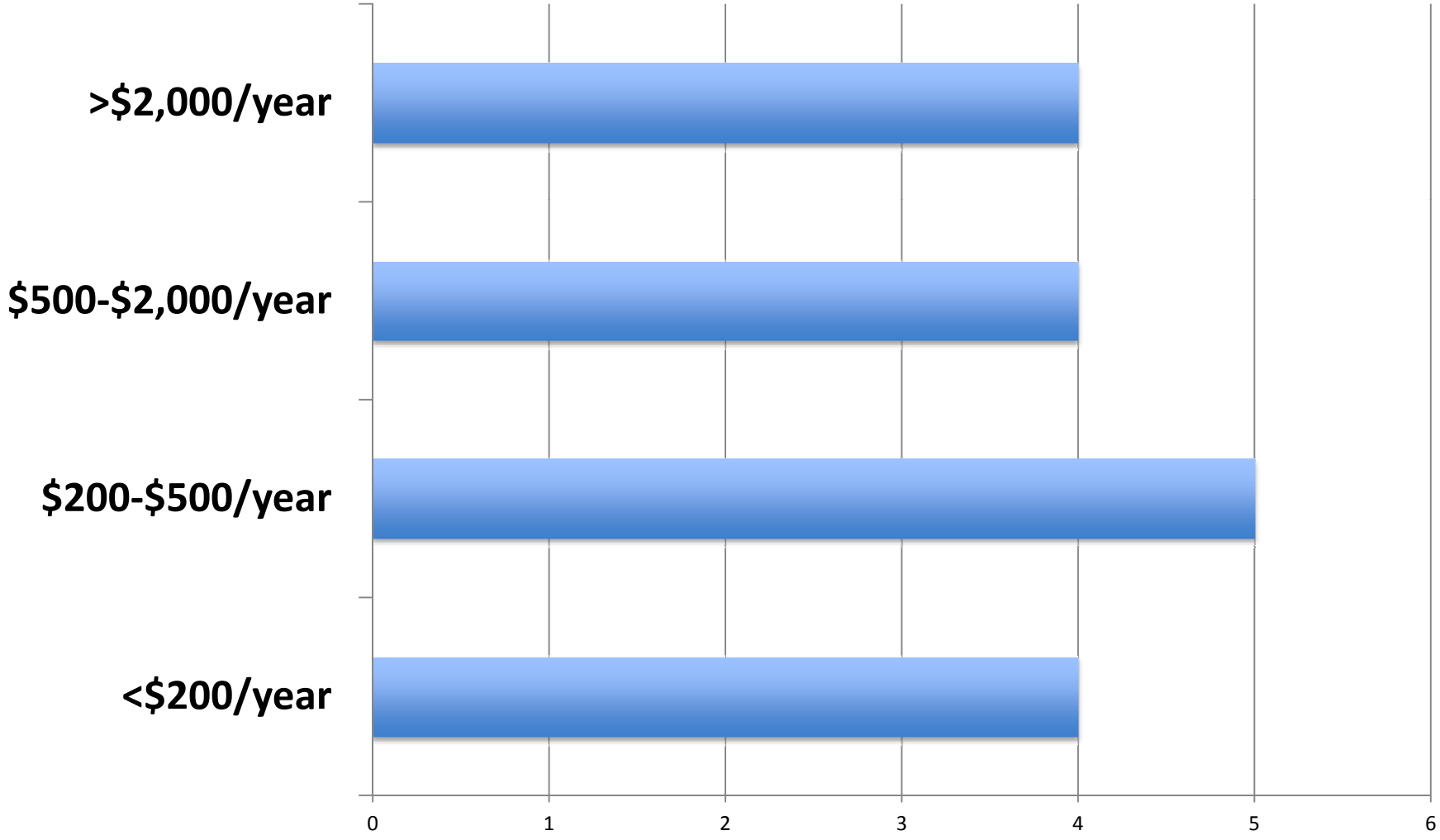
What website design software are you currently using for your Diabetes Center website?



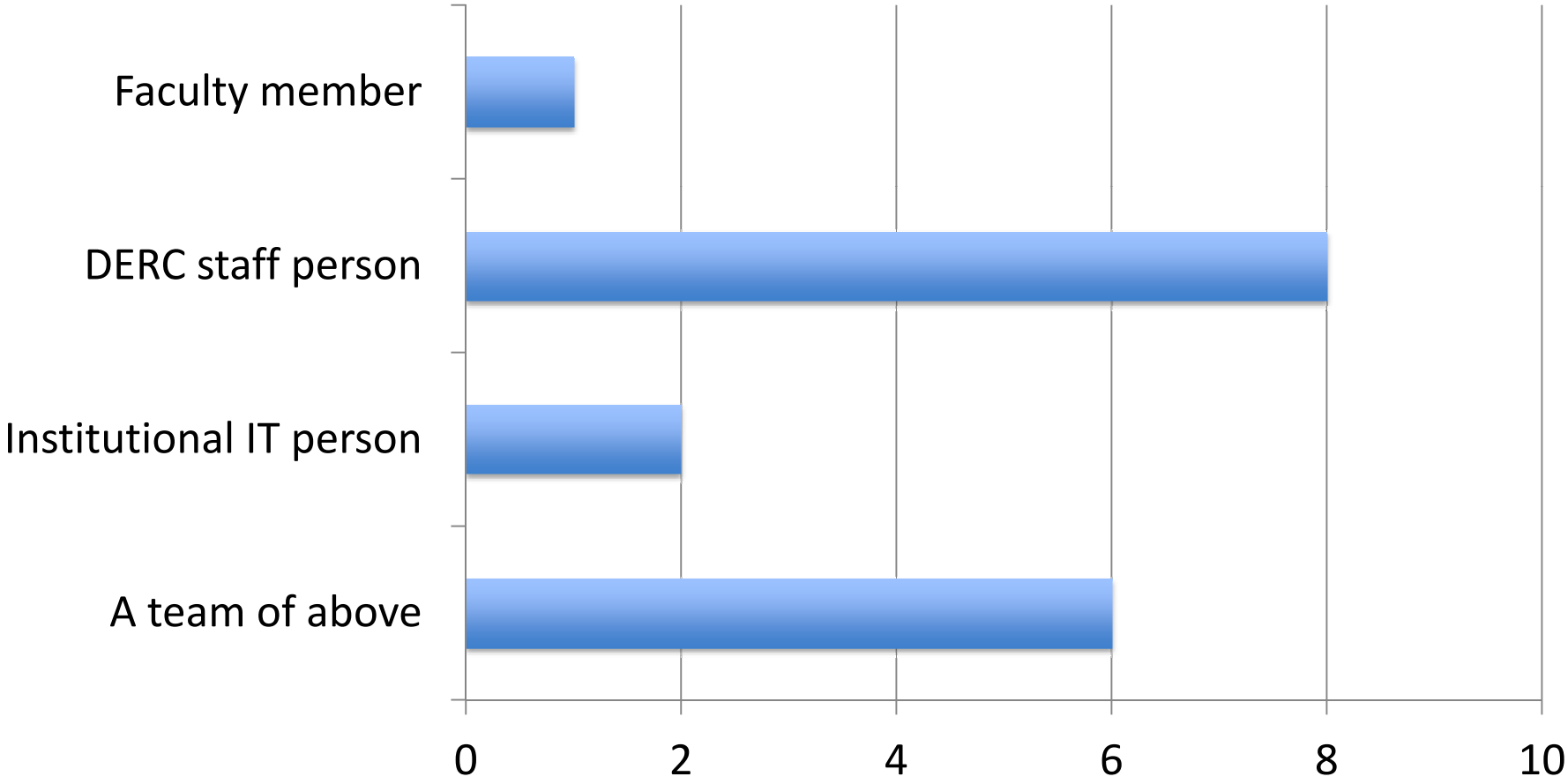
Where is your Diabetes Center website hosted?



What is the charge for hosting and maintenance for your site?



What type of individual maintains/curates your website?



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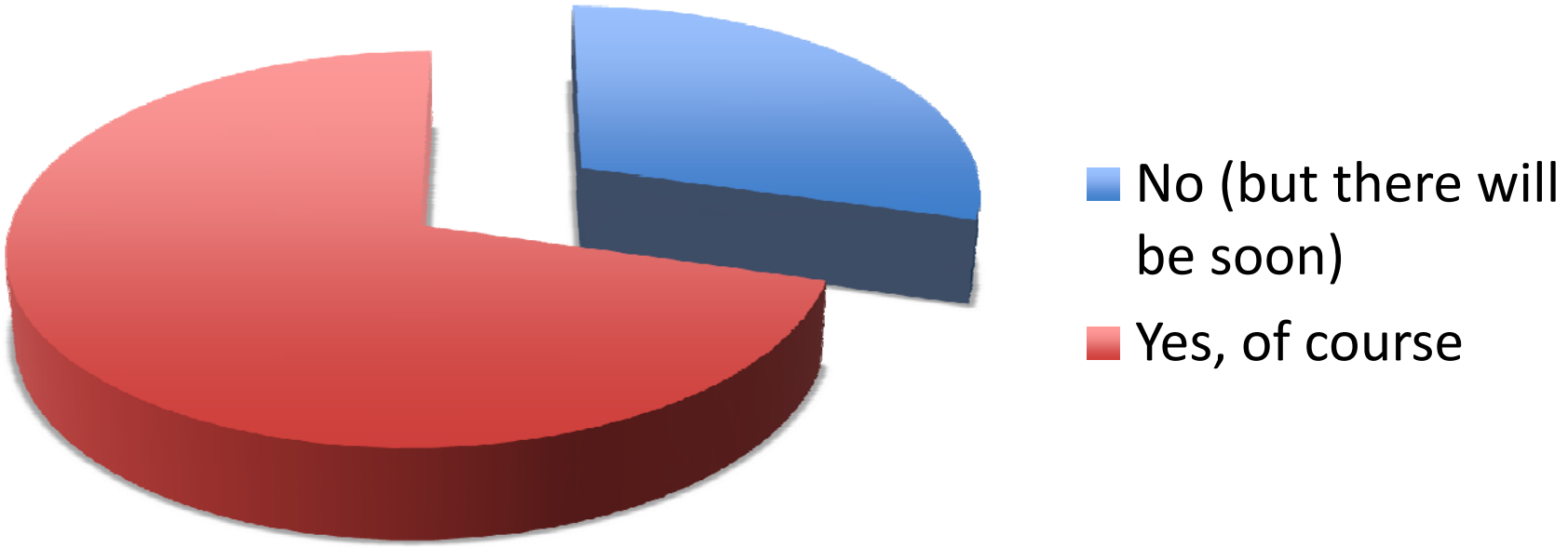
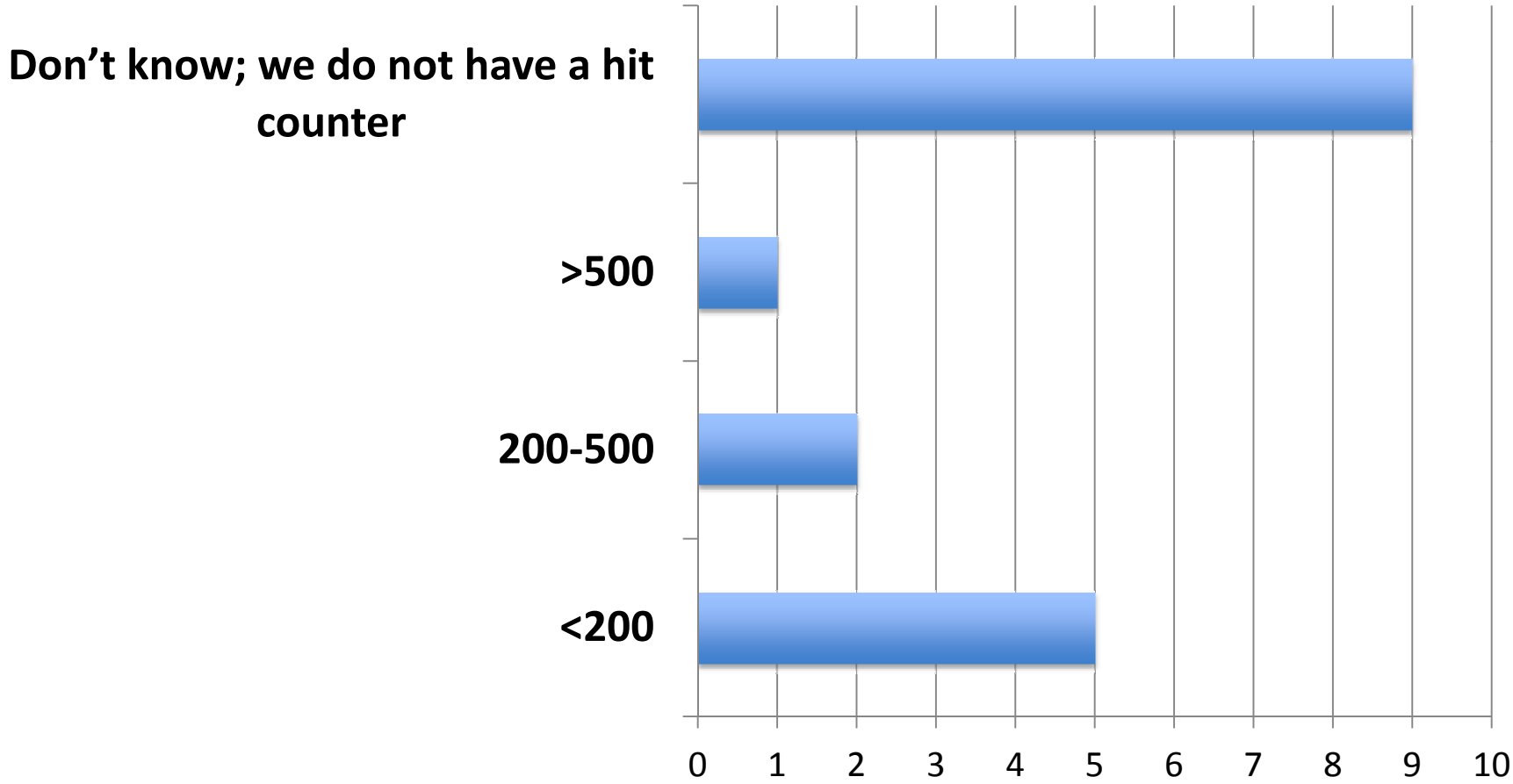
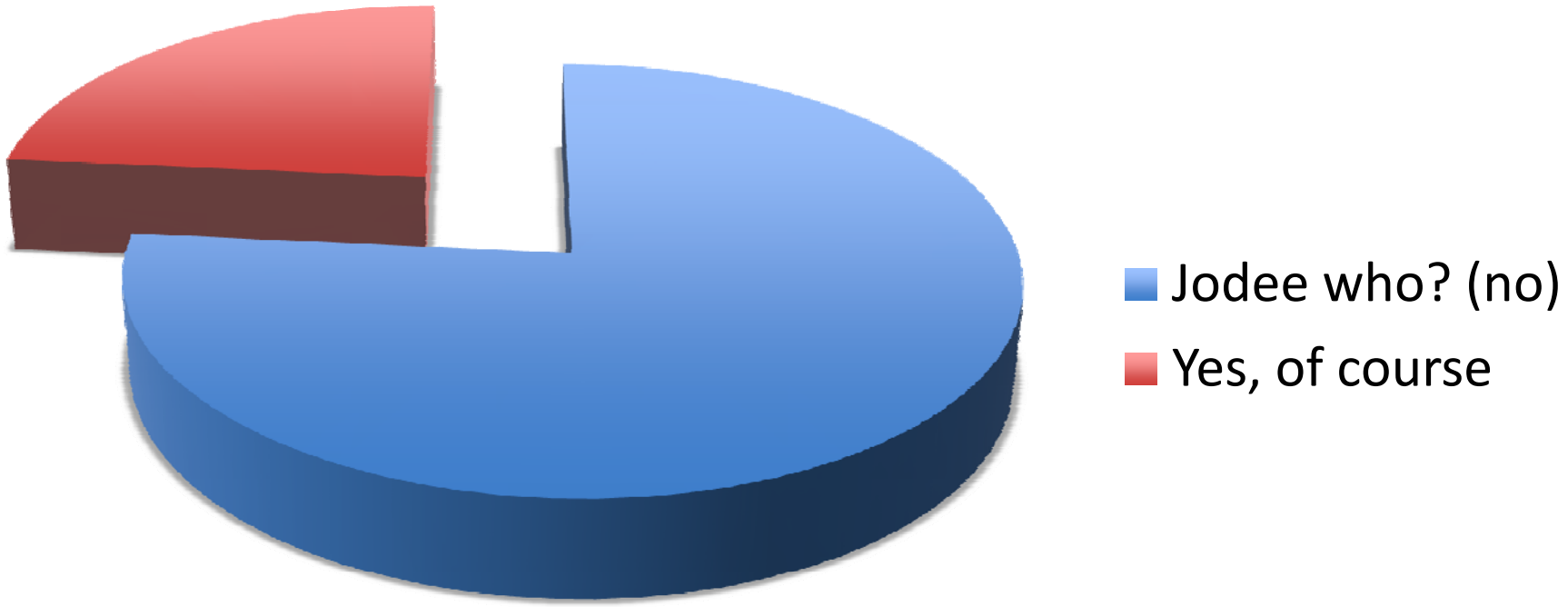


How many hits does your institutional Diabetes Center website have in an average month?



Does your Diabetes Center website manager communicate with Jodee Allen (central Diabetes Center website manager)?



Conclusions

- The cost of operating the websites is substantial
- Diabetes Center Websites are highly Diverse
- Each Center operates independently
- There is little or no interconnectedness
- Sites use different software for websites
- There is little connection to the “mother ship”
- Activity on most of these sites is robust

How can we improve?

- Define our goals!
- Consider expanding into social media
- Platform harmonization
- More uniformity (without losing originality and creativity)
- Interconnectedness
- Across-sites searchability
- Potential added features
  - bulletin boards
  - technical Q&A
  - “shop for services”
- Inclusion in DRC renewals?

DIABETES RESEARCH CENTERS

Diabetes Center Research Cores 2011

Albert Einstein College of Medicine

Analytical Imaging Core

Animal Physiology Core

Epigenomics Core

Flow Cytometry Core

Hormone Assay Core

Stable Isotopes and Metabolomics Core

Prevention & Control Core: Clinical Research Methodology, Behavioral Intervention/Evaluation Methodology, Social-Environmental Research Methodology

Baylor College of Medicine

Gene Vector Core

Microarray Resource, Biostatistics, and Bioinformatics Core

Mouse Metabolism Core

Mouse MRI Core

Proteomics Core

RNA In Situ Hybridization Core

Boston Area

Cell Biology and Morphology Core

Flow Cytometry Core

Animal Metabolic Physiology Core

Molecular Biology Core

Transgenic Core

Columbia University

Animal Phenotyping Core

Genomics Core

Histopathology Core

Hormone/Metabolites Core

Protein Production Core

Johns Hopkins University/Univ. Maryland (Baltimore Area)

Cell Biology Core

Integrated Physiology Core

Transgenic/ES Cell Core

Genetics Core

Clinical Investigation Core

Prevention & Control Core

Joslin Diabetes Center

Advanced Microscopy Core

Animal Physiology Core

Bioinformatics Core

Flow Cytometry Core

Genetics Core

Genomics Core

Proteomics Core

Specialized Assay Core

University of Alabama

Animal Physiology Core

Bio-Analytical Redox Biology Core

Community Engagements Core

Human Physiology Core

Metrics and Health Services Resources Core

UCSD/UCLA

Human Genetics Core (Cedars-Sinai Medical Center)

Inflammation Core

Mouse Phenotyping Core (UCLA)

Transcriptional Genomics Core

Transgenic and KO Mice Core

UCSF

Cytometry and Cell Sorting Core

Islet Metabolism Core

Microscopy Core

Mouse Genetics Core

University of Chicago

Islet Cell Biology Core

Ligand Assay Core

Molecular Biology and Genetics Core

Physiology Core

Animal Models Core

Prevention & Control Core

University of Michigan

Animal Phenotyping Core

Behavioral, Clinical and Health Systems (BCHS) Intervention Research Core

Biostatistics and Economic Modeling Core

Cell and Molecular Biology Core

Measurement Core

Morphology and Image Analysis Core

Peptide and Proteomics Core (Wayne State University)

University of Pennsylvania

Functional Genomics Core

Islet Cell Biology Core

Mouse Phenotyping, Physiology and Metabolism Core

Radioimmunoassay/Biomarkers Core

Transgenic and Chimeric Mouse Core

Viral Vector Core

University of Washington

Cellular and Molecular Imaging Core

Clinical Research Core

Islet Cell and Functional Analysis Core

Immunology and Inflammation Core

Mass Spectrometry Core

Virus, Molecular Biology and Cell Core

Vanderbilt University

Cell Imaging Shared Resource Core

Hormone Assay and Analytical Services Core

Islet Procurement and Analysis Core

Metabolic Physiology Shared Resource Core

Transgenic/ES Cell Shared Resource Core

Prevention, Control and Translation Core

Washington University

Immunoassay Core

Immunology of Type 1 Diabetes Core

Mass Spectrometry Core

Morphology and Bioimaging Core

Mouse Phenotyping Core

Transgenic and ES Cell Core

Prevention & Control Core

Yale University

Cell Biology Core

Clinical Metabolism Core

Diabetes Translational Core

Molecular Core

Physiology Core

Animal/Transgenic Core

DIABETES RESEARCH CENTERS

Diabetes Center Research Cores 2011

Animal Physiology and Phenotyping Cores (13 Diabetes Centers)

AECOM (Animal Physiology), Baltimore Area (Integrated Physiology), Baylor (Mouse MRI/Metabolism), Boston Area (Animal Metabolic Physiology), Columbia (Animal Phenotyping), Joslin (Animal Physiology), UAB (Animal Physiology), UCSD/UCLA (Mouse Phenotyping), U Michigan (Animal Phenotyping), U Penn (Mouse Phenotyping, Physiology & Metabolism), Vanderbilt (Metabolic Phenotyping), Washington U (Mouse Phentoyping), Yale (Animal Physiology)

Molecular Biology, Genetics and Genomics Cores (12 Diabetes Centers)

AECOM (Epigenomics Core), Baylor (Microarray Resource, Biostatistics and Bioinformatics), Boston Area (Molecular Biology), Columbia (Genomics), Baltimore Area (Genetics), Joslin (Genetics, Genomics, Bioinformatics), UCSD/UCLA (Transcriptional Genomics; Human Genetics), U Chicago (Molecular Biology and Genetics), U Michigan (Cell and Molecular Biology), U Penn (Functional Genomics; Viral Vector), U Washington (Virus, Molecular Biology and Cell); Yale (Molecular)

Histology, Morphology and Image Analysis Cores (11 Diabetes Centers)

AECOM (Analytical Imaging), Baylor (RNA In Situ Hybridization), Boston Area (Cell Biology and Morphology), Columbia (Histopathology), Joslin (Advanced Microscopy), UCSF (Microscopy), U Michigan (Morphology and Image Analysis), U Washington (Cellular and Molecular Imaging), Vanderbilt (Cell Imaging), Washington U (Morphology and Bioimaging), Yale (Cell Biology)

Transgenic, Specialized Animal & Gene Modification Cores (10 Diabetes Centers)

Baylor (Gene Vector Core), Boston Area (Transgenic), Baltimore Area (Transgenic/ES Cell), UCSD/UCLA (Transgenic and KO Mice), UCSF (Mouse Genetics), U Chicago (Animal Models), U Penn (Transgenic and Chimeric Mouse); Vanderbilt (Transgenic/ES Cell); Washington U (Transgenic and ES Cell; Immunology of T1D Core); Yale (Animal/Transgenic)

Assay Cores – Human and Laboratory Animal (10 Diabetes Centers)

AECOM (Hormone Assay), Columbia (Hormone/Metabolites), Joslin (Specialized Assay), UAB (Bio-Analytical Redox Biology), UCSD/UCLA (Inflammation), U Chicago (Ligand Assay), U Michigan (Chemistry Laboratory in Measurement Core), U Penn (Radioimmunoassay/Biomarkers), U Washington (Immunology & Inflammation), Vanderbilt (Hormone Assay and Analytical Services), Washington U (Immunoassay)

Protein, Proteomics, Metabolomics and Mass Spectrometry Cores (7 Diabetes Centers)

AECOM (Stable Isotopes and Metabolomics), Baylor (Proteomics), Columbia (Protein Production) Joslin (Proteomics), U Michigan (Peptide and Proteomics), U Washington (Mass Spectrometry), Washington U (Mass Spectrometry)

Clinical Investigation Cores (6 Diabetes Centers)

Baltimore Area (Clinical Investigation), UAB (Human Physiology), U Chicago (Physiology), U Michigan (Measurement; Behavioral, Clinical and Health Systems); U Washington (Clinical Research), Yale (Clinical Metabolism; Diabetes Translational)

Prevention and Control Cores (7 Diabetes Centers; multiple sub-cores; also support clinical investigation)

AECOM, Baltimore Area, UAB, U Chicago, U Michigan, Washington U, Vanderbilt

Islet Biology, Cell Biology and Metabolism Cores (6 Diabetes Centers)

Baltimore Area (Cell Biology), UCSF (Islet Metabolism), U Chicago (Islet Cell Biology), U Penn (Islet Cell Biology), U Washington (Islet Cell and Functional Analysis), Vanderbilt (Islet Procurement and Analysis)

Flow Cytometry Cores (4 Diabetes Centers)

AECOM (Flow Cytometry), Boston Area (Flow Cytometry), Joslin (Flow Cytometry), UCSF (Cytometry and Cell Sorting)

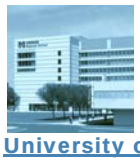
http://www.mmpc.org/

The MMPC is sponsored by the National Institutes of Health as a resource to provide services to the community of scientists who use mice to study diabetes, obesity, diabetic complications, and other metabolic diseases.

Case Western Reserve University
DIRECTOR: Henri Brunengraber, M.D., Ph.D.
DIRECTOR EMAIL: henri.brunengraber@case.edu
GENERAL CONTACT EMAIL: mmpc@case.edu
Provides isotopic measurement of metabolic pathway fluxes such as lipid and protein turnover using mass spec.
See a complete list of Case Western Reserve University MMPC tests.

University of California Davis
DIRECTOR: K.C. Kent Lloyd, DVM, Ph.D.
DIRECTOR EMAIL: kclloyd@ucdavis.edu
GENERAL CONTACT EMAIL: mmpc@ucdavis.edu
Provides tests for metabolism, insulin resistance, glucose balance, energy balance, eating behavior and cardiovascular phenotyping,
See a complete list of University of California Davis MMPC tests.

University of Cincinnati Medical Center
DIRECTOR: Patrick Tso, Ph.D.
DIRECTOR EMAIL: patrick.tso@uc.edu
GENERAL CONTACT EMAIL: dana.lee@uc.edu
Tests for lipid metabolism, energy balance and eating behavior, myocardial and macrovascular disease.
See a complete list of University of Cincinnati Medical Center MMPC tests.



University of Massachusetts Medical School
DIRECTOR: Jason Kim, Ph.D.
DIRECTOR EMAIL: Jason.Kim@umassmed.edu
GENERAL CONTACT EMAIL: elana.hastings@umassmed.edu
Tests for insulin resistance, glucose metabolism, insulin secretion, energy balance, obesity, and serum/tissue factors of diabetes.
See a complete list of University of Massachusetts Medical School MMPC tests.

Vanderbilt University School of Medicine
DIRECTOR: David Wasserman, Ph.D.
DIRECTOR EMAIL: david.wasserman@vanderbilt.edu
GENERAL CONTACT EMAIL: mmpc@vanderbilt.edu
Tests for insulin resistance and glucose metabolism, heart and kidney function as well as energy balance and feeding studies.
See a complete list of Vanderbilt University School of Medicine MMPC tests.

http://www.mmpc.org/

Yale University School of Medicine
DIRECTOR: Gerald Shulman, M.D., Ph.D.
DIRECTOR EMAIL: gerald.shulman@yale.edu
GENERAL CONTACT EMAIL: mmpc@yale.edu
Tests for insulin resistance and glucose balance, as well as energy balance, eating behavior and activity.
See a complete list of Yale University School of Medicine MMPC tests.

Georgia Health Sciences University - Coordinating and Bioinformatics Unit
DIRECTOR: Richard McIndoe, Ph.D.
DIRECTOR EMAIL: rmcindoe@georgiahealth.edu
GENERAL CONTACT EMAIL: jhigdon@georgiahealth.edu

The CBU coordinates the MMPC consortium activities and houses website and database. The CBU is shared with the NIH-sponsored Animal
Models of Diabetes Complications Consortium.

Mouse Metabolic Phenotyping Centers

Maren R. Laughlin, Ph.D.
Senior Advisor for Integrative Metabolism
National Institute of Diabetes & Digestive & Kidney Diseases

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

MMPC National Mouse Metabolic Phenotyping Centers

Mouse Metabolic Phenotyping Centers

www.mmpc.org

Mission

To advance medical and biological research by providing the scientific community with standardized, high quality metabolic and physiologic phenotyping services for mouse models of diabetes, diabetic complications, obesity and related disorders.

Centers 2011-15

Case Western Reserve University
DIRECTOR: **Henri Brunengraber, M.D., Ph.D.**

University of Cincinnati Medical Center
DIRECTOR: **Patrick Tso, Ph.D.**

Yale University School of Medicine
DIRECTOR: **Gerald Shulman, M.D., Ph.D.**

Vanderbilt University School of Medicine
DIRECTOR: **David Wasserman, Ph.D.**

University of Massachusetts Medical School
DIRECTOR: **Jason Kim, Ph.D.**

University of California, Davis
DIRECTOR: **Kent Lloyd, D.V.M., Ph.D.**

Coordinating and Bioinformatics Unit

Georgia Health Science University
DIRECTOR: **Richard McIndoe, Ph.D.**

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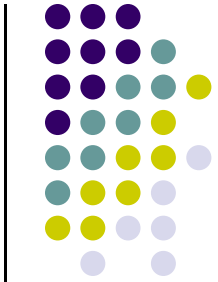
MMPC Administration and External Evaluation Committee



NIH

- Maren Laughlin, NIDDK
- Kristin Abraham, NIDDK
- Cristina Rabadan-Diehl, NHLBI
- Narasimhan Danthi, NHLBI

External Panel

- Joe Nadeau, Case Western Reserve
- Matthew Breyer, Lilly
- Thomas Gettys, Pennington
- Debbie Muoio, Duke University
- Kevin Hall, NIDDK intramural

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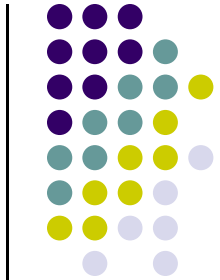
Major MMPC Activities



- Conduct TESTS on mice and isolated tissues
  – ship to MMPC, fee for service (~60% cost)
- Compile a DATABASE of phenotyping test data on background strains and genetic models
- STANDARDIZE and COMPARE existing methods
  (SOPs, research and opinion papers)
- DEVELOP and INTERROGATE methods *(collaborative projects)*
- EDUCATION
  - 3 annual 1-2 week courses plus visiting post-docs, etc.
  - Informative websites
  - Phone consults
- Research FUNDING programs (MICROMouse)
- Diabetic Complications Consortium (DCC) - phenotype NEW MODELS

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Phenotyping

In-depth metabolic/physiologic testing of mouse models

- PI sends mice, blood or tissues to a Center
- Fee-for-service testing
- Data returned to PI

Why?

- Lots of exciting expensive mouse models that deserve extensive phenotyping.
- High value on metabolic / physiological data that can elucidate function
- Many desired tests too difficult, expensive, for every lab to do
- MMPC allows wide sharing of technology through experiments, database, publications and training experiences.

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Address http://www.mmpc.org/index.aspx Go

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Welcome to the National Mouse Metabo Centers

The MMPC is sponsored by the National Institutes of H provide services to the community of scientists who use mice to study diabetes, obesity, dia nd other metabolic diseases.

NEW ACCOUNCEMENTS PAGE AVAILABLE - CLICK HERE

Case Western Reserve University

DIRECTOR: Henri Brunengraber, M.D., Ph.D.
DIRECTOR EMAIL: henri.brunengraber@case.edu
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Provides isotopic measurement of metabolic pathway fluxes such as lipid and protein turnover using mass spec.
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University of Cincinnati Medical Center

DIRECTOR: Patrick Tso, Ph.D.
DIRECTOR EMAIL: patrick.tso@uc.edu
GENERAL CONTACT EMAIL: dana.lee@uc.edu
Tests for lipid metabolism, energy balance and eating behavior, myocardial and macrovascular disease.
See a complete list of University of Cincinnati Medical Center MMPC tests.

University of Texas Southwestern Medical Center

DIRECTOR: Craig Malloy, M.D.
DIRECTOR EMAIL: craig.malloy@utsouthwestern.edu
GENERAL CONTACT EMAIL: shawn.burgess@utsouthwestern.edu
Provides isotopic measurement of metabolic pathway fluxes such as TCA cycle and gluconeogenesis using NMR.
See a complete list of University of Texas Southwestern Medical Center MMPC tests.

University of Washington, Seattle

DIRECTOR: Renee LeBoeuf, Ph.D.
DIRECTOR EMAIL: leboeuf@u.washington.edu
GENERAL CONTACT EMAIL: geronimo@u.washington.edu

Log In to MMPC

User Name
maren.laughlin@nih.gov

Password

Please save my user name.

Forgot Password | Create Login

GO

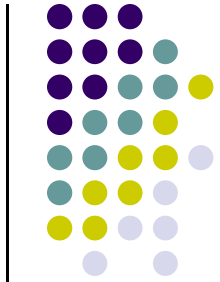
Whats New...

Morphology Services at University of Washington

The American Society of Nephrology (ASN) annual meeting will take place in Philadelphia, PA November 4-9.

http://www.mmpc.org/shared/catalog.aspx Microphone net

MMPC Quantitative Description



- 1114 members
- ~12 test cores
- >800 mouse strains studied (in database)
- >5000 mice studied (in database)
- 75,000 tests (2007-2009)
  - 3300 insulin clamps
  - 3600 indirect calorimetry
  - 3400 blood pressure
  - 13,600 body composition
  - 530 TCA cycle turnover flux
- 315 published papers (2006-2010)
- 87% of survey respondents report an overall positive experience

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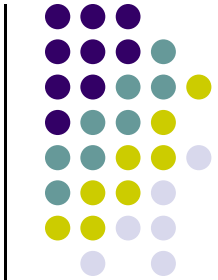
MMPC Test Classifications (Total of >200 tests)



- Energy Balance
  - Body Composition
  - Indirect Calorimetry
  - Temperature
  - Eating Behavior
  - Activity
  - Exercise tolerance

- Whole Body Glucose Metabolism, Insulin Resistance and Insulin Responsiveness
  - Hyperinsulinemic Euglycemic Clamp
  - Hyperglycemic Clamp
  - Hypoglycemic Clamp
  - Insulin and Glucose Tolerance Tests (oral, ip, iv)

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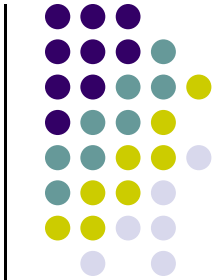
MMPC Test Classifications, continued



- Intermediary Metabolic Fluxes (liver, skeletal muscle, heart)
  - TCA cycle flux, anaplurosis, oxygen consumption, ATP turnover
  - Gluconeogenesis rate
  - Glycogen synthesis/turnover
  - Glucose, Protein, Ketone, Amino Acid Turnover
  - Lipid synthesis rates

- Lipid Metabolism
  - Lipid Absorption in gut
  - Lymph fistula (chylomicrons, lipoproteins)
  - Cholesterol, lipoprotein turnover
  - Fatty acid metabolism
  - Acyl-CoA concentrations

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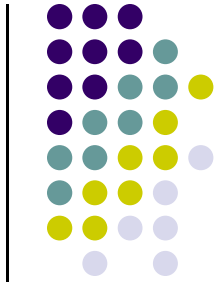
MMPC Test Classifications, continued



- Analyte and Hormone Measurements (plasma, lymph, tissues)
  - Isotopes (radioactive and stable by NMR and MS)
  - Hormones (insulin, glucagon, leptin, epinephrine, norepi)
  - Gut peptides (GLP-1, GIP, ghrelin, PYY)
  - Adipokines
  - Lipids, carbohydrates, amino acids
  - TCA cycle intermediates, etc.

- Histopathology / Morphometry
  - Kidney
  - Vasculature (atherosclerotic plaque)
  - Retina
  - Islet

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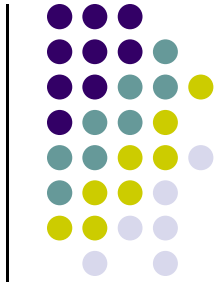
MMPC Test Classifications, continued



- Cardiovascular Function
  - Echocardiography, Systolic and Diastolic Function
  - Blood Pressure
  - Heart rate variability

- Renal Function
  - Glomerular Filtration Rate
  - Renal blood flow (Doppler)

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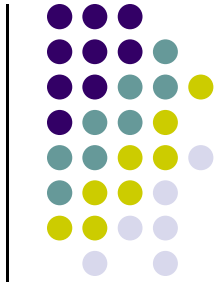
Future MMPC Test Classifications



- Imaging Metabolism
  - High energy status
  - Liver glycogen and lipid metabolism
  - Brain function

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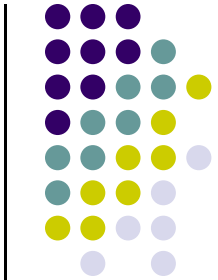
Example 1: Lymph Fistula model



- Awake, active animal
- Lipids, proteins from gut (diet, secreted)
- Transport into lymph can be monitored continuously
  - Lymph collection rate
    - 2 - 3 ml/h for rats
    - 0.2 - 0.3 ml/h for mouse
- Less protein modification in lymph vs. plasma
- Patrick Tso, Univ. Cincinnati MMPC

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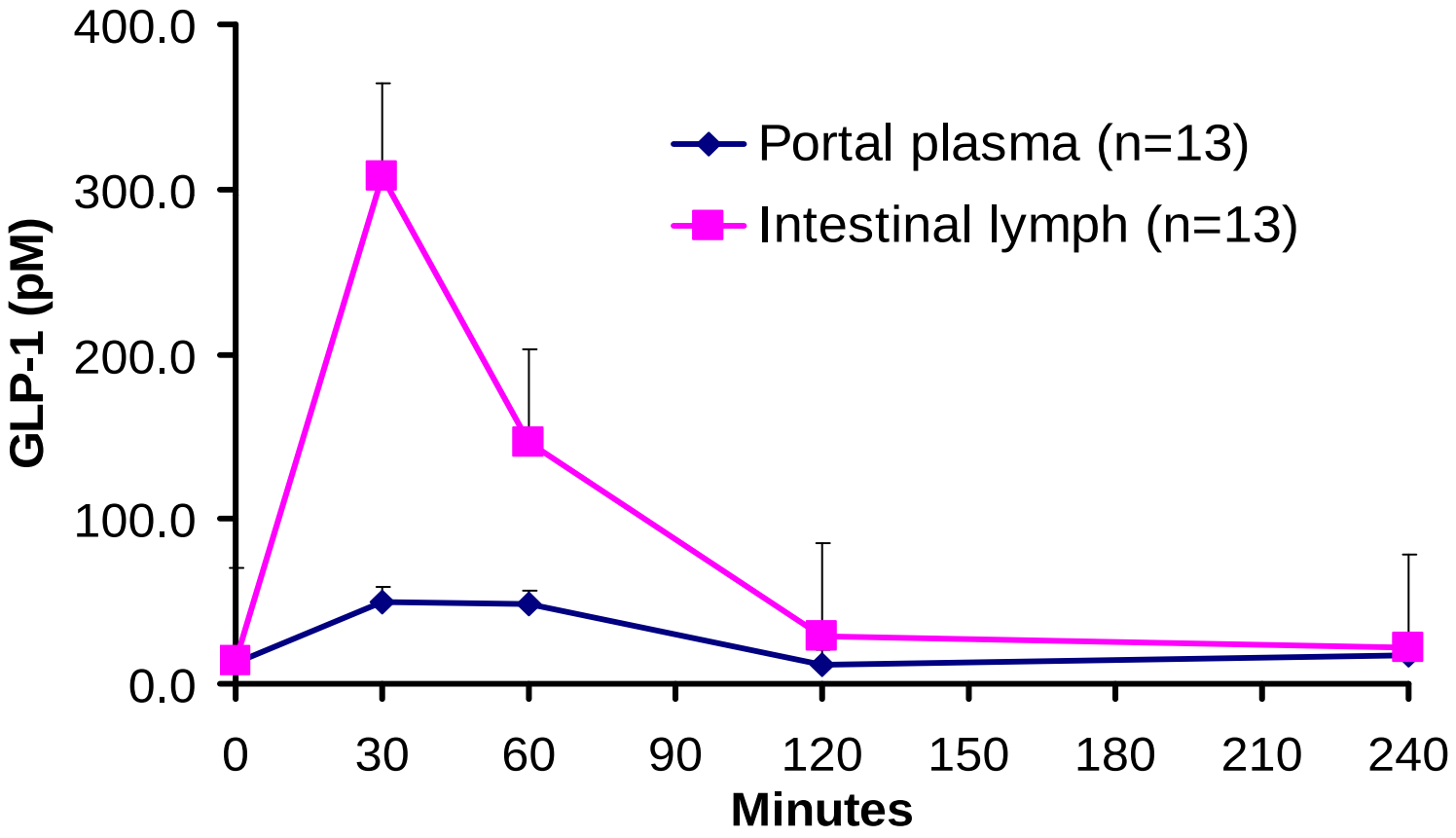
Effect of Ensure on Lymph & Portal Plasma GLP-1



D'Alessio et al., Am J Physiol, 2007

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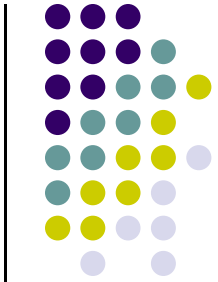
Example 2:
Enzyme concentration vs pathway flux



- Measure gluconeogenesis flux in perfused liver with stable isotopes and MR analysis

- Mice with variable concentrations of liver PEPCK used to assess control strength of a ‘rate determining’ step

- Shawn Burgess, U Texas Southwestern Medical Center MMPC in collaboration with Vanderbilt and Case researchers

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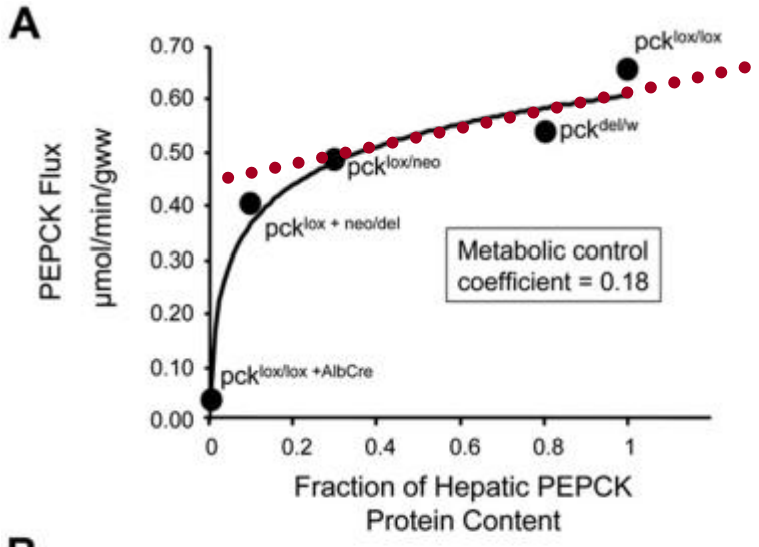

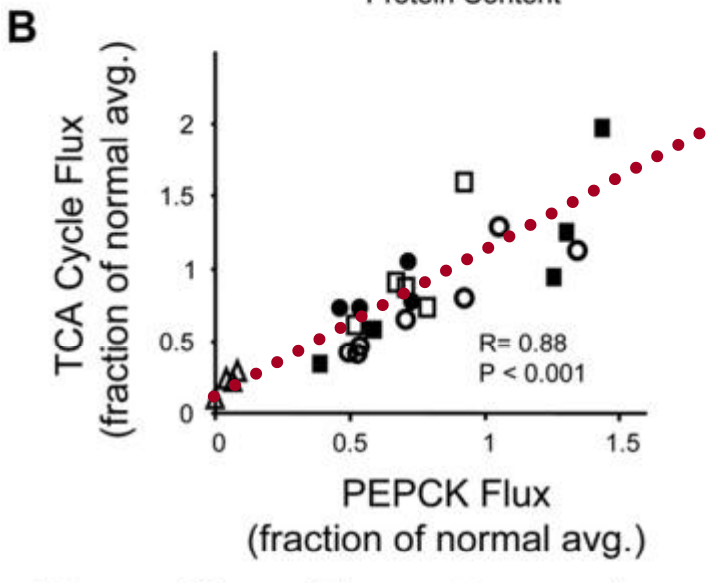

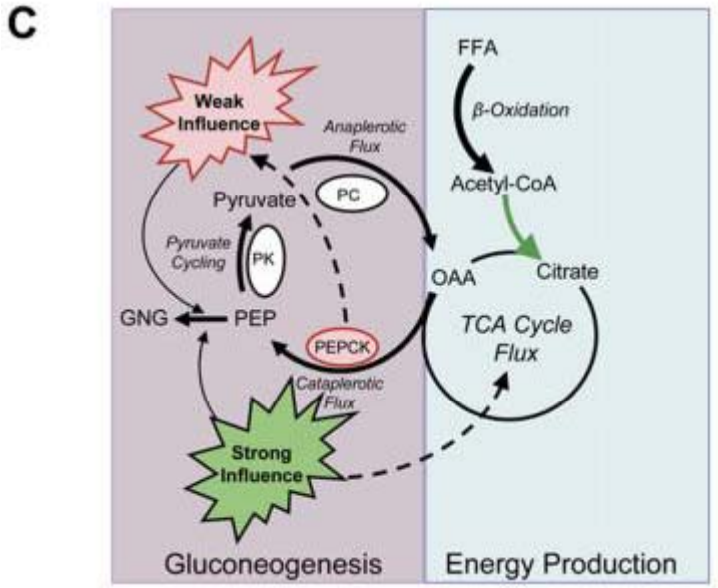

Burgess et al, Cell Metab 2007

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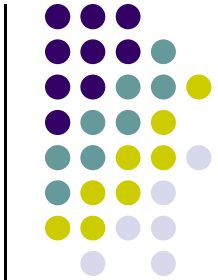
MMPC Database
Includes all MMPC data after publication or 2 years



Hyperinsulinemic Clamp, Insulin Dose = 4 U/kg/min

Test Group	Glucose (mg/dL)	Clamp Glucose (mg/dl)	Average GIR (mg/kg/min)	Basal Insulin (ng/ml)	Exper. Insulin (ng/ml)	Basal Ra (mg/kg/min)	Clamp Rd (mg/kg/min)	Clamp Endo Ra (mg/kg/min)
FVB/NJ 5 Hour Fasted/CHOW	144 ± 7 (7)	160 ± 5 (7)	56.6 ± 3.1 (7)	0.860 ± 0.218 (7)	2.08 ± 0.18 (7)	19.8 ± 1.3 (7)	61.0 ± 3.9 (7)	4.03 ± 2.35 (7)
FVB/NJ 5 Hour Fasted/CHOW Male	144 ± 7 (7)	160 ± 5 (7)	56.6 ± 3.1 (7)	0.860 ± 0.218 (7)	2.08 ± 0.18 (7)	19.8 ± 1.3 (7)	61.0 ± 3.9 (7)	4.03 ± 2.35 (7)
DBA/2J 5 Hour Fasted/CHOW	137 ± 6 (10)	153 ± 3 (10)	46.7 ± 2.2 (10)	1.270 ± 0.094 (10)	2.87 ± 0.29 (10)	18.6 ± 2.3 (10)	43.3 ± 1.8 (10)	-3.81 ± 0.92 (10)
DBA/2J 5 Hour Fasted/CHOW Male	137 ± 6 (10)	153 ± 3 (10)	46.7 ± 2.2 (10)	1.270 ± 0.094 (10)	2.87 ± 0.29 (10)	18.6 ± 2.3 (10)	43.3 ± 1.8 (10)	-3.81 ± 0.92 (10)
C57BL/6J 5 Hour Fasted/CHOW	143 ± 7 (8)	156 ± 4 (8)	54.4 ± 2.2 (8)	0.620 ± 0.086 (8)	1.96 ± 0.16 (8)	18.4 ± 1.9 (8)	49.1 ± 1.9 (8)	-5.56 ± 1.91 (8)

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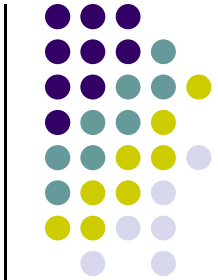
Standardize & Compare Tests



NIH Experiment in Centralized Mouse Phenotyping: the Vanderbilt Experience and Recommendations for Evaluating Glucose Homeostasis in the Mouse Owen P. McGuinness, Julio E. Ayala, Maren R. Laughlin, and David H. Wasserman. AJP - Endo October (2009) 297: E849-E855

Considerations in the Design of Hyperinsulinemic-Euglycemic Clamps in the Conscious Mouse
Julio E. Ayala, Deanna P. Bracy, Owen P. McGuinness, and David H. Wasserman. Diabetes: 55, 2006

Glucose Metabolism in Vivo in Four Commonly Used Inbred Mouse Strains
Eric D. Berglund, Candice Y. Li, Greg Poffenberger, Julio E. Ayala, Patrick T. Fueger, Shannon E. Willis, Marybeth M. Jewell, Alvin C. Powers, and David H. Wasserman. Diabetes (2008) 57:1790-1799

Long Chain Fatty Acid Uptake *in Vivo*: Comparison of [^{125}I]-BMIPP and [^{3}H]-Bromopalmitate
Jane Shearer, Kimberly Coenen, R. Richard Pencek, Larry L. Swift, David H. Wasserman, Jeffrey N. Rottman. Lipids. (2008) 43(8):703-11

Lost in Translation (A Perspective on the Current State of the Mouse Glucose Clamping Field - 2009)
David H. Wasserman, Julio E. Ayala, and Owen P. McGuinness. Diabetes (2009) 58:1947

Assessment of Feeding Behavior in Laboratory Mice
Kate L.J. Ellacott, Gregory J. Morton, Stephen C. Woods, Patrick Tso, and Michael W. Schwartz. Cell Metabolism (2010) 12:10-17.

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Standardize & Compare Tests

Assessment of Different Bariatric Surgeries in the Treatment of Obesity and Insulin Resistance in Mice
Deng Ping Yin, MD, PhD1,3; Qiang Gao, PhD1; Lian Li Ma1; Wenwei Yan, PhD1; Phillip E. Williams1; Owen P. McGuinness
David H. Wasserman, PhD2 and Naji N.Abumrad. Ann Surg. 2011 Jul;254(1):73-82

Markers of Glycemic Control in the Mouse: Comparisons of 6-h and Overnight-fasted Blood Glucose to HbA1c
Byoung Geun Han, Chuan-Ming Hao, Elena E. Tchekneva, Ying-Ying Wang, Chieh Allen Lee, Benyamin Ebrahim, Raymond C. Harris, Timothy S. Kern, David H. Wasserman, Matthew D. Breyer and Zhonghua Qi. Am J Physiol Endocrinol Metab 295: E981–E986, 2008.

Characterization of Susceptibility of Inbred Mouse Strains to Diabetic Nephropathy
Zhonghua Qi, Hiroki Fujita, Jianping Jin, Linda S. Davis,Yihan Wang,2Agnes B. Fogo, and Matthew D. Breyer. DIABETES (2005) 54:2628-2637.

Echocardiographic Evaluation of Ventricular Function in Mice.
Jeffrey N. Rottman, Gemin Ni, and Michael Brown. ECHOCARDIOGRAPHY (2007) 24:83-89/

Temporal Changes in Ventricular Function Assessed Echocardiographically in Conscious and Anesthetized Mice
Jeffrey N. Rottman, MD, Gemin Ni, MD, Michelle Khoo, MBBS, Zhizhang Wang, BS, Wei Zhang, MS, Mark E. Anderson, MD, PhD, and Ernest C. Madu, J Am Soc Echocardiogr 2003;16:1150-7.

Effect of murine strain on metabolic pathways of glucose production after brief or prolonged fasting.
Burgess SC, Jeffrey FM, Storey C, Milde A, Hausler N, Merritt ME, Mulder H, Holm C, Sherry AD, Malloy CR. Am J Physiol Endocrinol Metab. 2005 Jul;289(1):E53-61

MMPC
National Mouse Metabolic Phenotyping Centers

Standardize & Compare Tests

The intestinal lymph fistula model--a novel approach to study ghrelin secretion.
Tong J, Tschöp MH, Aulinger BA, Davis HW, Yang Q, Liu J, Gaylinn BD, Thorner MO, D'Alessio D, Tso P. Am J Physiol Gastrointest Liver Physiol. 2010 Mar;298(3):G474-80.

A novel, noninvasive method for the measurement of intestinal fat absorption.
Jandacek RJ, Heubi JE, Tso P. Gastroenterology. 2004 Jul;127(1):139-44.

Toward a more complete (and less controversial) understanding of energy expenditure and its role in obesity pathogenesis.
Kaiyala KJ, Schwartz MW. Diabetes. 2011 Jan;60(1):17-23.

Direct animal calorimetry, the underused gold standard for quantifying the fire of life.
Kaiyala KJ, Ramsay DS. Comp Biochem Physiol A Mol Integr Physiol. 2011 Mar;158(3):252-64.

Identification of body fat mass as a major determinant of metabolic rate in mice.
Kaiyala KJ, Morton GJ, Leroux BG, Ogimoto K, Wisse B, Schwartz MW. Diabetes. 2010 Jul;59(7):1657-66.

Standard Operating Procedures for Describing and Performing Metabolic Tests of Glucose Homeostasis in Mice
Julio E. Ayala, Varman T. Samuel, Gregory J. Morton4 Silvana Obici, Colleen M. Croniger, Gerald I. Shulman3 David H. Wasserman and Owen P. McGuinness for the NIH Mouse Metabolic Phenotyping Center Consortium. Disease models and mechanisms (2010) 3: 1-10.

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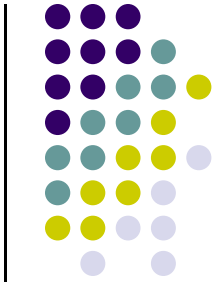
National Mouse Metabolic Phenotyping Centers

Develop & Interrogate Methods



New program to support collaboration among MMPCs:

MMPC Working Group Collaborative Projects (MMPC WGCP)

- ~$100K DC/year for consortium-designed projects
- Projects arise from MMPC working groups
- Members: MMPC personnel, advisors, NIH staff, other experts

Past projects:

Strategies to reduce quarantine time
Insulin Clamp Technology for the Mouse
Mouse Models of Bariatric Surgery
Energy Balance: indirect calorimetry vs double-labeled water

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Education—Annual MMPC Courses

I. Tracers in Metabolic Research:
Principles and Practice of Kinetic Analysis

March 12-16, 2012, The Peabody Hotel, Little Rock, AR
Organizers: Henri Brunengraber and Robert Wolf
held annually, 100 students contact: Deb Viane djviane@uams.edu

An annual week-long course in the theory and practice of isotopic tracers (stable and radioactive) for the study of metabolism in man and animals. The course includes isotopomer analysis for metabolic flux rates and metabolic regulation.

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National Mouse Metabolic Phenotyping Centers

Education--Annual MMPC Courses

II. Glucose Clamping The Conscious Mouse: A Laboratory Course

David Wasserman

Held annually at Vanderbilt University School of Medicine (10 students/year)

Practice, protocols and quantitative tools—everything needed to learn how to conduct a glucose clamp in mice.

III. An Organ Systems Approach to Experimental Targeting of the Metabolic Syndrome

Held annually at Vanderbilt University School of Medicine (20 students/year)
http://www.mc.vanderbilt.edu/medschool/mpb/

Owen McGuinness

Intensive two week annual laboratory and classroom experience for 20 students to provide the tools needed to assess whether an experimental intervention (pharmacologic, genetic, dietary, or environmental) alters macronutrient metabolism, energy balance, cardiovascular homeostasis or animal behavior.

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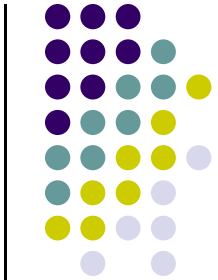
MMPC Research Funding Opportunities



MMPC Initiative for Collaborative Research: MICROmouse

- $75,000 TC (total available funds: ~$300K/yr, average 4-5/year)
- applications due 4x/year
- open to all US investigators, including post docs
- peer reviewed
- 1 year, possible renewal for second year

Comes in 2 flavors:

- **Pilot and Feasibility Studies**

  Develop new or miniaturized tests to meet identifiable, outstanding needs necessary to phenotype mouse models of metabolic disease, and augment the mission of a Center

- **Collaborative research projects**

  Research projects with, or between MMPCs – requires 2 or more PIs, one at an MMPC

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In vivo assessment of mouse muscle oxidative capacity by 31P-MRS
Douglas Befroy, Yale University (New Haven, CT)

Interplay between circadian disorganization and obesity
Shin Yamazaki, Academy College (Nashville, TN)

Impact of Bariatric Surgery on Adipose Tissue Inflammation
Alyssa Hasty, Vanderbilt University (Nashville, TN)

Metabolic Fluxes During Hyperinsulinemic Euglycemia in Insulin Resistant Mice
Nishanth Sunny, University of Texas Southwestern (Dallas, TX)

MRI Phenotyping of Murine Diabetic Retinopathy
Bruce Berkowitz, Wayne State University (Detroit, MI)

MICROmouse 2009-2011

Remodeling of Hepatic Lipid Metabolism by Dietary Methionine Restriction
Thomas W Gettys, Pennington Biomedical Research Center (Baton Rouge, LA)

Role of Melanocortin Signaling in Gastric Bypass-induced Feeding Behavior
Amanda Vanhoose, Vanderbilt University (Nashville, TN)

ß-cell gap-junctional coupling effects on plasma insulin oscillations
Richard Benninger, Vanderbilt University (Nashville, TN)

A novel AMPK-interacting protein in cardiac metabolism and function
Brian Iritani, University of Washington (Seattle, WA)

Characterization of A New Murine Model of Cardiomyopathy in Type 2 Diabetes
Kevin O'Brien, University of Washington (Seattle, WA)

Effects of acute deletion of selective transcription factors on mouse islets
Craig Nunemaker, University of Virginia Health System (Charlottesville, VA)

Increased risk for diabetes and obesity in offspring of multiparous mice
Laura Woollett, University of Cincinnati (Cincinnati, OH)

Long Chain Fatty Acids and Mineralocorticoid Receptor Upregulation in a Mouse Model of Diabetic Nephropathy
Bardia Askari, University of Washington (Seattle, WA)

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National Mouse Metabolic Phenotyping Centers

Department of Health and Human Services

Part 1. Overview Information

Participating Organization(s)	National Institutes of Health (NIH)
Components of Participating Organizations	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Funding Opportunity Title	**Collaborative Interdisciplinary Team Science in NIDDK Research Areas (R24)**
Activity Code	R24 Resource-Related Research Projects
Announcement Type	Reissue of PAR-08-182
Related Notices	None
Funding Opportunity Announcement (FOA) Number	**PAR-11-221**
Companion FOA	None
Number of Applications	See Section III. 3. Additional Information on Eligibility.
Catalog of Federal Domestic Assistance (CFDA) Number(s)	93.847
FOA Purpose	Collaborative Interdisciplinary Team Science Awards will foster the application of interdisciplinary, integrative and/or paradigm-shifting approaches to address complex challenges in biomedical research relevant to the National Institute of Diabetes and Digestive and Kidney Diseases. The NIDDK supported R24 grant mechanism is designed to apply the flexibility of the Research Resource Project Grant mechanism (R24) to accommodate many forms of approaches including discovery-based or resource-generating and hypothesis-driven or hypothesis-generating science. Information regarding the mission of NIDDK and its constituent Extramural Scientific Divisions, including Diabetes, Endocrinology and Metabolic Diseases (DEM), Digestive Diseases and Nutrition (DDN), and Kidney, Urology and Hematologic Diseases (KUH) may be found at:

	http://www2.niddk.nih.gov/Funding/default.htm .

Key Dates

Posted Date	June 2, 2011
Letter of Intent Due Date	Not Applicable
Application Due Date(s)	November 15, 2011, November 15, 2012, and November 15, 2013
AIDS Application Due Date(s)	Not Applicable
Scientific Merit Review	February/March 2012, February/ March 2013, and February/March 2014
Advisory Council Review	May 2012, May 2013, and May 2014
Earliest Start Date(s)	July 1, 2012, July 1, 2013, and July 1, 2014
Expiration Date	November 16, 2013
Due Dates for E.O. 12372	Not Applicable

Required Application Instructions

It is critical that applicants follow the instructions in the PHS398 Application Guide except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts*). Conformance to all requirements (both in the Application Guide and the FOA) is required and strictly enforced. While some links are provided, applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV. When the program-specific instructions deviate from those in the Application Guide, follow the program-specific instructions. **Applications that do not comply with these instructions may be delayed or not accepted for review.**

Looking ahead: NIH is committed to transitioning all grant programs to electronic submission using the SF424 Research and Related (R&R) format and is currently investigating solutions that will accommodate NIH's multi-project programs. NIH will announce plans to transition the remaining programs in the *NIH Guide to Grants and Contracts* and on NIH's Applying Electronically website.

Note: A new version of the paper PHS 398 application form and instructions (revised 6/2009) must now be used.

Download the new application form and instructions from http://grants.nih.gov/grants/forms.htm.

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Part 2. Full Text of Announcement

Section I. Funding Opportunity Description

The complexity of biomedical science often requires the input and expertise of multiple collaborating investigators working as an investigative team. Currently, support for collaborative research may take the form of a large R01 with a Program Director/Principal Investigator (PD/PI) and one or more key personnel and/or collaborators; a multi-PD/PI R01 where credit and responsibility for a project are shared; a Program Project grant (P01) with 3 or more individual and independent projects, with cores, centered on a common theme; or a Center supporting a focused set of resource-related activities. While the R01, P01 and Centers mechanisms can foster certain kinds of collaborations, their structures cannot always readily accommodate interdisciplinary team science that synergizes around a single, critically important research challenge; for example, an unanswered, critical question or construction of a unique resource. In recognition of the need to provide a flexible mechanism to support interdisciplinary team science, the current initiative will support a Collaborative Interdisciplinary Research Program designed to provide flexible support for research teams focused on innovative approaches to a single research challenge relevant to understanding biology that falls within the research mission of the NIDDK. This includes, for example, research related to diabetes, endocrine and metabolic diseases, digestive diseases and nutrition, and kidney, urologic and hematologic diseases and the development of new approaches to prevent, treat and cure these diseases. Collaborative interdisciplinary teams could support basic, or integrated basic and clinical studies that have a potential to move forward the NIH agenda on translation.

The Collaborative Interdisciplinary Research program is designed to support: (a) A team of independent investigators with complementary expertise that will develop a synergistic approach to investigate a single, critically

important research challenge; (b) Research projects of high scientific quality supported by strong preliminary data; and that might include the development or use of unique resources.

Investigators, who are considering pursuing a collaborative approach to their research problem, are strongly encouraged to contact NIDDK program staff prior to the projected receipt date to discuss a potential application. The discussion could include relevance of the topic to the NIDDK mission, the scope and approach of the project, and the choice of funding mechanism.

Funding decisions will be based on criteria for programmatic relevance and scientific quality. Successful research should have a major impact on areas relevant to the mission of NIDDK. A description of NIDDK scientific program areas can be found at http://www2.niddk.nih.gov/Funding/default.htm .

Teams that are at a formative stage and are addressing studies in the research areas of Kidney, Urology and Hematologic Diseases (KUH) have the opportunity to submit a "Seeding R24". For details of this mechanism, follow this link to the KUH webpage (http://www2.niddk.nih.gov/KUH/KUHHome/default.htm).

Section II. Award Information

Funding Instrument	Grant
Application Types Allowed	New Renewal Resubmission The OER Glossary and the PHS398 Application Guide provide details on these application types.
Funds Available and Anticipated Number of Awards	The number of awards is contingent upon NIH appropriations, and the submission of a sufficient number of meritorious applications.
Award Budget	Applications that are submitted in response to this FOA must have budgets greater than or equal to $500,000 in direct costs per year. Facilities and administrative costs requested by consortium participants are not included in the direct cost limitation, see http://grants.nih.gov/grants/guide/notice-files/NOT-OD-05-004.html.
Award Project Period	The scope of the proposed project should determine the project period (the maximum project period for an R24 award is five years). As it is anticipated that the underlying science will change over time, only one competitive renewal is allowed. Thus, the maximum project period for these R24s is 10 years.

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

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions:

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

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

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions

Nonprofits Other Than Institutions of Higher Education

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

For profit Organizations

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

Governments

- State Governments
- County Governments
- City or Township Governments
- Special District Governments
- Indian/Native American Tribal Governments (Federally Recognized)
- Indian/Native American Tribal Governments (Other than Federally Recognized)

- Eligible Agencies of the Federal Government
- U.S. Territory or Possession

Other

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

Non-domestic (non-U.S.) Entities (Foreign Organizations) are eligible to be represented by members of a collaborative team but may not apply for funding under this FOA.

Foreign (non-U.S.) components of U.S. Organizations are allowed.

Required Registrations

Applicant organizations must complete the following registrations as described in the PHS398 Application Guide to be eligible to apply for or receive an award. Applicants must have a valid Dun and Bradstreet Universal Numbering System (DUNS) number in order to begin each of the following registrations.

- Central Contractor Registration (CCR) – must maintain an active registration, to be renewed at least annually
- eRA Commons

All Program Directors/Principal Investigators (PD/PIs) must also work with their institutional officials to register with the eRA Commons or ensure their existing eRA Commons account is affiliated with the eRA Commons account of the applicant organization.

All registrations must be completed by the application due date. Applicant organizations are strongly encouraged to start the registration process at least four (4) weeks prior to the application due date.

Eligible Individuals (Program Director/Principal Investigator)

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

The R24 is a flexible “R” type grant mechanism that can be used to encourage a multi-disciplinary, team based approach to complex challenges in biomedical science in ways that are not often the case with the traditional R01. The PD/PI will submit the R24 from his/her institution (hereby termed applicant institution). Other members of the team may serve as co-investigators; or as a group of multiple-PDs/PIs. For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the PHS398 Application Guide. Funding to PDs/PIs at institutions other than the applicant institution will be administered according to the guidelines of a consortium. The Program Director/Principal Investigator of an R24 grant may be located at one institution while other members of the collaborative team may be located at the same, affiliated, or other institutions. Members of the team need not have interacted previously on this, or other problems.

2. Cost Sharing

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

3. Additional Information on Eligibility

Number of Applications

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

NIH will not accept any application in response to this FOA that is essentially the same as one currently pending initial peer review unless the applicant withdraws the pending application. NIH will not accept any application that is essentially the same as one already reviewed. Resubmission applications may be submitted, according to the NIH Policy on Resubmission Applications from the PHS398 Application Guide and must include an introduction addressing the previous peer review critique from the Summary Statement.

Applicants may submit Renewal applications for up to a total of 10 years of support.

Institutions may submit more than one application.

Individual PDs/PIs may submit one application.

Section IV. Application and Submission Information

1. Address to Request Application Package

Applicants are required to prepare applications according to the current PHS 398 application forms in accordance with the PHS 398 Application Guide.

2. Content and Form of Application Submission

It is critical that applicants follow the instructions in the PHS398 Application Guide, except where instructed in this funding opportunity announcement to do otherwise. Conformance to the requirements in the Application Guide is required and strictly enforced. Applications that are out of compliance with these instructions may be delayed or not accepted for review.

Application Submission

Applications must be prepared using the PHS 398 research grant application forms and instructions for preparing a research grant application. Submit a signed, typewritten original of the application, including the checklist, and three signed photocopies in one package to:

Center for Scientific Review
National Institutes of Health
6701 Rockledge Drive, Room 1040, MSC 7710
Bethesda, MD 20892-7710 (U.S. Postal Service Express or regular mail)
Bethesda, MD 20817 (for express/courier service; non-USPS service)

At the time of submission, two additional paper copies of the application and all copies of the appendix must be sent to:

Dr. Francisco Calvo
Chief, Review Branch
Division of Extramural Activities
National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK)
6707 Democracy Boulevard, Room 752, MSC 5452
Bethesda, MD 20892-5452
(for express/courier service: Bethesda, MD 20817)
Telephone: 301-594-8897
Email: fc15y@nih.gov

Page Limitations

All page limitations described in the PHS398 Application Guide and the Table of Page Limits must be followed.

Research Plan

All instructions in the PHS398 Application Guide must be followed, with the following additional instructions:

A clear plan of operation should be provided for the administrative structure and proposed interactions among the investigators. Research related to any resources that are needed to enhance the capabilities of the team should be clearly articulated. The coordinated use of shared resources that could increase the efficiency of the entire team, as well as facilitate the use of new technologies and the pursuit of new lines of investigation should be defined. The plan for development and use of resources should help to promote the interdisciplinary and collaborative research around which the team has formed. Shared research resources and activities can include services (e.g., cell isolations, patient recruitment, statistical or bioinformatics support); equipment (e.g., confocal microscope, scanning electron microscope); or other types of key facilities, cores, or reagents (e.g., use of transgenic facilities, access to batch preparation of reagents, clinical research resources), as needed by the collaborative team. Since the overall goal of the R24 is to bring together investigators from varied disciplines to attack a single research challenge in a coherent fashion, the justification for drawing investigators from varied disciplines (e.g., chemistry, physics, biological computation, imaging, molecular biology, physiology, etc) should be well defined. The role(s) for each member of the team and how the team will provide the requisite synergies for answering the complex problem should be clearly articulated. These activities should significantly enhance the investigators' existing capabilities and introduce new approaches to the research aims of the objective of the collaborative team. The collaborative research plan must facilitate a synthesis of information that would not be possible otherwise.

This mechanism is not intended to support core facilities or ongoing resources. Furthermore, it is not designed to support groups of investigators at the same institution who would normally interact and collaborate in the absence of a collaborative grant.

See Administrative Guidelines for R24 Collaborative, Interdisciplinary Team Science (Research Resource) Grants at the following link: http://www2.niddk.nih.gov/Funding/Grants/ApplicantGuidelines/CollaborativeR24

Resource Sharing Plan

Individuals are required to comply with the instructions for the Resource Sharing Plans (Data Sharing Plan, Sharing Model Organisms, and Genome Wide Association Studies (GWAS) as provided in the PHS398 Application Guide.

Appendix

Do not use the appendix to circumvent page limits. Follow all instructions for the Appendix (please note all format requirements) as described in the PHS398 Application Guide

Foreign Organizations

Foreign (non-US) organizations must follow policies described in the NIH Grants Policy Statement, and procedures for foreign organizations described throughout the PHS398 Application Guide.

3. Submission Dates and Times

Part I. Overview Information contains information about Key Dates.

Information on the process of receipt and determining if your application is considered “on-time” is described in detail in the PHS398 Application Guide.

Applicants may track the status of the application in the eRA Commons, NIH’s electronic system for grants administration.

4. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review.

5. Funding Restrictions

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

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

6. Other Submission Requirements and Information

Applications must be postmarked on or before the due dates in Part I. Overview Information.

If an application is received after that date, it will not be reviewed.

Upon receipt, applications will be evaluated for completeness by the Center for Scientific Review , NIH. Applications that are incomplete will not be reviewed.

Applicants should include a plan for the collaborative team science approach under the Research Strategy Section of the application, including plans for enhancing communication between and among members of the team. For purposes of evaluating the suitability of the R24 and the strengths of the collaborative team science approach to the central problem, the following criteria will apply in addition to those defined in the enhanced peer review criteria:

1) Does the team that has been assembled have the appropriate mix of expertise necessary to achieve the objectives of the work proposed?

2) Does the team apply appropriate and state-of-the-art approaches to the problem in such a way as to create a level of synergy that will significantly enhance the outcome?

3) Has an administrative organization been delineated, including plans for a) the coordination of ongoing research, b) the establishment and maintenance of internal communication and cooperation among investigators, c) the prioritization of usage of shared resources?

4) Are the proposed budgets appropriate for the work to be done? Is there an appropriate institutional commitment to the program, including lines of accountability regarding management of the R24 grant?

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

Applicants requesting $500,000 or more in direct costs in any year (excluding consortium F&A) must contact NIH program staff at least 6 weeks before submitting the application and follow the Policy on the Acceptance for Review of Unsolicited Applications that Request $500,000 or More in Direct Costs as described in the PHS398 Application Guide.

The Program Director/Principal Investigator must include a cover letter with the application that identifies the program staff member and Institute or Center that has agreed to accept assignment of the application. An application received without indication of prior staff concurrence and identification of program staff contacted will be returned to the applicant without review. See the following link for detailed instructions: http://grants2.nih.gov/grants/guide/notice-files/NOT-OD-02-004.html

It is **strongly encouraged** that applicants contact program staff early in the process (6 to 9 months ahead of application due date). Discussions could include appropriateness of the R24 mechanism, budgetary considerations, and programmatic relevance and are beneficial to both the applicant as well as program staff for planning purposes.

These instructions apply to all new, renewal, and resubmission applications.

Post Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in NOT-OD-10-115.

Section V. Application Review Information

1. Criteria

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

This R24 is not intended to support more traditional investigator-initiated and highly focused studies best supported through the R01 or P01 mechanisms. Nor is it intended to provide core type infrastructure for the R24 application or for already existing and funded projects.

Overall Impact - Overall

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

Scored Review Criteria - Overall

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

Significance

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

Investigator(s)

Are the PD/PIs, collaborators, and other researchers well suited to the project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project? Is the proposed research topic best addressed by an interdisciplinary approach as presented by this particular team? Do team members have other research support as well as a publication record that supports their particular expertise and field of research? Team Science: Does the team that has been assembled have the appropriate mix of expertise necessary to achieve the objectives of the work proposed?

Innovation

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

concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed? Is the planned research substantially different from that already being pursued in the laboratories of team members or elsewhere?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?

If the project involves clinical research, are the plans for 1) protection of human subjects from research risks, and 2) inclusion of minorities and members of both sexes/genders, as well as the inclusion of children, justified in terms of the scientific goals and research strategy proposed? Are paradigm-shifting approaches and/or technologies being used to address the problem? Are the unique strengths of each team member being appropriately used to address the question? Synergy: Does the team apply appropriate and state-of-the-art approaches to the problem in such a way as to create a level of synergy that will significantly enhance the outcomes?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements? Has a plan been developed to facilitate the interaction of PD/PIs and key personnel at different institutions? Will data and resources be easily shared in order to address the application in an integrated, interdisciplinary way? Administration organization: Does the administrative organization reflect a coordination of ongoing research and establish and maintain internal communication and cooperation among investigators? Are mechanisms to prioritize the usage of shared resources provided?

Additional Review Criteria - Overall

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

Protections for Human Subjects

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

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

Inclusion of Women, Minorities, and Children

When the proposed project involves clinical research, the committee will evaluate the proposed plans for inclusion of minorities and members of both genders, as well as the inclusion of children. For additional information on review of the Inclusion section, please refer to the Human Subjects Protection and Inclusion Guidelines.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following five points: 1) proposed use of the animals, and species, strains, ages, sex, and numbers to be used; 2) justifications for the use of animals and for the appropriateness of the species and numbers proposed; 3) adequacy of veterinary care; 4) procedures for limiting discomfort, distress, pain and injury to that which is unavoidable in the conduct of scientifically sound research including the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices; and 5) methods of euthanasia and reason for selection if not consistent with the AVMA Guidelines on Euthanasia. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

Biohazards

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

Resubmissions

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

Renewals

For Renewals, the committee will consider the progress made in the last funding period and justification for continuing need for support using the R24 as opposed to other grant mechanisms. Only a single renewal will be allowed for an R24 project.

Revisions

Not Applicable

Additional Review Considerations - Overall

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

Applications from Foreign Organizations

Reviewers will assess whether the project presents special opportunities for furthering research programs through the use of unusual talent, resources, populations, or environmental conditions that exist in other countries and either are not readily available in the United States or augment existing U.S. resources.

Select Agent Research

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

Resource Sharing Plans

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

Budget and Period of Support

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

2. Review and Selection Process

Applications will be evaluated for scientific and technical merit by (an) appropriate Scientific Review Group(s) convened by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) , in accordance with NIH peer review policy and procedures, using the stated review criteria. Review assignments will be shown in the eRA Commons.

As part of the scientific peer review, all applications:

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

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

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

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons.

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

Section VI. Award Administration Information

1. Award Notices

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

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

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

Any application awarded in response to this FOA will be subject to the DUNS, CCR Registration, and Transparency Act requirements as noted on the Award Conditions and Information for NIH Grants website.

2. Administrative and National Policy Requirements

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

Cooperative Agreement Terms and Conditions of Award

Not Applicable.

3. Reporting

When multiple years are involved, awardees will be required to submit the Non-Competing Continuation Grant Progress Report (PHS 2590) annually and financial statements as required in the NIH Grants Policy Statement.

A final progress report, invention statement, and Financial Status Report are required when an award is relinquished when a recipient changes institutions or when an award is terminated.

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

Section VII. Agency Contacts

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

Investigators who are considering pursuing a collaborative approach to their research problem, are strongly encouraged to contact NIDDK program staff at least 9 months prior to the projected receipt date to discuss a potential application. The discussion should include the choice of funding mechanism, relevance of the topic to the NIDDK mission and the scope and approach of the project. Further information on the NIDDK R24 Collaborative Team Science award is available at http://www2.niddk.nih.gov/Funding/Grants/ApplicantGuidelines/CollaborativeR24.

Application Submission Contacts

GrantsInfo (Questions regarding application instructions and process, finding NIH grant resources)
Telephone 301-435-0714
TTY 301-451-5936
Email: GrantsInfo@nih.gov

eRA Commons Help Desk(Questions regarding eRA Commons registration, tracking application status, post submission issues)
Phone: 301-402-7469 or 866-504-9552 (Toll Free)
TTY: 301-451-5939
Email: commons@od.nih.gov

Scientific/Research Contact(s)

Louis Martey
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK
6707 Democracy Boulevard, Room 687
Bethesda, MD 20892-5460
Telephone: 301-594-7733
Email:Louis.Martey@nih.gov

Chris Mullins, Ph.D.
Director of Basic Cell Biology Programs
Division of Kidney, Urologic and Hematologic Diseases
NIDDK, National Institutes of Health
2 Democracy Plaza, Room 637
6707 Democracy Blvd.

Bethesda, MD 20892-5458
Telephone: 301-451-4902
Fax: 301-480-3510
Email: mullinsC@extra.niddk.nih.gov

Edward Doo, M. D.
Division of Digestive Diseases and Nutrition
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
6707 Democracy Boulevard, Room 651
Bethesda, MD 20892
Tel: 301-451-4524
Fax: 301-480-8300
Email: dooe@niddk.nih.gov

Peer Review Contact(s)

Dr. Francisco Calvo
Chief, Review Branch
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
6707 Democracy Boulevard, Room 752, MSC 5452
Bethesda, MD 20892-5452
(for express/courier service: Bethesda, MD 20817
Telephone: 301-594-8897
Email: fc15y@nih.gov

Financial/Grants Management Contact(s)

Todd Le
Grants Management Specialist
NIH\NIDDK\DEA
6707 Democracy Blvd, Room 726
Bethesda, MD 20892-5456 (Fedex use zip 20817)
Phone: (301) 594-7794
Fax: (301) 594-9523
Email: toddle@mail.nih.gov

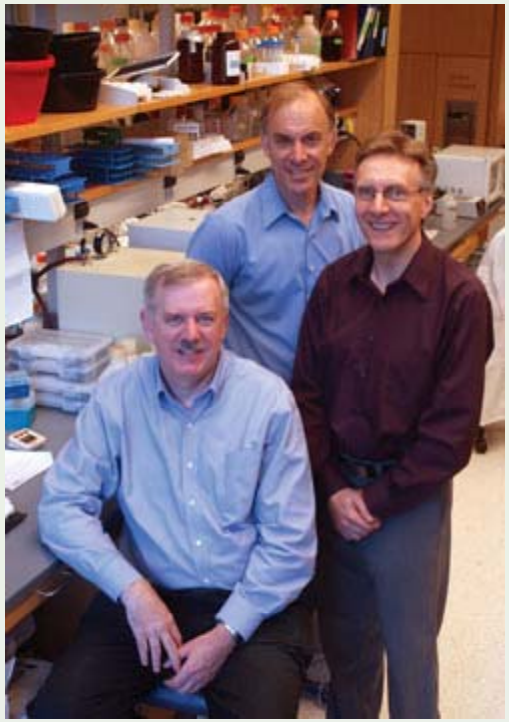
Section VIII. Other Information

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

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Parts 74 and 92.

NIDDK Collaborative, Interdisciplinary Team Science (R24) PAR-11-221



Google hit

Philip Smith, PhD

DEM/NIDDK

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Other collaborative mechanisms

- *Transformative R01 (NIH Common Fund)*
  - One or many PIs/collaborators
- *A multi-PD/PI R01*
  - Two or more PIs share leadership on an R01
- *Program Project Grant (P01)*
  - 3 or more individual and independent projects
  - Centered on a common theme
  - http://www2.niddk.nih.gov/Funding/Grants/GrantReview/P01Guidelines
- *Center*
  - Supports focused set of resource-related activities

R24
(Resource-Related Research Project)

- *Define an innovative solution to a complex problem relevant to NIDDK*
  - Discovery-based, resource-generating
  - hypothesis -driven or -generating science
- *Assemble a collaborative team around the problem*
  - Multi-disciplinary expertise
  - Individual strengths but synergistic approach

Characteristics of an R24

- Team of independent investigators
  - Funded and published
  - Complementary expertise (distinct disciplines)
- Synergistic approach
  - Single, critically important question
- Projects of high scientific quality
  - Supported by strong preliminary data
  - Could include development of new resources

Characteristics of a P01

- Team of independent investigators
  - Track record of funding and productivity
- Broad Theme with complementary projects
- Projects of high scientific quality
  - At least 3 projects for full duration of award
  - Supported by strong preliminary data
  - Each project can stand alone as an independent project (R01 submission encouraged)
- Cores
  - Each core must support at least 2 projects

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Support of collaborative R24s and P01s- single pool

- 7% of Research Project Budget allocated to these mechanisms (~$80M in FY11)
- P01s capped at $1.25M DC/yr up to 5 yr
- R24s must be >$500K DC/yr up to 5 yr
- Both mechanisms now limited to 1 competitive renewal
- R24 has 1 receipt date (Nov 15)
- P01 has 3 receipt dates each year (Jan 25, May 25, Sept 25)

R24 Application Process

- Strongly encouraged to contact program staff ***early*** in the process
  - 6-9 months prior to submission
- Single deadline per fiscal year
  - ***November 15***, 2012/2013
- Budget ≥ $500K (DC per year)
  - ***Requires*** pre-approval from NIDDK
  - ***No later than*** 6 weeks prior to submission
  - ***Cover letter*** stating approval of program staff

Application Specifics

- PHS398 Paper Application/12 pages
  - Only 1 renewal allowed (maximum 10 years)
- Team
  - Varied disciplines needed and why
  - Role of each member/synergies
- Clear plan of operation
  - Administrative structure/proposed interactions
- Resources
  - To be developed to enhance capabilities
  - Coordinated use of shared resources

Review

- First level: SRG convened by NIDDK
  - Scientific and technical merit
    - Significance, Investigators, Innovation, Approach, Environment
- Second Level: NIDDK Advisory Council
  - Relevance to program priorities
  - Availability of funding

http://commonfund.nih.gov/TRA/fundedresearch.aspx

- *~80 awards over three years*
  - *11 awards for NIDDK*
- *Managed by 13 NIH Institutes*
- *Span a diverse range of biomedical and behavioral research*

Phenotype of NIDDK T-R01s

- Average annual direct cost ~$700K
- Range of topics (examples)
  - RNA as a Hormone: Systemic Signaling in Mammals via Circulating, Cell-Free Small miRNAs
  - Oral Delivery Vehicles for RNAi Therapies
  - Three-dimensional Scaffold-based Systems for Primary Human Intestinal Culture
  - Open Source Science: Transforming Chronic Illness Care
  - Disappearing gastrointestinal microbiota in epidemic obesity.
  - Human Pharmacogenetics and Human Liver Regeneration

T-R01 Application Specifics

- FY12 receipt date: January 12, 2012
- $25M common fund committed FY12
- No budget cap (1/3 committed to>$1M apps)
- No need for preapproval
- Research Strategy limited to 12 pages
- Editorial review

And Finally

- Read Guidelines before submission
  - NIDDK R24: **PAR11-221**
  - NIDDK P01: **PAR11-043**
  - NIH Common Fund T-R01: RFA **RM11-006**

- Contact Program Staff early!!!
  - Your Program Officer or
  - Corinne Silva, silvacm@mail.nih.gov

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NIDDK

Centers for Diabetes Translation Research (CDTR): P30

Christine Hunter, Ph.D.

CDTR: Purpose / Background

- Advance “bedside to practice” research in diabetes
  - Research resources to close the gap between efficacy research and practice
  - Support consultation/resource Cores in areas relevant to the NIDDK translation research program (e.g. R34/R18s)
  - Expand the potential pool of Center expertise
  - Encourage broad use of Centers—regionally and nationally
- Presented to Council in May 2010—supportive of CDTRs

CDTR Specifics

- Mechanism: P30 x 5 years (renewable)
- RFA published in August 2010
  - Eligibility: strong base in diabetes bedside to practice translational research
- Budget : $200K DC for cores with additional funds possible
  - Pilot and feasibility program ($50K)
  - Plan to serve as a regional/national resource ($100K)
  - Subcontract to a minority serving institution (no cap)

PI	Institution	Core Highlights
William Herman	University of Michigan	HIT (communication, pt empowerment), community engagement
Julie Schmittdiel	Kaiser, USCF, MN, Harvard	Health economics, HIT for adherence, prevention (women and children)
Richard Davis and Michael Pignone	University of North Carolina	Access technologies, community engagement with child/family focus
Tom Elasy	Vanderbilt	Health literacy/numeracy, methodologies, and health disparities
Marshall Chin	University of Chicago	Quantitative analysis, PBRNs, health disparities
Spero Manson	University of Colorado	Focus on American Indians: engagement, sustainability, and mobile/health technology
Debra Haire-Joshu	Washington University	Health economics, policy evaluation, partnerships with Alaskan Natives, D & I

CDTR Plans

- Funded for just over 1 month
- Plans to link with P & C Cores from DRTCs
- First annual meeting will be held in 2012

Questions / Discussion

Training for Behavioral Scientists in Type 1 Diabetes

Christine Hunter, Ph.D.
National Institute of Diabetes & Digestive & Kidney Diseases

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T1D Training for Behavioral Scientists: **Rationale**

- Optimal T1D treatment requires a complex set of behaviors
  - e.g., self-monitoring, medication adherence, lifestyle choices
- Skills and support needs vary over the life course
- Management is influenced by psychosocial factors
  - e.g., peer and familial support, mental health status, health literacy/numeracy, stress management, communication skills
- Behavioral scientists role in T1D:
  - Identify barriers and facilitators to good diabetes management in individuals, families, and the healthcare team or system
  - Develop and test novel / improved approaches for optimizing glucose control and quality of life

T1D Training for Behavioral Scientists: **Current Pipeline Challenge**

- Relatively small numbers of trained behavioral scientists focused on T1D research
- Currently funded behavioral T1D researchers
  - Mostly quite senior / only a few emerging leaders
  - Few focused on young adult or adult populations
  - Largely publish in diabetes specific journals
  - Low visibility in broad behavioral medicine conferences and journals

T1D Training for Behavioral Scientists: **T32 and K12 Goals**

- Develop a highly trained workforce of behavioral scientists to assume leadership roles in behavioral T1D research
- Provide exposure to the rich scientific opportunities in T1D
- Support the transition to an independent research career
- Supervision and mentorship should include a diabetologist and behavioral scientist
  - Expands pool of mentors to top behavioral scientist working areas relevant to T1D and assures grounding in the specifics of T1D
  - Encourages a multi-disciplinary approach to research / care
- Supported under the Special Statutory Program for T1D

T1D Training for Behavioral Scientists: **T32 Specifics**

- RFA-DK-11-027:

- Diabetes Research Training for Behavioral Scientists (T32)
  - Prepare predoctoral and/or postdoctoral behavioral scientists for behavioral research careers in type 1 diabetes
  - Provide each trainee with up to 2 years of full-time predoctoral or three years of postdoctoral support
  - Funding for years 3, 4, and 5 of the awards, are contingent upon the availability of T1D funds
  - Up to 5 awards with 2-3 slots per award, corresponding to a total of $1 million, for fiscal year 2012.
  - NINR also on the RFA
  - **Receipt Date: March 2, 2012**

T1D Training for Behavioral Scientists: **K12 Specifics**

- RFA-DK-11-028:

- Career Development Programs in Diabetes Research for Behavioral Scientists (K12)
  - Prepare postdoctoral behavioral scientists for behavioral research careers in type 1 diabetes (within 5 years of their terminal degree)
  - Allows Scholars at least 2 years of supervised research experience
  - Contingent on the availability of funds, awards may be extended to 5 years
  - Up to 5 awards with 2-3 slots per award, corresponding to a total of $2.5 million, for fiscal year 2012
  - **Receipt Date: March 2, 2012**

Questions / Discussion

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

Training for Engineers in Type 1 Diabetes

Art Castle, Ph.D.
Program Director, NIDDK

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- New applications will be solicited to support research training of engineers
- Joint mentorship of diabetologists and bioengineers
- Research relevant to type 1 diabetes
  - i.e. Artificial Pancreas

The NIH Common Fund
WE ACCELERATE DISCOVERY.

The NIH Common Fund Metabolomics Program is soliciting information on specific needs for reference standards to facilitate research using metabolomic approaches. Please respond to this Request for Information before November 21, 2011.

Overview

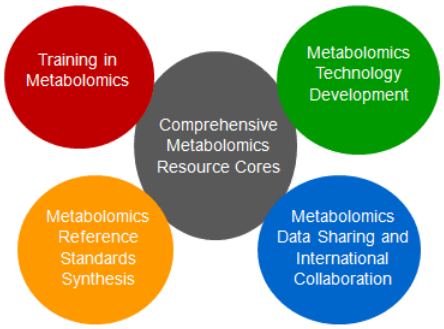
Metabolomics is the study of low molecular weight molecules or metabolites found within cells and biological systems. The metabolome is a measure of the output of biological pathways and, as such, is often considered more representative of the functional state of a cell than other 'omics measures such as genomics or proteomics. In addition, metabolites are conserved across various animal species, facilitating the extrapolation of research findings in laboratory animals to humans. Common technologies for measuring the metabolome include mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR), which can measure hundreds to thousands of unique chemical entities (UCE).

Despite early promise, challenges remain before the full potential of metabolomics can be realized. Existing metabolomics facilities are at capacity, with relatively few scientists who possess in-depth expertise in metabolomics, and a derth of training opportunities to gain that expertise. Some companies provide metabolomics services and limited standards; however, issues with cost, intellectual property rights, and limited profit incentives minimize their use in basic, clinical, and translational research.

To address these challenges, the Common Fund's Increasing Metabolomics Research Capacity program is developing the following program components:

Comprehensive Metabolomics Resource Cores
Goal: To create National Comprehensive Metabolomics Resource Cores, expanding on existing nationally funded metabolomics resources. This initiative will allow institutions to expand and improve their capacity to conduct comprehensive metabolomics studies by adding and improving instrumentation, expanding faculty expertise, and developing new training programs to meet the need for expertise.

Training in Metabolomics
Goal: To increase the number of investigators with metabolomics expertise by supporting interdisciplinary training involving a diverse set of training vehicles that match career stage and goals. This initiative will support early and mid-career development awards with an emphasis on encouraging collaborations between basic and clinical investigators.

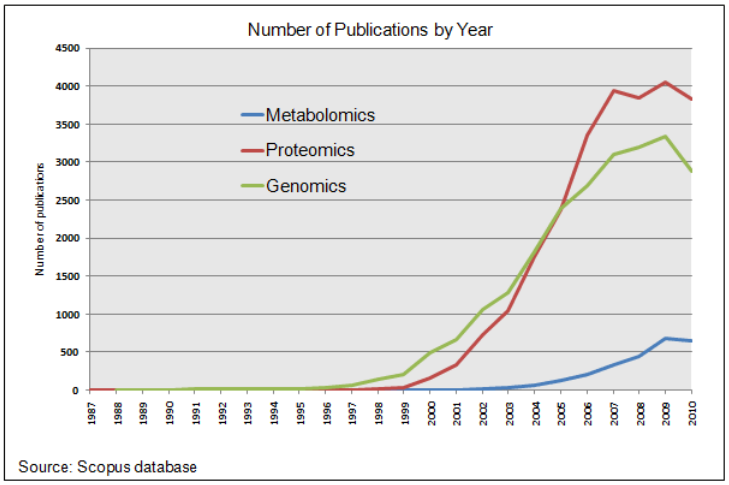


Increasing Metabolomics Research Capacity Program Components

Metabolomics Technology Development
Goal: To address current limitations in metabolomics technologies so they can be easily adapted by other laboratories. Areas addressed may include, but are not limited to: increasing the number, quantitative accuracy, specificity, and throughput of molecular identification; increasing the identification of specific classes of metabolites including lipids and non-polar molecules; increasing the ability to measure more UCEs; and decreasing sample volume, costs, and time to make accurate metabolomics measurements.

Metabolomics Reference Standards Synthesis
Goal: To increase the repertoire of chemically identifiable metabolites through the synthesis of reliable metabolic standards. Data generated from these standards can be deposited into existing databases to expand the identities of the metabolite repertoire and serve as a resource for the entire metabolomics community.

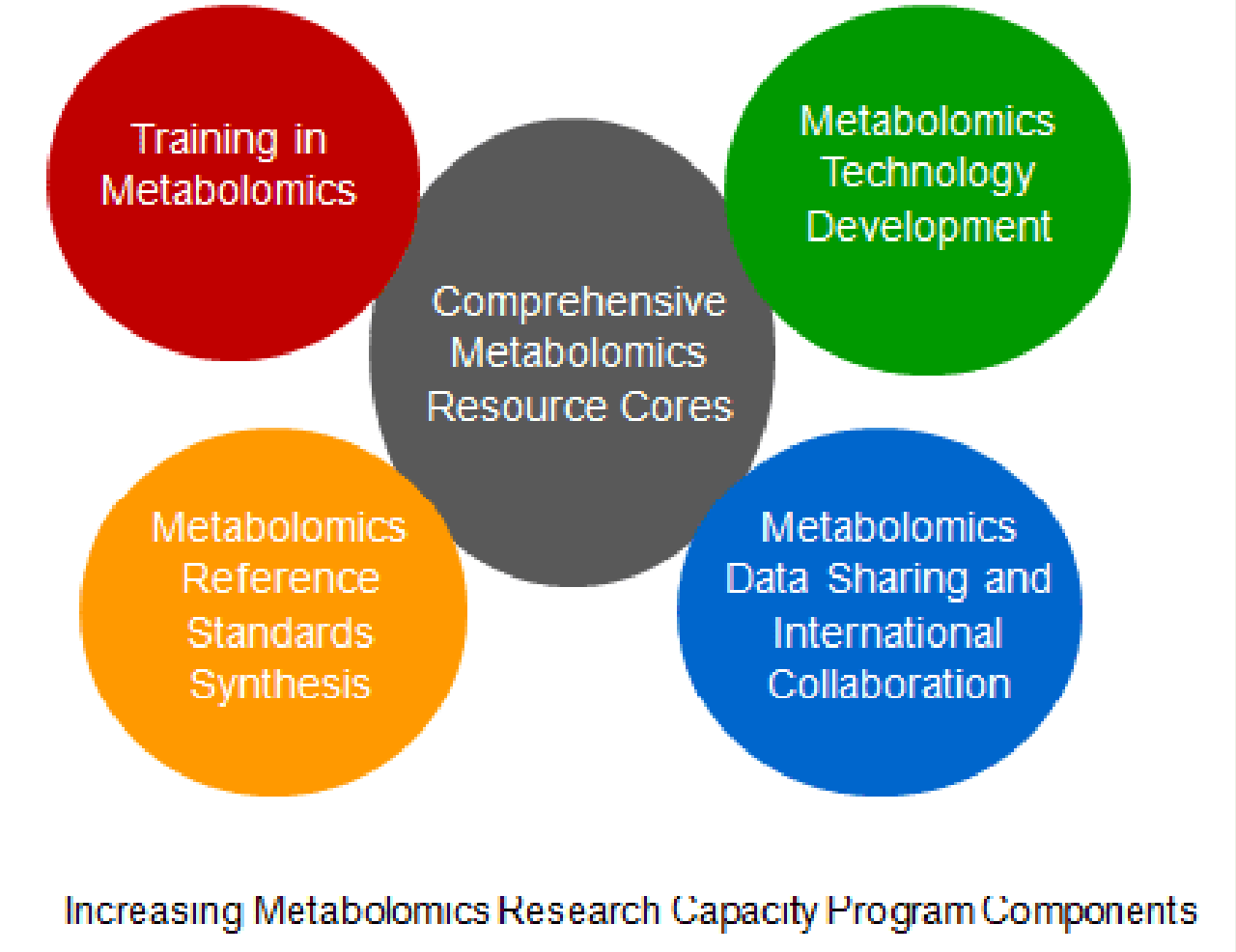
Data Sharing and International Collaboration will be important aspects of this program.



Common Fund FOAs: Metabolomics

Art Castle, Ph.D.
Program Director, NIDDK

Metabolomics Planned Initiatives



Increasing Metabolomics Research Capacity Program Components

http://commonfund.nih.gov/Metabolomics/

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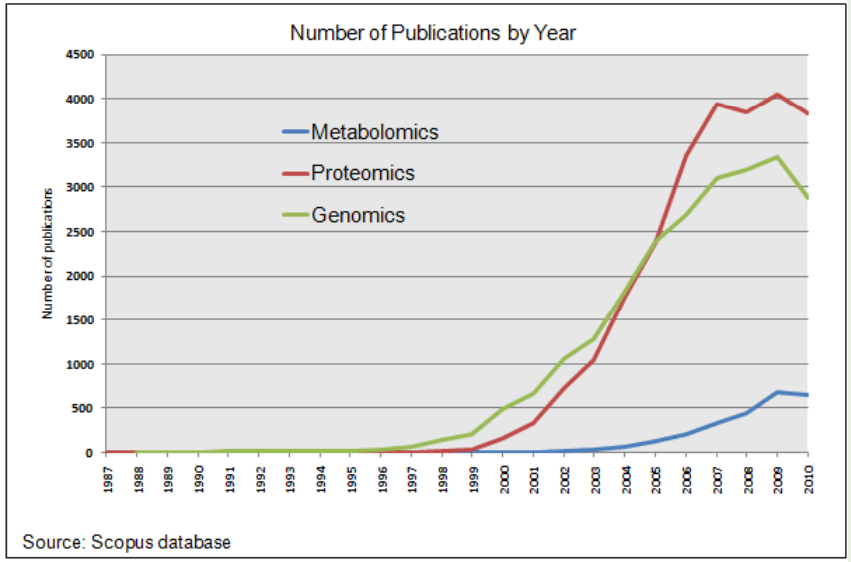
NEED for Increasing Metabolomics Capacity

• Metabolomics research has grown substantially in the US over the last five years but has not keep pace with genomics and proteomics research.



• Most of the growth is in basic research; however, metabolomics presents unique opportunities for advances in translational research as the metabolome represents a more current view of physiological/pathological conditions.

• An extramural virtual workshop was conducted with leaders in the field to explore the needs. Initiatives have been planned and will be released as FOAs

Comprehensive Metabolomics Resource Cores

• Goal: To create National Comprehensive Metabolomics Resource Cores

• Expand on existing nationally funded metabolomics resources.

• Coordinate sharing of data and resources among national resource cores and other national and international efforts.

• Provide additional training in metabolomics

• Invest in technology development.

NIDDK and NIH are particularly interested in translational potential of metabolomics

Future Metabolomics Comprehensive Research Cores should have a close relationship with large centers such as CTSAs, diabetes centers and other core facilities with translational efforts.

The **NIH Common Fund Metabolomics** Program is soliciting information on specific needs for reference standards to facilitate research using metabolomic approaches. Please respond to this Request for Information before November 21, 2011

NIDDK DIABETES CENTERS
NON-COMPETING RENEWALS
(TYPE 5 PROGRESS REPORT)
INSTRUCTIONS
2011-2012

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.

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 citing center support during the past year (include PMCID#)
- 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
- 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 (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)

(Table or Text) C. Awards funded 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 1-2 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)
- 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**

TABLE:
PUBLICATIONS CITING SUPPORT FROM THIS CORE CENTER GRANT

Core or P&F/P.I. Name)	Publications	Primary	Secondary
Core A/ Brown	Brown, A.C; Jones R.C.; Smith, A.J. Metformin reduces hepatic glucose output. Diabetes, 2008 volume; page # PMCID#	Core A	
	Brown, A.C.; Cheng, A.G.; Anderson, J.C. Results of Islet Transplantation. Diabetes, 2010, volume: page# PMCID#	Core A	Cores B,C
Core B/ Cheng,	Cheng, A.C.; Meyer, G.C. Linkage Studies in Animal Models for Diabetes Nature Genetics, 2009, volume: page#, PMCID#.	Core B	Core D
	*Smith, F.G.; Cheng, A.C.; Tissue Specific Knockout of Glut 4 PNAS, 2008, V: page#, PMCID#	Core B	
P&F/Smith	Smith, F.L.; Davis, S.E.; Morris, J.L. Role of macrophages in hypothalamic inflammation. J. Clin. Inv., 2009, volume: page#, PMCID#.	Core C	
P&F/Jones	Jones, T.L; Hathaway, J.B; Clemmons, A.H.; Akt and hepatic lipid accumulation. J. Biol. Chem., 2010, volume Page#, PMCID#.	NONE	

Instructions: List each publication only once under the Core (or P&F project PI name) most significantly contributing to the work. Each publication listed should cite the Diabetes Center grant number. For any publications that received Center grant support but did not cite the Center grant number, use an asterisk (*) at the beginning of the publication listing (see example above). The research core most significantly contributing to the work should be signified as "Primary." All other contributing research cores are designated as "Secondary." Use separate headings for each research core (i.e. publications supported by each 'primary' core should be grouped together), followed by the P&F projects at the end of the listing of Center publications.

Department of Health and Human Services

Part 1. Overview Information

Participating Organization(s)	National Institutes of Health (NIH)
Components of Participating Organizations	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Funding Opportunity Title	**Diabetes Research Centers (P30)**
Activity Code	P30 Center Core Grants
Announcement Type	Reissue of RFA-DK-11-002
Related Notices	None
Funding Opportunity Announcement (FOA) Number	**RFA-DK-11-015**
Companion FOA	None
Number of Applications	Only one application per institution is allowed, as defined in Section III. 3. Additional Information on Eligibility.
Catalog of Federal Domestic Assistance (CFDA) Number(s)	93.847
FOA Purpose	This Funding Opportunity Announcement (FOA) invites applications for Diabetes Research Centers, formerly named Diabetes Endocrinology Research Centers (DERCs) and Diabetes Research and Training Centers (DRTCs). Diabetes Research Centers are designed to support and enhance the national research effort in diabetes and related endocrine and metabolic diseases. Diabetes Research Centers support three primary research-related activities: Research Core services, a Pilot and Feasibility (P&F) program, and an Enrichment program. All activities pursued by Diabetes Research Centers are designed to enhance the efficiency, productivity, effectiveness and multidisciplinary nature of research in Diabetes Research Center topic areas. The NIDDK Diabetes Centers program in 2011 consists of 16 Centers each located at outstanding research institutions with documented programs of

	research excellence in diabetes, endocrine and metabolic diseases. Information about the NIDDK Diabetes Research Centers may be found at the following URL: http://www2.niddk.nih.gov/Research/Centers/CenterPrograms/.

Key Dates

Posted Date	August 1, 2011
Letter of Intent Due Date	January 31, 2012
Application Due Date(s)	February 29, 2012
AIDS Application Due Date(s)	Not Applicable
Scientific Merit Review	June/July, 2012
Advisory Council Review	October, 2012
Earliest Start Date(s)	December, 2012
Expiration Date	March 1, 2012
Due Dates for E.O. 12372	Not Applicable

Required Application Instructions

It is critical that applicants follow the instructions in the PHS398 Application Guide except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts*). Conformance to all requirements (both in the Application Guide and the FOA) is required and strictly enforced. While some links are provided, applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV. When the program-specific instructions deviate from those in the Application Guide, follow the program-specific instructions. **Applications that do not comply with these instructions may be delayed or not accepted for review.**

Note: A new version of the paper PHS 398 application form and instructions (revised 6/2009) must now be used.

Download the new application form and instructions from http://grants.nih.gov/grants/forms.htm.

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Part 2. Full Text of Announcement

Section I. Funding Opportunity Description

I. PROGRAM OBJECTIVES

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) invites applications for Diabetes Research Center grants to support research in diabetes mellitus and its complications, and related areas of endocrinology and metabolism.

The prevalence of diabetes mellitus in the United States is reaching epidemic proportions and accounts for a huge national burden of morbidity, mortality, and health care expenditures. The mission of the Diabetes Centers is to serve as a key component of the NIDDK-supported research effort to develop new therapies and improve the health of Americans with, or at risk for, diabetes and related endocrine and metabolic disorders. The Centers promote new discoveries and enhance scientific progress through support of cutting-edge basic and clinical research related to the etiology and complications of diabetes, with the goal of rapidly translating research findings into novel strategies for the prevention, treatment and cure of diabetes and related conditions.

To accomplish this mission, the Diabetes Research Centers:

- Create an environment that supports important and innovative research;
- Raise awareness and interest in fundamental and clinical diabetes research at their institutions as well as locally, regionally, and nationally;

- Enhance diabetes research education and training opportunities for patients, students, scientists, and clinicians;
- Attract and retain early stage investigators and investigators new to diabetes research;
- Provide core services that leverage funding and unique expertise;
- Foster interdisciplinary collaborations, especially in the emerging areas of research, to catalyze new ideas and scientific approaches;
- Promote the translation of scientific discoveries from bench to bedside to community to improve public health.

II. CENTER STRUCTURE AND ACTIVITIES

The NIDDK Diabetes Research Centers are part of an integrated program of research support designed to enhance multidisciplinary cutting-edge research in diabetes and in related areas of endocrinology and metabolism. Diabetes Research Centers are intended to improve the efficiency and collaborative nature of diabetes research by providing shared access to specialized technical resources and expertise. In addition, Centers enhance translational research by providing a framework for fostering synergy between basic scientists and clinical investigators, with the goal of promoting rapid progress toward a treatment or cure for diabetes and its complications. Diabetes Research Centers support three primary research-related activities: (1) Research Core Services that provide resources to enhance the efficiency, productivity, and multidisciplinary nature of research in designated topic areas; (2) a Pilot and Feasibility Program designed to foster the development of new investigators and to provide seed-support for innovative high-risk projects; and (3) an Enrichment Program to promote interdisciplinary interaction and training of investigators in areas of NIDDK interest.

Institution and Research Base

A Diabetes Research Center must be an identifiable unit within a single institution such as a university medical center, or within a consortium of cooperating institutions. In either case, Diabetes Center applications must be associated with an existing program of excellence in biomedical research in diabetes and in related areas of metabolism and endocrinology. Program excellence is measured through a consistent and outstanding record of productivity and peer-reviewed research funding in diabetes and related research areas. A high level of integration and close collaboration among Center personnel from diverse scientific disciplines is an important feature of a successful Diabetes Research Center. Accordingly, the applicant should clearly state considerations for Center membership with specific reference to the potential of members to form interactive, collaborative and synergistic relationships. Center applicants should identify one or more central themes or focus areas that link Center investigators and their research programs.

Diabetes research often requires the use of specialized technologies and resources to support a cohesive research effort. The goal of the Diabetes Research Center program is to make state-of-the art technologies and resources readily accessible to a broad spectrum of investigators who are pursuing studies in relevant topic areas.

Administrative Core

Diabetes Research Center applications must include an administrative core that will be responsible for allocation and oversight of Center resources. The Administrative core will also be responsible for planning an enrichment program and for implementing a process for solicitation, review and selection of projects for the Pilot and Feasibility Program within the Center. In addition, all Diabetes Research Centers will be required to maintain a Center website, with the administrative core taking primary responsibility for its curation and oversight, as well as for ensuring proper and seamless integration of the Center website with the NIDDK Center program website. The Core Center Director should provide at least 1.2 person months (calendar year) effort on the Administrative Core and a total of 2.4 person months (calendar year) effort distributed among the Administrative and other components of the Center. One or more Associate Directors should be named who will be involved in the administrative, scientific, or training efforts of the Center and who will serve as Acting Center Director in the absence of the Director. A process must be in place that would be used to recommend a successor to the Director, if needed. An administrative assistant may also be proposed.

Biomedical Resource Cores

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. In a Diabetes Research Center, cores are intended to facilitate basic, clinical and translational research in diabetes, endocrinology and metabolic diseases in order to accomplish the stated goals of the individual Center and of the NIDDK Centers program.

Each research core should provide state-of-the art services to multiple funded research projects. A Center may support research at a single or set of cooperating institutions through an Institutional Core. In addition, Diabetes Center applicants may propose to share core services or functions with other Centers in the Diabetes Center program in order to expand, enhance, or increase the cost-effectiveness of research activities at the Center institution. Examples of biomedical cores that would be considered responsive to this Request for Applications include, but are not limited to:

- Genetics/genomics (including epigenetics)
- Proteomics
- Metabolomics
- Islet isolation and function
- Transgenic and ES-cell technology
- Protein chemistry and macromolecular structure
- Analytical biochemistry
- Integrative physiology
- Imaging

- Translational research (bench to bedside; bedside to community/practice)
- Clinical research
- Bioinformatics
- Biostatistics

These cores are not listed in any particular order, nor do they represent a comprehensive list of possibilities. In responding to this FOA, applicants are encouraged to propose cores that address specific objectives based on the unique requirements of investigators at the applicant institutions. Particular emphasis should be placed on services that support and foster interdisciplinary, integrated and translational approaches to research in Diabetes Center topic areas. 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. As with other research cores, details about access and prioritization of center members to the shared research core(s) should be provided. Moreover, the applicant should document that the Diabetes Center will be in a position to have some input to, and oversight of, the shared institutional core with respect to its management, planning for future changes and improvements, etc.

The need for core support from the Diabetes Research Center must be well justified, with clear documentation of a broad user base of NIDDK-funded investigators pursuing research activities in Center topic areas, as well as diabetes investigators with other sources of peer-reviewed support. 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 the clinical research homes being established by the Clinical and Translational Science Awards supported by the National Institutes of Health (http://ctsaweb.org/) and other related NIH roadmap activities, and any related NIDDK-funded Center programs such as the Nutrition Obesity Research Center (NORC) Program http://www3.niddk.nih.gov/centers/norc.shtml) and the Centers for Diabetes Translation Research (CDTR; http://grants.nih.gov/grants/guide/rfa-files/RFA-DK-10-009.html). Applicants should provide information on other programs supporting related resources at their institution and describe the nature of synergy and integration between the Diabetes Research Center and these other activities. Applicants must also clearly describe how duplication or redundancies of effort, services and resources will be avoided.

Pilot and Feasibility Program

The Diabetes Research 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. Typically at least 20-25% of the Center direct costs, exclusive of equipment, should be for support of P&F projects.

Funding and Duration of Support: It is anticipated that up to $50,000 in direct costs per year for up to two years will be provided for the majority of approved P&F projects. However, a limited number of applications may be selected for support as enhanced P&F awards with prior NIDDK approval. Enhanced P&F awards require prior approval from NIDDK and will be selected from worthy proposals in the following three project categories: clinical and translational research awards, clinical and basic research innovative partnership awards, or technology research and development awards. These enhanced awards may be funded at up to $100,000 direct costs per year and for up to 2 years. Efforts to increase the number of P&F awards and availability of funds for the program through the use of program income or alternative funding sources are particularly encouraged.

Eligibility: The P&F program is particularly directed at new investigators and 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 are also eligible for support under the Diabetes Research Center P&F program. 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. While the distribution of P&F funds to be used in each award category is ultimately at the discretion of the Center P&F committee, it is expected that the Center P&F program will, where possible, place particular emphasis on funding innovative clinical and translational research projects.

Named New Investigator [optional]

Each Diabetes Research Center may provide salary support for a P&F project recipient whom they designate a Named New Investigator. Support for this individual is generally for 2 years, and cannot exceed $90,000 per year, additional appropriate fringe benefits, and 9.0 to 12.0 calendar months effort. These funds are included in the Administrative Core budget, and the initial Named New Investigator should be clearly identified in the application. The individual selected should be a New Investigator who meets the P&F project eligibility criteria and is a permanent resident or US citizen. Individuals are eligible only once for this support. Subsequent candidates for this position are nominated by the Center and reviewed by its External Advisory Board. Appointment of the Named New Investigator is contingent upon the concurrence of the External Advisory Board and the NIDDK program director.

Enrichment Program

The Diabetes Research Center enrichment program should be designed to advance translational research in diabetes, endocrinology and metabolism and promote scientific exchange among investigators with research interests in these topic areas, and to enhance interactions between diabetes researchers and investigators from other fields with relevant expertise. The enrichment program can support activities such as seminars, guest speakers, visiting scientists, consultants, and workshops. Applicants should describe any training opportunities afforded by the Diabetes Research Center for Center participants, and document ways the Center may facilitate, enhance or foster the institutional training environment. Specifically, Center applicants should provide information on related NIDDK T32 training programs at the Center institution(s), and describe how the Diabetes Center will help

to integrate, facilitate and enhance activities of T32-supported trainees. A letter from the PD/PI of any related NIDDK-funded T32 at the Center institution should be included that acknowledges and details how the PD/PI of the T32 intends to promote cohesive interactions between the two programs.

Training postdoctoral fellows to conduct research in diabetes is an associated activity of a Diabetes Research Center. While stipends for fellows cannot be funded from the Center, the establishment of a Center should provide an enhanced environment for research training. Just as in the case of funding for individual research projects, funding for fellowships should be sought from NIH NRSA institutional training grants (e.g. T32, T35) and individual fellowships (e.g. F30, F32), and other sources such as private foundations, and commercial companies.

III. ADDITIONAL OPPORTUNITIES FOR RESOURCE CORES [optional; opportunities to exceed the $1M direct costs cap, but not to exceed $1.25M in direct costs]

The principal goal of the opportunities listed below is to provide NIDDK Diabetes Center research core services (and pilot and feasibility grant opportunities) to diabetes researchers at institutions that are not currently served by an NIDDK Diabetes Research Center.

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).

2) A Diabetes Research Center Core may 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 the applicant institution or an affiliated hospital. Such a Regional/National Shared Resource Core should provide a plan for expanding core services to investigators outside of the parent academic institution and its affiliated hospitals. Applicants should document that there is sufficient demand by the wider scientific community for the expansion (or establishment) of the proposed core services. The research base in diabetes at the institution(s) that would use the regional core(s) should also be documented. Plans for prioritization of research core services, as well as training to the broader research community, should be provided. Support for the expansion of the Center P&F program to the partnering institution(s) may also be requested (see below).

3) To broaden the scope and reach of the Diabetes Research Center P&F program, a Center may propose to serve a wider scientific community by expanding the Diabetes Center P&F program to a different institution(s). Expansion of the P&F program to an affiliated institution/hospital is encouraged, but will not be considered a Regional/National program for purposes of expanding the allowable requested funds. In general, NIDDK currently expects Diabetes Research Centers to allow investigators at affiliated hospitals or institutions to participate in the Center P&F program. Applicants may request funds to expand their P&F program to researchers at non-Diabetes Research Center institutions, and the applicant should provide details on how F&A costs for P&F grants will be handled with the partnering institution(s).

IV. SUBCONTRACTS TO SUPPORT UNDERSERVED OR HEALTH DISPARITY POPULATIONS [optional; subcontracts for this funding opportunity have no direct costs cap]

Diabetes Research Centers may propose partnerships that establish research cores and/or P&F programs at institutions of higher education (i.e., rural institutions, historically black colleges and universities (HBCUs), Tribally Controlled Colleges and Universities (TCCUs) and Hispanic-serving Institutions (HSIs) and Alaska Native and Native Hawaiian Serving Institutions), or other agencies that focus on underserved or health disparity populations. The primary goal of such partnerships is to foster scientific collaborations and to provide access to the Diabetes Research Center infrastructure to investigators at these institutions or organizations in order to foster health disparities research in populations disproportionately affected by diabetes. All funds exceeding the cap proposed for this purpose must be awarded to the institution that serves underserved or health disparity populations. Funding for activities supporting the collaboration at the Diabetes Center institution must be included with the Diabetes Research Center cap.

V. ADDITIONAL FEATURES

Cooperation, Coordination and Integration: applicants from institutions with an NIH Clinical and Translational Science Award program (http://www.ctsaweb.org/) are strongly encouraged to utilize the CTSA as a resource for enhancing clinical research programs within the Diabetes Research Center. In such cases, appropriate letters of support from the CTSA program director or principal investigator should be included with the application detailing plans for appropriate integration and synergy of the Diabetes Research Center and CTSA activities. In addition, applicants should address the potential for integration, harmonization, and enhancement of Diabetes Research Center activities through cooperation with other NIH-supported core facilities at the applicant institution. Other NIH-supported Centers and associated cores at the institution should be identified, and assurances provided that overlap or redundancy in core services will be avoided unless expressly required to fulfill the mission of the Diabetes Research Center.

The Diabetes Research Center must provide support for enrichment activities to foster multidisciplinary approaches to diabetes research and to attract new investigators or investigators with relevant expertise to diabetes research. While many of these activities occur at the grantee institution, applicants are encouraged to suggest coordinated

efforts, such as educational activities, that might operate on a regional or national level and involve multiple Diabetes Research Centers. The application should include a statement regarding willingness to participate in such activities.

The proposed budget should include travel for the Program Director/Principal Investigator (PD/PI) and Associate Center Director, or other key personnel, to attend an annual Diabetes Research Centers meeting. The application should include a statement of willingness to attend this annual meeting of Diabetes Research Center Directors.

Core access and Cost Recovery: core resources must precisely define issues regarding access to core services, including investigator eligibility requirements for services, and policies and procedures for prioritization of services when demand exceeds capacity. Financial considerations such as calculations that justify investment of funds in core services (e.g. comparative costs of other sources of proposed core services) and policies for cost recovery from investigators for use of services should also be included.

Center Evolution: Centers must document policies and procedures for ensuring continuing evolution of core services in response to changing needs. New technologies or services might appear that should be supported, existing technologies might become less important, or economic changes might obviate the need for core services, such as the availability of cost-effective commercial services or core services provided by the research institution. Cores should address the issue of allocation of resources to development of new technologies versus provision of services with existing technologies. In addition, cores must have well-defined policies to insure that intellectual property is identified and appropriately protected, but that intellectual property issues do not impede sharing of resources.

Section II. Award Information

Funding Instrument	Grant
Application Types Allowed	New Resubmission Renewal, including active Diabetes Research and Training Center (DRTC) grants that currently use the P60 grant mechanism. The OER Glossary and the PHS398 Application Guide provide details on these application types.
Funds Available and Anticipated Number of Awards	The number of awards is contingent upon NIH appropriations, and the submission of a sufficient number of meritorious applications. NIDDK intends to commit $15M in FY 2012.
Award Budget	Application budgets are limited to $1.0M per year in direct costs unless the applicant organization proposes to provide regional or national core services as described in the

	Funding Opportunity Announcement. Applications proposing a Regional/National Shared Research Resource Core are limited to $1.25M per year in direct costs. These budget limits are exclusive of: (a) first year equipment costs, (b) direct costs on subcontracts, health departments, community health centers or other agencies that focus on underserved or health disparity populations for the purpose of establishing collaborations and providing access to the research infrastructure to investigators at these institutions to foster health disparities research in populations disproportionately affected by diabetes, and (c) F&A costs on consortium and subcontract arrangements. It is anticipated that the award budget will be directly correlated to the breadth, quality and relevance to diabetes and related areas of the research base being served by the Center.
Award Project Period	The project period for an application submitted in response to this funding opportunity may not exceed five years.

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

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions

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

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

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions

Nonprofits Other Than Institutions of Higher Education

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

For-Profit Organizations

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

Governments

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

Other

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

Common Fund/Roadmap text, Collaborative Research, or Projects Greater than 5 years Duration: See instructional documents in the NIH Guide Publishing System for the text to insert.

Foreign Institutions

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

Required Registrations

Applicant organizations must complete the following registrations as described in the PHS398 Application Guide to be eligible to apply for or receive an award. Applicants must have a valid Dun and Bradstreet Universal Numbering System (DUNS) number in order to begin each of the following registrations.

- Central Contractor Registration (CCR) – must maintain an active registration, to be renewed at least annually

- eRA Commons

All Program Directors/Principal Investigators (PD/PIs) must also work with their institutional officials to register with the eRA Commons or ensure their existing eRA Commons account is affiliated with the eRA Commons account of the applicant organization.

All registrations must be completed by the application due date. Applicant organizations are strongly encouraged to start the registration process at least four (4) weeks prior to the application due date.

Eligible Individuals (Program Director/Principal Investigator)

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

For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the PHS398 Application Guide.

Because a Diabetes Research Center has a large and complex administrative structure, the Project Director/Principal Investigator (PD/PI) must have strong leadership abilities and demonstrated proficiency in managing large, multi-component projects.

2. Cost Sharing

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

3. Additional Information on Eligibility

Number of Applications

Only one application per institution is allowed.

NIH will not accept any application in response to this FOA that is essentially the same as one currently pending initial peer review unless the applicant withdraws the pending application. NIH will not accept any application that is essentially the same as one already reviewed. Resubmission applications may be submitted, according to the NIH Policy on Resubmission Applications from the PHS398 Application Guide.

Research Base: Successful Diabetes Research Center applications require an existing program of excellence in biomedical research in the area of diabetes, its complications, and in related research in endocrine and metabolic diseases. To justify Center support, the Diabetes Research Center must serve a large research base of NIDDK-funded investigators pursuing research activities in Center topic areas, as well as diabetes investigators with other sources of peer-reviewed support. Suggestions for describing and presenting this research base in the application are included in the Administrative Guidelines for NIDDK Diabetes Research Centers (http://www2.niddk.nih.gov/Research/Centers/CenterPrograms/).

Section IV. Application and Submission Information

1. Address to Request Application Package

Applicants are required to prepare applications according to the current PHS 398 application forms in accordance with the PHS 398 Application Guide.

2. Content and Form of Application Submission

It is critical that applicants follow the instructions in the PHS398 Application Guide, except where instructed in this funding opportunity announcement to do otherwise. Conformance to the requirements in the Application Guide is required and strictly enforced. Applications that are out of compliance with these instructions may be delayed or not accepted for review.

Letter of Intent

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

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

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

The letter of intent should be sent to:

Francisco O. Calvo, Ph.D.
Chief, Review Branch
National Institute of Diabetes and Digestive and Kidney Diseases
6707 Democracy Boulevard, Rm. 752
Bethesda, MD 20892-5452
(for express/courier service: Bethesda, MD 20817)
Telephone: 301-594-8897
Email: fc15y@nih.gov

Application Submission

Applications must be prepared using the PHS 398 research grant application forms and instructions for preparing a research grant application. Submit a signed, typewritten original of the application, including the checklist, and three signed photocopies in one package to:

Center for Scientific Review
National Institutes of Health
6701 Rockledge Drive, Room 1040, MSC 7710
Bethesda, MD 20892-7710 (U.S. Postal Service Express or regular mail)
Bethesda, MD 20817 (for express/courier service; non-USPS service)

At the time of submission, two additional paper copies of the application and all copies of the appendix files must be sent to:

Francisco O. Calvo, Ph.D.
Chief, Review Branch
National Institute of Diabetes and Digestive and Kidney Diseases
6707 Democracy Boulevard, Rm. 752
Bethesda, MD 20892-5452
(for express/courier service: Bethesda, MD 20817)
Telephone: 301-594-8897
Email: fc15y@nih.gov

Page Limitations

All page limitations described in the PHS398 Application Guide and the Table of Page Limits must be followed, with the following requirements:

- Introduction to a Resubmission Application is limited to 1 page.

- Research Strategy for the Center Overview is limited to 12 pages.
- Research Strategy for the Administrative Component is limited to 6 pages.
- Research Strategy for the Center Research Base is limited to 6 pages.
- Research Base Investigator Project Description is limited to 1 page for each Center investigator to describe funded research, collaborations, and use of Diabetes Research Center resources
- Research Strategy for each Research Core is limited to 12 pages.
- Research Strategy for the Center Research Base is limited to 6 pages.
- Research Strategy for each Pilot & Feasibility Program is limited to 12 pages.
- Research Strategy for the Enrichment Program is limited to 6 pages.

Supplemental Instructions for the Preparation of Diabetes Research Center Applications
Research Plan

All instructions in the PHS398 Application Guide must be followed, with the following additional instructions:

Content and order of information to be provided should be presented in the form and format as described in the Diabetes Center administrative guidelines (http://www.niddk.nih.gov/fund/other/guidelines.pdf) with adjustments as indicated in this FOA. An overview of the structure of a responsive Center application is provided below. Every effort should be made to provide information in tabular or chart form where indicated in the guidelines to facilitate application preparation and review. Failure to comply with these instructions may result in return of the application without review.

SECTION 1: CENTER OVERVIEW

- Face Page, Descriptive Abstract, Key Personnel and Table of Contents should be prepared as per standard instructions.
- Budgets

1. Detailed Budget for Initial Budget Period (398- Form Page 4)

2. Budget for Entire Proposed Project Period (398- Form Page 5);

3. Consolidated budget for first year of requested support (See Guidelines Illustration I;

   budgets for each individual Core should immediately precede the narrative for each Core)

4. Distribution of Professional Effort (see Guidelines Illustration II)

- Biographical Sketches (in alphabetical order): for all Diabetes Research Center investigators (key personnel, research base investigators, consultants and collaborators (PHS 398- Form Pages)

Biographical sketches for principal investigators on proposed P&F projects should be included within the P&F program section.

- Summary of total current and pending support of all Center investigators. Some institutions may have more than one NIDDK-funded Center grant. In such cases, research grants of investigators who participate in more than one NIDDK-funded Center should be noted when the research grant listed in the Diabetes Research Center application is also included as part of the research base of another NIDDK Center (see Guidelines Illustration III).
- Resources Format Page (PHS 398 Form Page):

Facilities and Major Equipment: general overall description of research facilities (space, equipment, collaborations, etc.) and the major, shared pieces of equipment to be used by Center members should be provided.

Note: Specific core facilities, equipment, and special resources should also be listed in each proposed core component.

- Specific Aims (limited to 1 page): Provide the broad, long-range objectives and goals of the proposed Diabetes Research Center.
- Research Strategy (limited to 12 pages): This narrative section summarizes the overall plan for the proposed or established multi-component Center. The multi-component application should be viewed as a confederation of interrelated research resources that are complementary to one another. This is an important section for it provides the group of investigators an opportunity to give conceptual wholeness to the overall Center – by giving a statement of the general problem area and by laying out a broad strategy for attacking the problems. As the strategy develops, each individual research component/core should be cited briefly as to its place in the overall scheme. Provide a general overall description of the facilities and institutional commitment; summarize the special features in the environment and/or resources that make this application strong or unique. Other Considerations: include listing of other relevant Centers and cores at the institution and affiliated hospitals, and plans to integrate, harmonize and reduce redundancies in activities. For Renewals: the Center Overview section should also highlight past performance and the major accomplishments from the prior funding period as described in the PHS 398 Instructions; changes from the original Center design should be highlighted.

SECTION 2: ADMINISTRATIVE COMPONENT

- Description (PHS 398- Form Page 2)

- Key personnel (PHS 398- Form Page 2 cont'd)
- Budget with comprehensive budgetary justifications (PHS 398- Form Page 4); funds requested for the P&F and enrichment programs should be included in the "other expenses" category of the budget for the Administrative Core.
- Biographical Sketches: Director and Associate Director(s) (PHS 398 Form page)
- Specific Aims (limited to 1 page): Describe the broad, long-range objectives and goals of the Administrative structure within the context of the proposed Center.
- Research Strategy (limited to 6 pages): Presentation of the administrative structure; Relationship and lines of authority and sanction by appropriate institutional officials; Description of the process that would be used to recommend a successor to the Director, if needed; Committee structure (include External and Internal advisory boards and the pilot and feasibility program oversight committee; Description of plans for website development, maintenance and curation.

SECTION 3: BIOMEDICAL RESEARCH COMPONENT

- Center Biomedical Research Base, Research Strategy (limited to 6 pages): Provide an overview of ongoing research and the impact of the Center on this research. Include an overview of the current research in diabetes, its complications, and in related endocrine and metabolic diseases at the institution(s). An appropriate and clear presentation of the ongoing research base is critical since it will show the research focus of the Diabetes Research Center and the interrelationships and potential for collaborations among investigators. Since the research base projects will already have been peer-reviewed, the quality of the individual funded projects will have been established and will not be re-evaluated. Provide sufficient detail to assist reviewers in judging the extent and the interrelatedness of ongoing research. Grouping the research base into areas of emphasis for the Center is advised.

New applications: Emphasize the anticipated impact of the establishment of a Diabetes Research Center on the research base. Include an indication of how the establishment of a Diabetes Research Center will provide added dimensions and new opportunities for diabetes and related research, along with increased cooperation, communication, and collaboration among investigators.

For Renewals: Progress including description of significant findings and new participants.

- Description of biomedical research base investigators: Organize the presentation of the research base to emphasize the focus of the research and the interrelationships of the Diabetes Research Center investigators. Provide a narrative description of no more than one page per research base investigator; try to limit each to less than one page. These narratives should include: (1) the grant number(s), title(s), and a few descriptive sentences, and (2) a list of the core(s) used with a brief sentence indicating what aspect of the research justifies the use of each core. Include ONLY those grants awarded, or subcontracted, to investigators at the applicant institution or consortium, not to

investigators at other locations, in the description of the research base. It is particularly important to provide a few sentences indicating the relatedness of a cited grant to research in diabetes, its complications, or related endocrine and metabolic diseases when this is not readily apparent from the title of the grant.

- Document collaborative efforts using a format such as Guidelines Illustration IV to aid in the review process.
- Biomedical Research Cores (present each core separately; Research Strategy limited to 12 pages per core)

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (PHS 398- Form Page 4)

4. Biographical sketches: Core Director and key personnel (PHS 398- Form Page)

5. Specific Aims (limited to 1 page): List in priority order, the broad, long-range objectives and goals of the proposed core. In addition, state the core's relationship to the Center goals and how it relates to the other cores at the applicant institution and in the application.

6. Research Strategy, including: Objectives of the core; Core function, including quality control; Benefits from core; Proposed developmental research or training; Future directions and plans to ensure continuing evolution & relevance of the core; For renewals: Core progress and productivity (include 2-3 examples of literature citations, grant awards, and 2-3 key advances supported by core activity); to assist reviewers, for each core also refer to the page numbers of the individual core-specific research publications in Guidelines Illustration VII; if applicable, describe any recharge system that may be in place to allow investigators to utilize a core, including information on any proposed F&A charges to outside users of the core.

7. New applications: Funded investigators who will use the core and proposed extent of use

(see Guidelines Illustration V). For Renewals: Core Use during the last grant period (see Guidelines Illustration V)

- Pilot and Feasibility Program

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (to be included in the Administrative Component budget; justify any changes for future years)

4. Biographical sketches: Program Director and Committee (PHS 398- Form Page)

5. Specific Aims (limited to 1 page):

6. Research Strategy (limited to 12 pages): Management of the pilot and feasibility program; Program progress and productivity (include key publications supported by the P&F program, grant awards resulting directly from P&F awards, and 2-3 key advances supported by the P&F program); Future directions and plans; For initial applications include: eligibility requirements, selection process, abstracts of proposed P&F awards, and justification for core usage by P&F awards; For competing renewal applications include: Total number of all P&F submissions received each year during the prior project period, selection process and funding success rates, single paragraph synopses of Pilot & Feasibility studies awarded during the last project period. Clearly indicate the Named New Investigator, if such a position is being requested, and how he/she was selected. Include salary support for this position in the Administrative Core personnel section.

7. For Renewals: Pilot and Feasibility Project Outcomes (see Guidelines Illustration VI)

- Enrichment Program

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-contd)

3. Budget with justifications (to be included in Administrative Component budget)

4. Biographical sketches: Program director and key personnel (PHS 398- Form Page)

5. Specific Aims (limited to 1 page)

6. Research Strategy (limited to 6 pages): New applications: Describe plans for the enrichment program; Renewal applications: Describe the enrichment program and indicate the program's value to Center members. Indicate how the program has grown or been adapted to better serve Center members' needs during the past funding period; Future directions and plans to ensure continuing evolution and relevance of the enrichment program; Other considerations (include plans to enhance interactions with relevant NIDDK supported T32 training programs; letters of acknowledgment and support from T32 PD/PIs should be provided separately)

SECTION 4: REGIONAL/NATIONAL SHARED RESOURCE CORES & EXPANSION OF THE PILOT & FEASIBILITY PROGRAM [OPTIONAL]

- Biomedical research cores (two opportunities): 1) a Regional/National Shared Resource Core that is located at a different institution, and/or 2) expansion of research core services at the applicant organization to serve a wider scientific community on a geographic or national level; present each core separately; Research Strategy limited to 12 pages per core)

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (PHS 398- Form Page 4)

4. Biographical sketches: Core Director and key personnel (PHS 398- Form Page)

5. Specific Aims (limited to 1 page): List in priority order, the broad, long-range objectives and goals of the proposed core. In addition, state the core's relationship to the Center goals and how it relates to the other cores at the applicant institution and in the application.

6. Research Strategy, including: Objectives of the core; Core function, including quality control; Benefits from core to current Center members and/or the wider scientific community; Proposed developmental research or training; Future directions and plans to ensure continuing evolution & relevance of the core; if applicable, describe any recharge system that may be in place to allow investigators to utilize a core, including information on any proposed F&A charges to outside users of the core.

7. New Cores: Funded investigators who will use the core and proposed extent of use (see Guidelines Illustration V). For Existing Cores: Core use during the last grant period (see Guidelines Illustration V).

- Expansion of the Diabetes Research Center Pilot and Feasibility Program to diabetes researchers at additional institutions

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (PHS 398- Form Page 4); justify any changes for future years; provide details on how F&A costs for P&F grants will be handled with the partnering institution(s).

4. Biographical sketches: Program Director and Committee (PHS 398- Form Page); provide

details on how F&A costs for P&F grants will be handled with the partnering institution.

5. Specific Aims (limited to 1 page)

6. Research Strategy (limited to 12 pages): Management of the expanded pilot and feasibility program; plans for advertizing and solicitation; For applications proposing a new, expanded P&F program: eligibility requirements, review and selection process, abstracts of proposed P&F awards, and plans for research core access and usage by P&F awardees; For competing renewal applications with an existing, expanded P&F program: Total number of all P&F submissions received each year during the prior project period, selection process and funding success rates, single paragraph synopses of Pilot & Feasibility studies awarded during the last project period.

7. For applications proposing a new, expanded P&F program: Biographical sketches of proposed P&F Awardees (PHS 398- Form Page)

SECTION 5: SHARED RESEARCH CORES AND/OR EXPANSION OF THE PILOT & FEASIBILITY PROGRAM TO SUPPORT UNDERSERVED OR HEALTH DISPARITY POPULATIONS [OPTIONAL]

- Subcontracts for Research/Resource Cores at institutions of higher education (i.e., rural institutions, historically black colleges and universities (HBCUs), Tribally Controlled Colleges and Universities (TCCUs) and Hispanic-serving Institutions (HSIs) and Alaska Native and Native Hawaiian Serving Institutions), or other agencies/organizations that focus on underserved or health disparity populations. (subcontracts for this opportunity have no direct costs cap); Research Strategy is limited to 12 pages per proposed core/activity.

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (PHS 398- Form Page 4)

4. Biographical sketches: Core Director and key personnel (PHS 398- Form Page)

5. Specific Aims (limited to 1 page): List in priority order, the broad, long-range objectives and goals of the proposed core. In addition, state the relationship of the proposed core to the Center goals and how it relates to the current cores at the applicant institution and in the application.

6. Research Strategy, including: Objectives of the core; Core function, including quality control; Benefits of the core to current Center members and the investigators at the minority-serving institution or organization; Plans for evaluating the objectives of the proposed partnership; Proposed developmental research or training; Future directions and plans to ensure continuing evolution & relevance of the core; if applicable, describe any recharge system that may be in place to allow investigators to utilize a core, including information on any proposed F&A charges to outside users of the core.

7. New Cores: Funded investigators who will use the core and proposed extent of use (see Guidelines Illustration V). For Existing Cores: Core Use during the last grant period (see Guidelines Illustration V).

- Subcontracts for expansion of the Diabetes Research Center Pilot and Feasibility Program to support investigators at institutions of higher education (i.e., rural institutions, historically black colleges and universities (HBCUs), Tribally Controlled Colleges and Universities (TCCUs) and Hispanic-serving Institutions (HSIs) and Alaska Native and Native Hawaiian Serving Institutions), or other agencies/organizations that focus on underserved or health disparity populations.

1. Description (PHS 398- Form Page 2)

2. Key Personnel (PHS 398- Form Page 2-cont'd)

3. Budget with justifications (PHS 398- Form Page 4); justify any changes for future years; provide details on how F&A costs for P&F grants will be handled with the partnering

institution/organization(s).

4. Biographical sketches: Program Director and Committee (PHS 398- Form Page)

5. Specific Aims (limited to 1 page):

6. Research Strategy (limited to 12 pages): Management of the expanded pilot and feasibility program; plans for advertizing and solicitation; For applications proposing a new, expanded P&F program at a subcontracting institution include: eligibility requirements, review and selection process, abstracts of proposed P&F awards, and plans for research core access and usage by P&F awardees; For competing renewal applications with an existing P&F program at a subcontracting institution: Total number of all P&F submissions received each year during the prior project period, selection process and funding success rates, single paragraph synopses of Pilot & Feasibility studies awarded during the last project period.

7. For applications proposing a new, expanded P&F program: Biographical sketches of proposed P&F Awardees (PHS 398- Form Page)

SECTION 6: CENTER-RELATED INFORMATION (suggested Illustrations only)

- Suggested Illustration for Renewal Applications: Publications Citing Support from this Center during the past project period. List only those publications that clearly used Center resources (e.g. core or P&F support); do not list all publications from Center members (see Guidelines Illustration VII; include PMCID numbers).
- Checklist (PHS 398- Form Page)

Resource Sharing Plan

Individuals are required to comply with the instructions for the Resource Sharing Plans (Data Sharing Plan, Sharing Model Organisms, and Genome Wide Association Studies; GWAS) as provided in the PHS398 Application Guide, with the following modifications:

- Generally, Resource Sharing Plans (Data Sharing Plan, Sharing Model Organisms, and GWAS Sharing Plan) are expected, but they are not applicable for this FOA.

Appendix

Do not use the appendix to circumvent page limits. Follow all instructions for the Appendix (please note all format requirements) as described in the PHS398 Application Guide.

3. Submission Dates and Times

Part I. Overview Information contains information about Key Dates.

Information on the process of receipt and determining if your application is considered "on-time" is described in detail in the PHS398 Application Guide.

Applicants may track the status of the application in the eRA Commons, NIH's electronic system for grants administration.

4. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review.

5. Funding Restrictions

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

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

6. Other Submission Requirements and Information

Applications must be received on or before the due dates in Part I. Overview Information. If an application is received after that date, it will not be reviewed.

Upon receipt, applications will be evaluated for completeness by the Center for Scientific Review and responsiveness by components of participating organizations, NIH. Applications that are incomplete and/or nonresponsive will not be reviewed.

The Diabetes Research Center must be an identifiable organizational unit within a single university, medical school, or within a consortium of cooperating institutions with a university affiliation. To qualify for a Diabetes Research

Center grant, just as with all other P30 grants, the applicant institution must already have a substantial base of ongoing, independently supported, peer-reviewed research projects in diabetes mellitus and its complications, and related areas of endocrinology and metabolic diseases.

The research base must exist prior to the submission of an application and it is a critical element considered during the peer review process. The currently funded research base provides the major support for a group of investigators who would benefit from shared resources. The body of research described as the research base may include only currently funded, peer-reviewed research grants awarded to the applicant institution/consortium. These may be federally or privately funded awards. Training grants and fellowship awards are not considered part of the research base. Focus, relevance, interrelationships, quality, productivity, and, to some extent, quantity, are all considered in judging the adequacy of the research base. Although collaborations with investigators outside the applicant institution/consortium are encouraged, the research base includes ONLY support for the investigators at the applicant institution/consortium.

Post Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in NOT-OD-10-115.

Section V. Application Review Information

1. Criteria

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

Overall Impact - Overall

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

Scored Review Criteria - Overall

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

Significance

Does the Center address an important problem or a critical barrier to progress in the field? If the aims of the Center are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field? What are the strengths of the Center's research base (its breadth and depth) and the relevance and interrelation of the separately funded research projects to the focus/theme(s) of the Center? Is there a strong scientifically excellent research base in diabetes, its complications, and related endocrinology and metabolic diseases at the Center, which would benefit by the services/programs supported through the Diabetes Research Center? What is the likelihood that the Diabetes Research Center will increase efficiency; promote new research directions and meaningful collaborations among Center investigators; facilitate interactions and collaborations among the investigators; and prove cost-effective? In renewal applications, have the benefits of the Center been documented in the form of increased collaborations, new research directions, and cost savings?

Investigator(s)

Are the PD/PIs, collaborators, and other researchers well suited to the Center? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project? Are the Center investigators responsible for the individual research projects willing to interact with each other and contribute to the overall objectives of the Diabetes Research Center? What are the scientific and administrative leadership abilities of the proposed center Director and Associate Director(s) and their commitment and ability to devote adequate time to the effective management of the Center program? If applicable, are the P&F studies submitted for evaluation from applicants eligible for P&F funding? If requested, does the Named New Investigator appear well qualified and eligible for support?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed? Does the selection process by which the individual Pilot & Feasibility (P&F) studies were selected appear appropriate; does the Center encourage ‘high-risk’, innovative ideas through their P&F program? Have the cores provided new methods, techniques, and/or resources and developed ways to support investigators in new areas of diabetes and its complications, and related areas of endocrinology and metabolism research, as appropriate to the purpose of the core and the research supported by the Center?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the Center? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?

If the Center involves clinical research, are the plans for 1) protection of human subjects from research risks, and 2) inclusion of minorities and members of both sexes/genders, as well as the inclusion of children, justified in terms of the scientific goals and research strategy proposed? How appropriate and relevant are the proposed cores and the modes of operation (such as potential utilization, prioritization of requests for services, cost-recovery, and quality control monitoring)? Will the cores provide opportunities not otherwise available to the investigators through other available federally funded and/or institutional resources; represent appropriate cost savings/cost sharing advantage; and stimulate the development of new approaches? Is appropriate administrative organization proposed for the following:(a) coordination of ongoing research between the separately funded projects and the Center, including mechanisms for internal monitoring;(b) establishment and maintenance of internal communication and cooperation among the Center investigators;(c) mechanism for selecting and replacing professional or technical personnel within the cores;(d) mechanism for reviewing the use of, and administering funds for, the P&F program;(e) management capabilities, including fiscal administration, procurement, property and personnel management, planning, budgeting, and other appropriate capabilities? Is there efficient and effective use and/or planned use of the limited enrichment funds, including the contribution of these activities to the stated goals of the Center?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements? Is there evidence of institutional commitment to the Center program, including lines of accountability, regarding management of the Center grant and the institution's contribution to the management capabilities of the Center? Is there clear potential for interaction with scientists from other departments and institutions?

Additional Review Criteria - Overall

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

The following additional review criteria apply to all new and renewal Diabetes Research Center applications. Foremost, does the research base to be supported by the Center show evidence of a strong and consistent record of productivity and peer-reviewed funding in Center-related research areas? Do the proposed cores fill a need present in the diabetes research community, and will they provide services that would otherwise be unavailable, or be more cost-effective to conduct centrally? Is the necessary technical and analytical expertise available? Does the application demonstrate ability to monitor use and utility of the cores, and provide approaches to ensure continuing development and evolution of services as needs of the community change? Does the existing Center show clear evidence of successful implementation of a recharge structure to support expanded and/or evolving Center activities? Do the new proposals document a clear intent to implement a recharge structure to support expanded and/or evolving Center activities?

Protections for Human Subjects

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

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

Inclusion of Women, Minorities, and Children

When the proposed Center involves clinical research, the committee will evaluate the proposed plans for inclusion of minorities and members of both genders, as well as the inclusion of children. For additional information on review of the Inclusion section, please refer to the Human Subjects Protection and Inclusion Guidelines.

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following five points: 1) proposed use of the animals, and species, strains, ages, sex, and numbers to be used; 2) justifications for the use of animals and for the appropriateness of the species and numbers proposed; 3) adequacy of veterinary care; 4) procedures for limiting discomfort, distress, pain and injury to that which is unavoidable in the conduct of scientifically sound research including the use of

analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices; and 5) methods of euthanasia and reason for selection if not consistent with the AVMA Guidelines on Euthanasia. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animal Section.

Biohazards

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

Resubmissions

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

Renewals

For Renewals, the committee will consider the progress made in the last funding period, as follows:

Research Base:

- Does the Center show evidence of a stable or growing research base with strong and consistent record of scientific excellence and achievement reflected in an outstanding level productivity and continuing success in securing peer-reviewed research funding?
- Does the Center show evidence of fostering multi-disciplinary collaborations among its Center investigators?

Biomedical Cores:

- Are the number and impact of research publications that acknowledge the Center sufficient to justify each core?
- Is there a significant fraction of papers that a) acknowledge the Center and b) do not have core personnel as co-authors?
- Are the number and listing of Center investigators who have used the core and resultant key advances consistent with the level of core investment?
- Do the number and listing of investigators who have used the core multiple times indicate satisfaction and continuing need for core services?
- Are there sufficient numbers of users who are not core personnel or their collaborators?
- Are the number and listing of users who are not Center personnel or members consistent with the best utilization of the core by the community?

- Are the numbers of services/tests completed by each core indicative of a growing need and sufficient to justify continued support?
- Is the capacity of each core with current resources sufficient to serve the needs of the Center community?
- Does the Center provide evidence of ability to evolve cores to meet changing needs of the research community?
- Does the Center provide evidence of Program Income and sufficient institutional support?
- Does the Center website show evidence of continuing maintenance and a high level of quality and usability?

Administrative Core:

- Has the administrative structure proven effective?
- Has the enrichment program been effective?
- Is(Are) the Center Director(s) appropriately qualified to lead the Diabetes Research Center?

Pilot & Feasibility Program:

- Are the numbers and types of P&F awards well justified?
- Are data provided to document the outcome of all P&F projects completed in the last five years, including those that failed to lead to further funding?
- Are papers generated under these awards, projects successfully funded with independent grants, and key advances linked to these awards well documented and consistent with the level of support provided?

Revisions

Not Applicable.

Additional Review Considerations - Overall

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

Applications from Foreign Organizations

Not Applicable.

Select Agent Research

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

Resource Sharing Plans

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

Budget and Period of Support

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

2. Review and Selection Process

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

As part of the scientific peer review, all applications:

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

Applications will compete for available funds with all other recommended applications submitted in response to this FOA. Following initial peer review, recommended applications will receive a second level of review by the National Diabetes and Digestive and Kidney Diseases Advisory Councill. The following will be considered in making funding decisions:

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

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons.

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

Section VI. Award Administration Information

1. Award Notices

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

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

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

Any application awarded in response to this FOA will be subject to the DUNS, CCR Registration, and Transparency Act requirements as noted on the Award Conditions and Information for NIH Grants website.

2. Administrative and National Policy Requirements

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

Cooperative Agreement Terms and Conditions of Award

Not Applicable.

3. Reporting

When multiple years are involved, awardees will be required to submit the Non-Competing Continuation Grant Progress Report (PHS 2590) annually and financial statements as required in the NIH Grants Policy Statement.

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

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

Section VII. Agency Contacts

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

Application Submission Contacts

GrantsInfo (Questions regarding application instructions and process, finding NIH grant resources)
Telephone 301-435-0714
TTY 301-451-5936
Email: GrantsInfo@nih.gov

eRA Commons Help Desk(Questions regarding eRA Commons registration, tracking application status, post submission issues)
Phone: 301-402-7469 or 866-504-9552 (Toll Free)
TTY: 301-451-5939
Email: commons@od.nih.gov

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Section VIII. Other Information

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

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Parts 74 and 92.

Diabetes Research Centers
Up-Coming RFAs

Jim Hyde, Ph.D.
National Institute of Diabetes & Digestive & Kidney Diseases

NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Fiscal Year 2013

- **RFA-DK-11-015**: Published in NIH Guide on August 1, 2011
- Application deadline: February 29, 2012
- Initial Review: June/July 2012
- Earliest Funding: December 2012 (FY2013)
- Renewal Applications:
  - Baltimore Area - U Chicago
  - Baylor - U Michigan
  - Columbia - U Washington
  - UAB - Washington U
  - UCSD/UCLA - Yale

Next RFA: Fiscal Year 2015

- RFA to be published in NIH Guide by early 2014 (late 2013)
- Application deadline: Early Summer 2014
- Initial Review: Fall 2014
- Earliest Funding: April 2015 (FY2015)
- Renewal Applications:
  - Albert Einstein COM
  - Boston Area
  - UCSF

NIDDK Center Programs (P-series grants)

- Diabetes Research Centers
- Centers for Diabetes Translation Research
- Molecular Therapy Centers
- Cystic Fibrosis Research and Translation Centers
- Digestive Diseases Research Centers
- Nutrition and Obesity Research Centers
- O'Brien Kidney Research Centers
- O'Brien Urology Research Centers
- Research Centers for Excellence in Pediatric Nephrology
- Polycystic Kidney Disease Research & Translation Centers
- Centers of Excellence in Molecular Hematology

Program Officers for NIDDK Centers

- All attended at least one of the five NIDDK Center site visits
- NIDDK program directors are currently having meetings to discuss areas for harmonization and improvement (RFAs, Center Administrative Guidelines, etc.)
- If you have suggestions for improving RFAs, Administrative Guidelines, etc., let Jim know.

Questions

- Should NIDDK consider organizing some future, joint meetings of Center PIs? For example, DRCs and NORCs.
- Would a presentation each year about other DK Center programs at the annual Diabetes Centers' Directors meeting be useful?

Questions

- Would it be worthwhile to create a 1-page, double-sided brochure to help advertize and promote the NIDDK Diabetes Research Centers program?

Cell Metabolism
Article

Autophagy in Hypothalamic AgRP Neurons Regulates Food Intake and Energy Balance

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SUMMARY

Macroautophagy is a lysosomal degradative pathway that maintains cellular homeostasis by turning over cellular components. Here we demonstrate a role for autophagy in hypothalamic agouti-related peptide (AgRP) neurons in the regulation of food intake and energy balance. We show that starvation-induced hypothalamic autophagy mobilizes neuron-intrinsic lipids to generate endogenous free fatty acids, which in turn regulate AgRP levels. The functional consequences of inhibiting autophagy are the failure to upregulate AgRP in response to starvation, and constitutive increases in hypothalamic levels of pro-opiomelanocortin and its cleavage product α-melanocyte-stimulating hormone that typically contribute to a lean phenotype. We propose a conceptual framework for considering how autophagy-regulated lipid metabolism within hypothalamic neurons may modulate neuropeptide levels to have immediate effects on food intake, as well as long-term effects on energy homeostasis. Regulation of hypothalamic autophagy could become an effective intervention in conditions such as obesity and the metabolic syndrome.

INTRODUCTION

Chronic overnutrition contributes to metabolic disturbances that predispose to the development of obesity and insulin resistance (Lionetti et al., 2009), hallmark of the metabolic syndrome (de Luca and Olefsky, 2008). The molecular mechanisms and cell-intrinsic pathways that govern neuronal regulation of food intake are unclear. The hypothalamic arcuate nucleus consists of neurochemically discrete and functionally antagonistic neurons, including agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons (Sainsbury and Zhang, 2010) that form a focal point for the integration of nutritional and metabolic cues, central and peripheral neural afferents (Morris and Rui, 2009), and action of adiposity hormones such as leptin and insulin (Belgardt and Bruning, 2010). Hypothalamic neurons release specific neuropeptides (Sainsbury and Zhang, 2010) that generate behavioral responses including food seeking and the initiation or cessation of feeding. AgRP increases food intake by acting as a natural antagonist for the melanocortin receptors (Garfield et al., 2009). POMC neurons express POMC that is processed further to secrete α-melanocyte-stimulating hormone (α-MSH) for regulating food intake and energy expenditure (Mountjoy, 2010). In addition to extrinsic nutritional signals, levels of intracellular metabolites, particularly the neuronal availability of free fatty acids, have been proposed to play a role in the hypothalamic regulation of food intake (Andrews et al., 2008). However, the intraneuronal mechanisms that regulate endogenous levels of free fatty acids within the hypothalamus, as well as mechanisms that link fatty acid availability to AgRP secretion, are not known.

Macroautophagy (hereafter autophagy) is a cellular process that recycles organelles and proteins to maintain cellular homeostasis (He and Klionsky, 2009). In addition, autophagy serves as an alternative energy source to sustain cellular function during starvation. Induction of autophagy requires the de novo formation of a double-walled limiting membrane that elongates and seals to form an autophagosome. This vesicle engulfs and targets cargo destined for degradation by fusing with the lysosome (He and Klionsky, 2009). An upstream negative regulator of autophagy is the nutrient-sensor mammalian target of rapamycin (mTOR) (Pattingre et al., 2008). Activation of hypothalamic mTOR has been shown to regulate food intake and energy homeostasis (Cota et al., 2006), but whether part of the mTOR effect is via its modulatory effect on autophagy remains unknown. We have recently reported a novel lipophagic function of autophagy, by which the activation of this pathway during starvation induces mobilization of lipid droplets to generate free fatty acids in liver (Singh et al., 2009a). Fasting increases circulating free fatty acids that are rapidly taken up by organs such as liver and esterified to triglycerides within lipid droplets. Lipolytic mechanisms, for instance lipophagy (Singh et al., 2009a), then break down these cellular lipid stores to provide endogenous free fatty acids for energy under nutrient-deficient conditions. In addition, lipophagy

Structural and functional characterization of a single-chain peptide–MHC molecule that modulates both naive and activated CD8$^+$ T cells

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Contributed by Stanley G. Nathenson, July 7, 2011 (sent for review May 31, 2011)

Peptide–MHC (pMHC) multimers, in addition to being tools for tracking and quantifying antigen-specific T cells, can mediate downstream signaling after T-cell receptor engagement. In the absence of costimulation, this can lead to anergy or apoptosis of cognate T cells, a property that could be exploited in the setting of autoimmune disease. Most studies with class I pMHC multimers used noncovalently linked peptides, which can allow unwanted CD8$^+$ T-cell activation as a result of peptide transfer to cellular MHC molecules. To circumvent this problem, and given the role of self-reactive CD8$^+$ T cells in the development of type 1 diabetes, we designed a single-chain pMHC complex (scK^d.IGRP) by using the class I MHC molecule H-2K^d and a covalently linked peptide derived from islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP$_{206–214}$), a well established autoantigen in NOD mice. X-ray diffraction studies revealed that the peptide is presented in the groove of the MHC molecule in canonical fashion, and it was also demonstrated that scK^d.IGRP tetramers bound specifically to cognate CD8$^+$ T cells. Tetramer binding induced death of naive T cells and in vitro- and in vivo-differentiated cytotoxic T lymphocytes, and tetramer-treated cytotoxic T lymphocytes showed a diminished IFN-γ response to antigen stimulation. Tetramer accessibility to disease-relevant T cells in vivo was also demonstrated. Our study suggests the potential of single-chain pMHC tetramers as possible therapeutic agents in autoimmune disease. Their ability to affect the fate of naive and activated CD8$^+$ T cells makes them a potential intervention strategy in early and late stages of disease.

CD8$^+$ cytotoxic T lymphocytes (CTLs) use their T-cell receptors (TCRs) to recognize peptides presented by class I MHC molecules, and this recognition can lead to the demise of the cell displaying the cognate peptide–MHC (pMHC) complex. As a result, CD8$^+$ T cells are important pathogenic effectors in a number of autoimmune diseases, including type 1 diabetes (1). The development of strategies to interfere with their function offers new therapeutic opportunities. Treatment of CTLs with multimers of pMHC complexes has shown promise in inhibiting CTL-mediated cytotoxicity (2–5). For example, pMHC multimers constructed with short flexible linkers cause rapid death of peptide-specific CTLs (3), whereas those with long rigid linkers inhibit CTL-mediated cytotoxicity by interfering with integrin-mediated CTL adhesion (2). In addition, dimeric Ig fusions of pMHC complexes have been shown to inhibit lysis of target cells by alloreactive CTLs (4, 5).

We reasoned that, in addition to their inhibition of already differentiated CTLs (2–5), pMHC multimers should also be effective against naive T cells, as they would present antigen in the absence of a second costimulatory signal and would be predicted to drive the T cells to apoptosis or anergy (6–9). This is a profoundly unexplored area, perhaps because of the early unexpected finding that pMHC tetramers could instead activate naive CD8$^+$ T cells (10). This behavior was subsequently found to result from the release of the peptide from the tetramers and its transfer to MHC molecules on T cells, which then acted as antigen-presenting cells capable of activating their naive counterparts (11, 12). Thus, the activity of pMHC multimers against CD8$^+$ T cells, both naive and antigen-experienced, requires reevaluation with the use of pMHC complexes in which the peptide is rendered nonexchangeable by virtue of covalent linkage to the complex (13, 14).

To this end, we used a disease-relevant model system consisting of autoreactive CD8$^+$ 8.3 T cells. The 8.3 T-cell clone was originally isolated from the pancreatic islets of a nonobese diabetic (NOD) mouse (15), a model system for type 1 diabetes in which CD8$^+$ T cells have an important pathogenic role (16). The 8.3 T-cell clone is specific for the peptide composed of residues 206 to 214 of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP$_{206–214}$) presented by H-2K^d (17), and its pathogenicity has been demonstrated by adoptive transfer studies (15) and the accelerated disease that occurs in NOD mice that transgenically express the 8.3 TCR (18). T cells specific for IGRP$_{206–214}$ represent a prevalent population in the islets of NOD mice (17, 19, 20), and the monitoring of their numbers in the blood can be used to predict disease (20). IGRP epitopes have also been found to be targeted by CD8$^+$ T cells in human type 1 diabetes (21–23).

We used 8.3 T cells to investigate whether a single multimeric pMHC reagent could be developed that would inactivate or eradicate both CTLs and naive CD8$^+$ T cells. We designed a single-chain pMHC complex in which IGRP$_{206–214}$ is covalently attached to β$_2$-microglobulin (β2m), which itself is covalently linked to the heavy chain of H-2K^d. X-ray diffraction analysis of the single-chain H-2K^d/IGRP$_{206–214}$ (scK^d.IGRP) demonstrated that the covalently linked peptide is presented in the canonical binding groove of the MHC molecule in a fashion that would support productive TCR engagement. Tetramers of scK^d.IGRP exhibit high-specificity binding for the cognate 8.3 TCR. Most importantly, scK^d.IGRP tetramers specifically induce apoptosis of naive CD8$^+$ 8.3 T cells, as well as of in vitro-generated CTLs and islet-infiltrating CTLs naturally differentiated in vivo. The tetramers also gain access to splenic and pancreatic T cells when administered in vivo. These characteristics support further exploration of the therapeutic potential of single-chain pMHC tetramers for type 1 diabetes and other conditions in which CD8$^+$ T cells contribute to the pathogenic process.

Author contributions: D.S., G.M., S.G.N., T.P.D., and S.C.A. designed research; D.S., G.M., U.A.R., and R.J.C. performed research; D.S. contributed new reagents/analytic tools; D.S., G.M., U.A.R., and T.P.D. analyzed data; and D.S., G.M., S.G.N., T.P.D., and S.C.A. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 3NWM).

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Neuron
Article

Neuropeptide Exocytosis Involving Synaptotagmin-4 and Oxytocin in Hypothalamic Programming of Body Weight and Energy Balance

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SUMMARY

Hypothalamic neuropeptides play essential roles in regulating energy and body weight balance. Energy imbalance and obesity have been linked to hypothalamic signaling defects in regulating neuropeptide genes; however, it is unknown whether dysregulation of neuropeptide exocytosis could be critically involved. This study discovered that synaptotagmin-4, an atypical modulator of synaptic exocytosis, is expressed most abundantly in oxytocin neurons of the hypothalamus. Synaptotagmin-4 negatively regulates oxytocin exocytosis, and dietary obesity is associated with increased vesicle binding of synaptotagmin-4 and thus enhanced negative regulation of oxytocin release. Overexpressing synaptotagmin-4 in hypothalamic oxytocin neurons and centrally antagonizing oxytocin in mice are similarly obesogenic. Synaptotagmin-4 inhibition prevents against dietary obesity by normalizing oxytocin release and energy balance under chronic nutritional excess. In conclusion, the negative regulation of synaptotagmin-4 on oxytocin release represents a hypothalamic basis of neuropeptide exocytosis in controlling obesity and related diseases.

INTRODUCTION

The hypothalamus in the central nervous system (CNS) is known as the central regulator of feeding, energy, and body weight homeostasis (Coll et al., 2007; Flier and Maratos-Flier, 1998; Mobbs, 2007; Park and Bloom, 2005; Schwartz et al., 2000; Ukropec et al., 2006). All these hypothalamic functions are critically mediated by various hypothalamic neuropeptides. Several well-appreciated examples of such neuropeptides include α-melanocyte stimulating hormone (α-MSH), cocaine and amphetamine regulated transcript (CART), neuropeptide Y (NPY), and agouti-related peptide (AGRP). These neuropeptides have been shown to be controlled at the gene transcriptional levels (Bates et al., 2003; Kim et al., 2006; Kitamura et al., 2006; Xu et al., 2005) by nuclear transcription factors that sense nutrient and metabolic cues of the body (Ahima et al., 1996; Air et al., 2002; Friedman and Halaas, 1998). Interestingly, recent research has begun to recognize the importance of neuropeptide posttranscriptional modulation (Plum et al., 2009), indicating that the control of neuropeptide gene expression represents only an initial step in the whole cascade of neuropeptide regulation. Logically, this process ultimately involves regulation of neuropeptide release to precisely control the biological functions of neuropeptides. However, how hypothalamic neuropeptide exocytosis is regulated and whether it is critical for metabolic physiology and disease have not been explored.

Recent research in basic science has obtained significant knowledge regarding the general principles of neuropeptide/neurotransmitter vesicular exocytosis (Stojilkovic, 2005). Studies based on synaptic neurotransmitter release have identified vesicular exocytosis as a process that is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex (Jahn and Scheller, 2006; Südhof and Rothman, 2009) under the regulation of synaptotagmins (Syts) (Chapman, 2008; Südhof, 2002). Syts are a group of Ca^{2+}-binding proteins that catalyze the formation of SNARE complex to provide the force and energy required for exocytosis. The mammalian Syt family is composed of 17 members. While most of them are predominantly present in the CNS, some of them are involved in the vesicular functions of endocrine cells such as pancreatic α and β cells (Fukuda and Mikoshiba, 1999; Gao et al., 2000; Gauthier et al., 2008; Iezzi et al., 2005) and glucose-transport metabolic cells (Hudson and Birnbaum, 1995; Li et al., 2007). These interesting studies, which were mainly based on peripheral endocrine systems, have raised the recent alarm on the potential implication of Syts in diabetes (Gauthier and Wollheim, 2008). However, research to date addressing Syts in hypothalamic neuroendocrine neurons is still missing.

Syt4 is an inducible Syt isoform detectable only in the brain and in the neuroendocrine system (Vician et al., 1995), hinting at a possible role in neuroendocrine physiology. Notably, compared to other Syt family members, the puzzling aspect of Syt4 is its lack of a critical Ca^{2+}-binding amino acid (von Poser et al., 1997) and related inability to induce Ca^{2+}-dependent exocytosis in biophysical models (Chapman et al., 1998; Thomas et al., 1999). Recent

Results of a Successful Telephonic Intervention to Improve Diabetes Control in Urban Adults

A randomized trial

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OBJECTIVE — To compare the effectiveness of a telephonic and a print intervention over 1 year to improve diabetes control in low-income urban adults.

RESEARCH DESIGN AND METHODS — A randomized trial in Spanish and English comparing a telephonic intervention implemented by health educators with a print intervention. Participants ($N = 526$) had an A1C ≥7.5% and were prescribed one or more oral agents. All were members of a union/employer jointly sponsored health benefit plan. Health coverage included medications. Primary outcomes were A1C and pharmacy claims data; secondary outcomes included self-report of two medication adherence measures and other self-care behaviors.

RESULTS — Participants were 62% black and 23% Hispanic; 77% were foreign born, and 42% had annual family incomes <$30 thousand. Baseline median A1C was 8.6% (interquartile range 8.0–10.0). Insulin was also prescribed for 24% of participants. The telephone group had mean ± SE decline in A1C of 0.23 ± 0.11% over 1 year compared with a rise of 0.13 ± 0.13% for the print group ($P = 0.04$). After adjusting for baseline A1C, sex, age, and insulin use, the difference in A1C was 0.40% (95% CI 0.10–0.70, $P = 0.009$). Change in medication adherence measured by claims data, but not by self-report measures, was significantly associated with change in A1C ($P = 0.01$). Improvement in medication adherence was associated ($P = 0.005$) with the telephonic intervention, but only among those not taking insulin. No diabetes self-care activities were significantly correlated with the change in A1C.

CONCLUSIONS — A 1-year tailored telephonic intervention implemented by health educators was successful in significantly, albeit modestly, improving diabetes control compared with a print intervention in a low-income, insured, minority population.

Diabetes Care 34:2–7, 2011

Improving glycemic control in type 2 diabetes significantly decreases the risk of serious chronic complications such as retinopathy, neuropathy, and nephropathy, as shown by large-scale clinical trials from the last 2 decades (1,2). These studies, along with smaller trials, set the stage for evidence-based medical management of diabetes (3). Although effective therapies for management have been developed, treatment goals are often not reached—especially in lower income and minority populations (4)—and many individuals find it challenging to perform routine self-management (5). Critical reviews of the scientific literature on interventions to improve glycemic control show promising results for improved processes of care, such as screening for complications and laboratory tests, as well as for behavioral interventions and self-management training (6,7).

Evidence is emerging for the use of telephonic interventions to improve diabetes self-care and health outcomes; studies include use of automated calls with nurse follow-up (8) or calls implemented by individuals with graduate degrees (9). Telephonic interventions may enhance self-care adherence (10) by offering the opportunity to customize information to individuals under real-world conditions. Nonetheless, the efficacy of telephonic interventions in all populations and settings has not been established, and improvements in health outcomes for patients remain challenging even with many new pharmaceutical agents becoming available and combinations of type 2 diabetes medications becoming a standard of care.

As an adjunct to diabetes self-management education and medical care, a telephonic intervention by health educators may provide the coaching and motivation needed for individuals to perform diabetes self-management activities over time, especially medication adherence. The Improving Diabetes Outcomes (I DO) study aimed to evaluate the incremental effect of a tailored telephone intervention, in English and Spanish, on the mean A1C levels and medication adherence beyond that achieved with the mailing of print self-management materials. The population is insured, lower-income, mostly minority individuals who had health care and medication benefits covered in full by their labor union/employer plan. However, the study protocol allowed only telephonic and print contact with participants so that individuals who might not have agreed to participate in more conventional in-person studies could take part. The main study outcomes were changes in A1C and medication adherence. The study also sought to determine what demographic and behavioral factors might mediate the effect of the interventions. We now report the main re-

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See accompanying editorial, p. 240.

Hepatocyte Growth Factor and Clinical Diabetes in Postmenopausal Women

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OBJECTIVE — To investigate the association between circulating levels of hepatocyte growth factor (HGF), a mesenchymal-derived pleiotrophic factor that is elevated in obesity, and the prevalence of type 2 diabetes.

RESEARCH DESIGN AND METHODS — A cross-sectional analysis among 892 postmenopausal women within the Women's Health Initiative Observational Study (WHI-OS).

RESULTS — HGF levels positively correlated with BMI and homeostasis model assessment for insulin resistance. In the multivariable analysis comparing the highest tertile with the lowest tertile of HGF, the odds ratio for prevalent diabetes was 2.47 (95% CI [1.12–5.47], *P* for trend = 0.014) after accounting for age, race, BMI, and other risk factors for diabetes.

CONCLUSIONS — HGF levels are associated with the presence of type 2 diabetes in postmenopausal women. Future studies should consider the prospective evaluation of the association of HGF with the development of type 2 diabetes.

Diabetes Care 33:2013–2015, 2010

Hepatocyte growth factor (HGF) is a mesenchymal-derived pleiotropic factor that regulates growth, motility, and morphogenesis of various cells (1). HGF is highly expressed in white adipose tissue (2,3), and it stimulates glucose uptake in cultured adipocytes (4). In humans, circulating HGF positively correlates with insulin and glucose (5) and is reported to be elevated in obesity (6), metabolic syndrome (5), hypertension (7), and coronary heart disease (8). Although HGF levels are observed to be elevated in these diabetes-associated conditions, the specific HGF-diabetes association has not yet been investigated. Hence, we conducted a study to examine the cross-sectional relationship between HGF and diabetes in a representative sample of postmenopausal women (n = 892) within the Women's Health Initiative Observational Study (WHI-OS).

RESEARCH DESIGN AND METHODS

— The WHI-OS is an ongoing prospective study of 93,676 postmenopausal women aged 50–79 years (9). At baseline, the women were queried about lifestyle factors, medical history, and personal habits, and a physical examination was performed to obtain height, weight, and blood pressure. Fasting blood samples were collected, centrifuged, frozen on site at −70°C, and stored in the specimen repository.

We conducted a study using data from a case-cohort study within the WHI-OS that aimed to evaluate the association of several adipokines and risk of cancers of the breast, colorectum, and endometrium (10). The study population for the current analysis included 892 women selected from the subcohort, a representative sample of WHI-OS women without cancer at baseline. Diabetes was defined as a history of treated diabetes or fasting glucose ≥126 mg/dl. Plasma HGF levels were measured by a multiplex assay (Human Adipokine Panel B; Millipore, Billerica, MA) based on Luminex xMAP technology (http://www.luminexcorp.com). The interassay coefficient of variation for HGF assay was 11.7%. We performed unconditional logistic regression analysis to evaluate the association between HGF tertiles and prevalent diabetes while accounting for potential confounders. Tests of linear trend across HGF tertiles were conducted by assigning a score for each tertile and including this variable as a continuous variable in the model. All statistical analyses were performed using SAS version 9.1 (Cary, NC), and *P* values <0.05 were considered statistically significant.

RESULTS

— In this population, HGF levels showed modest correlation with age (r = 0.20; $P < 0.0001$), BMI (r = 0.18; $P < 0.0001$), waist circumference (r = 0.19; $P < 0.0001$), and insulin resistance as measured by homeostasis model assessment for insulin resistance (r = 0.21; $P < 0.0001$). In addition, current postmenopausal hormone use and alcohol intake were associated with lower HGF levels (data not shown). In the age- and race-adjusted logistic regression model, the odds ratio (OR) for prevalent diabetes comparing women in the highest tertile of HGF with those in the lowest tertile was 3.63 (95% CI [1.83–7.19], *P*-trend <0.0001), which was attenuated to 2.78 (1.36–5.69), *P*-trend = 0.003 after additional adjustment for BMI (Table 1). This association remained significant when we further accounted for smoking, physical activity, family history of diabetes, alcohol intake, postmenopausal hormone use, and plasma levels of C-reactive protein (2.47 [1.12–5.47], *P*-trend = 0.014). To control for potential residual confounding by adiposity, we evaluated the effect of additional inclusion of waist circumference in the model. The results, however, were similar (2.34 [1.04–5.28], *P*-trend = 0.024). Additional adjustment for circulating insulin levels in the final multivariable model attenuated the results to borderline significance (1.95 [0.87–4.40], *P*-trend = 0.078). There

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PREVENTING CHRONIC DISEASE
PUBLIC HEALTH RESEARCH, PRACTICE, AND POLICY
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BRIEF

High Prevalence of Obesity Among Inner-City Adolescent Boys in the Bronx, New York: Forgetting Our Boys

Carmen R. Isasi, MD, PhD; Amy Whiffen; Eleanor Campbell; Yolanda Florez; Katherine Freeman; Judith Wylie-Rosett

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PEER REVIEWED

Abstract

We examined sex differences in overweight and obesity in a sample of 1,619 inner-city adolescents. Participants were enrolled from 11 public schools in the Bronx, New York. The prevalence of overweight and obesity was 21.7% and 22.5%, respectively; prevalence of obesity was significantly higher among adolescent boys than adolescent girls (24.9 vs 20.1%). Childhood obesity is a public health concern in the United States, and the higher prevalence of obesity in adolescent boys requires additional attention.

Objective

Childhood obesity is a growing concern in the United States. Data from the third National Health and Nutrition Examination Survey show that excess weight is associated with metabolic abnormalities such as dyslipidemia and insulin resistance (1). National surveys have reported a prevalence of overweight and of obesity of 34% and 18%, respectively, among youth aged 12 to 19 years (2). Low-income and minority youth, particularly Hispanics and African Americans, are the most affected (2-5).

Findings from recent studies challenge the common assumption that girls are at higher risk of overweight and obesity than boys (5,6). Among Mexican American adolescents, boys also have a higher prevalence of obesity than girls (6). A recent analysis of data from the National Longitudinal Study of Adolescent Health (Add Health), a national representative sample of adolescents in grades 7 through 12, found that during early adolescence, boys have a higher body mass index (BMI) than girls, although girls have a faster increase in BMI over the years (7). This disparity appears to persist as teenagers get older, but by age 20 the prevalence of obesity in adolescent girls gets closer to or higher than that of boys (6,7). Despite this reversal, a high prevalence of obesity among adolescent boys is still of concern. The Add Health study showed that 88% of adolescent boys remained obese as young adults (8).

The purpose of our study was to examine sex differences in overweight and obesity among inner-city adolescents in the Bronx, New York. Factors associated with excess weight may vary by sex, and treatment approaches may need to take into account these differences.

Methods

Study sample

This cross-sectional study took place during February through June and October through December of 2008. Inclusion criteria for this study included ability to speak and comprehend English and not being enrolled in special education classes. The Bronx has 350 public schools that enroll students in grades 7 through 10 (9). The 11

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Article

Snapin Mediates Incretin Action and Augments Glucose-Dependent Insulin Secretion

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SUMMARY

Impaired insulin secretion contributes to the pathogenesis of type 2 diabetes mellitus (T2DM). Treatment with the incretin hormone glucagon-like peptide-1 (GLP-1) potentiates insulin secretion and improves metabolic control in humans with T2DM. GLP-1 receptor-mediated signaling leading to insulin secretion occurs via cyclic AMP stimulated protein kinase A (PKA)- as well as guanine nucleotide exchange factor-mediated pathways. However, how these two pathways integrate and coordinate insulin secretion remains poorly understood. Here we show that these incretin-stimulated pathways converge at the level of snapin, and that PKA-dependent phosphorylation of snapin increases interaction among insulin secretory vesicle-associated proteins, thereby potentiating glucose-stimulated insulin secretion (GSIS). In diabetic islets with impaired GSIS, snapin phosphorylation is reduced, and expression of a snapin mutant, which mimics site-specific phosphorylation, restores GSIS. Thus, snapin is a critical node in GSIS regulation and provides a potential therapeutic target to improve β cell function in T2DM.

INTRODUCTION

Insufficient glucose-stimulated insulin secretion (GSIS) significantly contributes toward hyperglycemia in type 2 diabetes mellitus (T2DM) (Prentki and Nolan, 2006). Insulin is stored in secretory vesicles in pancreatic β cells and is secreted by exocytosis to precisely control blood glucose homeostasis (Gauthier and Wollheim, 2008; Lang, 1999). Upon stimulus by glucose, β cells secrete insulin in a biphasic manner, which is considered to be important for optimal glycemic control (Del Prato and Tiengo, 2001; Pimenta et al., 1995). An early, first-phase insulin release occurs during the first few minutes of glucose stimulus, whereas later time points comprise the second phase of GSIS. Humans at risk of developing T2DM or with established T2DM exhibit defective first-phase insulin release well before detectable changes in the second phase (Gerich, 2002; Lillioja et al., 1988; Vaag et al., 1995; Ward et al., 1984), and restoration of first-phase insulin secretion corrects glycemic control (Basu et al., 1996).

The incretin hormone glucagon-like peptide-1 (GLP-1) and its peptide analog exendin-4 (E4) improve metabolic control in T2DM predominantly by restoring first-phase and augmenting second-phase insulin secretion in humans with T2DM (Egan et al., 2002; Fehse et al., 2005). In addition to their secretagogue effects, GLP-1 and E4 stimulate proliferation and inhibit apoptosis in rodent β cells (Drucker, 2006). Most if not all effects of GLP-1 and E4 in β cells appear to require intracellular activation of the adenosine-3′-5′-cyclic monophosphate (cAMP) protein kinase A (PKA) signaling pathway by the G protein-coupled receptor of GLP-1, which is highly expressed on pancreatic β cells (Drucker and Nauck, 2006). A second mechanism of PKA-independent incretin potentiation of GSIS involves the cAMP-regulated guanine nucleotide exchange factor (cAMP-GEF) EPAC2 (Seino and Shibasaki, 2005). However, PKA activity appears to be essential for optimal incretin effects on stimulating insulin vesicle exocytosis (Chepurny et al., 2010; Doyle and Egan, 2007).

In β cells, insulin exocytosis is regulated in part by specific kinases, which by altering protein phosphorylation modify assembly of proteins associated to secretory vesicles (Foster et al., 1998; Kwan et al., 2006; Shimazaki et al., 1996). Appropriate assembly of vesicle-associated proteins prepares the secretory vesicle for exocytosis. In β cells, glucose metabolism-induced Ca^{2+} elevation is required for the final step of vesicle fusion to the cell membrane (Gauthier and Wollheim, 2008; Takahashi et al., 2010). While PKA signaling serves a central role in incretin GSIS potentiation (Kwan et al., 2006; Seino and Shibasaki, 2005), how PKA-dependent and -independent effects of cAMP signaling are coordinated and integrated is unclear. The node at which these two pathways converge, a protein likely to be the target of PKA-dependent phosphorylation and to participate in insulin vesicle exocytosis regulation, remains to be identified.

To examine specifically in vivo effects of PKA signaling in pancreatic β cells and to identify a PKA target protein important in mediating coordinated incretin effects on GSIS, we have generated a mouse model of disinhibited PKA activity by conditional ablation of the inhibitory PKA regulatory subunit 1A (prkar 1a). This mouse exhibits augmented GSIS and improved glucose tolerance in the absence of fasting hyperinsulinemia

Cell Metabolism
Article

Rescue of Obesity-Induced Infertility in Female Mice due to a Pituitary-Specific Knockout of the Insulin Receptor

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SUMMARY

Obesity is associated with insulin resistance in metabolic tissues such as adipose, liver, and muscle, but it is unclear whether nonclassical target tissues, such as those of the reproductive axis, are also insulin resistant. To determine if the reproductive axis maintains insulin sensitivity in obesity in vivo, murine models of diet-induced obesity (DIO) with and without intact insulin signaling in pituitary gonadotrophs were created. Diet-induced obese wild-type female mice (WT DIO) were infertile and experienced a robust increase in luteinizing hormone (LH) after gonadotropin-releasing hormone (GnRH) or insulin stimulation. By contrast, both lean and obese mice with a pituitary-specific knockout of the insulin receptor (PitIRKO) exhibited reproductive competency, indicating that insulin signaling in the pituitary is required for the reproductive impairment seen in DIO and that the gonadotroph maintains insulin sensitivity in a setting of peripheral insulin resistance.

INTRODUCTION

Nutritional status is tightly coupled to reproductive function. Short-term and chronic withdrawal of nutrients is known to inhibit reproductive function in mammals (Cameron and Nosbisch, 1991), likely an evolutionary adaptation to the large amount of energy required for reproduction. In addition, conditions of excess nutrition, such as obesity, have also been linked to reproductive dysfunction. Infertility is associated with conditions such as type 2 diabetes, metabolic syndrome, and polycystic ovarian syndrome (PCOS). These conditions are marked by obesity and a complex metabolic phenotype that includes hyperinsulinemia and hyperleptinemia as well as insulin and leptin resistance. Of these metabolic disorders linked to infertility, PCOS is the best characterized and is the most common cause of infertility in women. In addition to obesity and hyperinsulinemia, PCOS is associated with anovulation and elevated luteinizing hormone (LH) levels, suggesting that the central reproductive axis is tonically activated, leading to ovarian dysfunction.

We and others have shown that insulin and insulin-like growth factors can augment the effects of gonadotropin-releasing hormone (GnRH) on LH expression and secretion (Adashi et al., 1981; Buggs et al., 2006; Soldani et al., 1995) in vitro using LH-secreting gonadotroph cell lines, suggesting that direct insulin action in the gonadotroph may contribute to the elevated LH observed in women with PCOS. In contrast to studies showing that insulin can augment GnRH (Buggs et al., 2006), GnRH has also been shown to inhibit an insulin response in vitro (Navratil et al., 2009). Some groups have noted that in PCOS women, insulin injection caused circulating gonadotropin levels to decrease (Lawson et al., 2008; Mehta et al., 2005; Patel et al., 2003). This suggests that the dysregulation of LH synthesis and/or release in PCOS may be due to insulin dysregulation at the level of the gonadotroph; however, whether the central reproductive tissues maintain insulin sensitivity in the presence of hyperinsulinemia and peripheral insulin resistance remains unclear. While women with PCOS and infertility display insulin resistance in insulin target tissues such as liver and muscle, it is unknown whether the central reproductive axis is also insulin resistant. Approaching this question has been hampered by the difficulty in generating a rodent model to mimic the complex phenotype of PCOS. Mixed results have stemmed from attempts to model the obese, infertile state, and some mouse strains have reacted differently to the effects of a high-fat diet (HFD) (Tortoriello et al., 2004). Consequently, a clear view of nutritional regulation of the reproductive axis has yet to be defined.

Given the discrepant in vitro observations of the gonadotroph response to insulin, we chose to focus on isolating the role of insulin in vivo in the reproductive axis as an important signaling factor of overnutrition. To determine the direct effects of insulin on the gonadotroph, our laboratory has developed a pituitary-specific insulin receptor (IR) knockout (KO), or PitIRKO, mouse. In this study, we use the PitIRKO model to directly assess the role of insulin signaling in the pituitary gonadotroph in the context of infertility associated with diet-induced obesity (DIO).

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PLoS GENETICS

Genome-Wide Association Analysis Identifies Variants Associated with Nonalcoholic Fatty Liver Disease That Have Distinct Effects on Metabolic Traits

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Abstract

Nonalcoholic fatty liver disease (NAFLD) clusters in families, but the only known common genetic variants influencing risk are near *PNPLA3*. We sought to identify additional genetic variants influencing NAFLD using genome-wide association (GWA) analysis of computed tomography (CT) measured hepatic steatosis, a non-invasive measure of NAFLD, in large population based samples. Using variance components methods, we show that CT hepatic steatosis is heritable (~26%–27%) in family-based Amish, Family Heart, and Framingham Heart Studies (n = 880 to 3,070). By carrying out a fixed-effects meta-analysis of genome-wide association (GWA) results between CT hepatic steatosis and ~2.4 million imputed or genotyped SNPs in 7,176 individuals from the Old Order Amish, Age, Gene/Environment Susceptibility-Reykjavik study (AGES), Family Heart, and Framingham Heart Studies, we identify variants associated at genome-wide significant levels ($p<5\times10^{-8}$) in or near *PNPLA3, NCAN*, and *PPP1R3B*. We genotype these and 42 other top CT hepatic steatosis-associated SNPs in 592 subjects with biopsy-proven NAFLD from the NASH Clinical Research Network (NASH CRN). In comparisons with 1,405 healthy controls from the Myocardial Genetics Consortium (MIGen), we observe significant associations with histologic NAFLD at variants in or near *NCAN, GCKR, LYPLAL1*, and *PNPLA3*, but not *PPP1R3B*. Variants at these five loci exhibit distinct patterns of association with serum lipids, as well as glycemic and anthropometric traits. We identify common genetic variants influencing CT–assessed steatosis and risk of NAFLD. Hepatic steatosis associated variants are not uniformly associated with NASH/fibrosis or result in abnormalities in serum lipids or glycemic and anthropometric traits, suggesting genetic heterogeneity in the pathways influencing these traits.

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Racial Differences in Glycemic Markers: A Cross-sectional Analysis of Community-Based Data

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Abstract

Background—Although black and white differences in hemoglobin A_{1c} (HbA_{1c}) values are well established, recent studies suggest that the difference might not reflect differences in glycemia.

Objective—To investigate racial disparities in glycemic markers, including those that reflect biological processes independent of hemoglobin glycation and erythrocyte turnover.

Design—Cross-sectional.

Setting—Community-based.

Participants—1376 nondiabetic and 343 diabetic adults in a substudy of the Atherosclerosis Risk in Communities Study.

Measurements—Hemoglobin A_{1c}, fasting glucose, glycated albumin, fructosamine, and 1,5-anhydroglucitol levels.

Results—In persons with and without diabetes, black persons had significantly higher values of HbA_{1c}, glycated albumin, and fructosamine levels compared with white persons before and after adjustment for covariates and fasting glucose concentration. Serum 1,5-anhydroglucitol, which is reduced in the setting of hyperglycemia-induced glycosuria, was lower in black persons compared with white persons, although this difference was statistically significant only in nondiabetic adults.

Disclosure: The authors have no relevant financial relationships to disclose.

Trends in the Prevalence of Type 2 Diabetes in Asians Versus Whites

Results from the United States National Health Interview Survey, 1997–2008

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OBJECTIVE—To examine trends in the prevalence of type 2 diabetes and related conditions in Asian Americans compared with non-Hispanic whites.

RESEARCH DESIGN AND METHODS—We analyzed data from the National Health Interview Survey (NHIS) from 1997 to 2008 to construct a nationally representative sample of 230,503 U.S. adults aged ≥18 years. Of these adults, 11,056 identified themselves as Asian Americans and 219,447 as non-Hispanic whites.

RESULTS—The age- and sex-adjusted prevalence of type 2 diabetes was higher in Asian Americans than in whites throughout the study period (4.3–8.2% vs. 3.8–6.0%), and there was a significant upward trend in both ethnic groups ($P < 0.01$). BMI also was increased in both groups, but age- and sex-adjusted BMI was consistently lower in Asian Americans. In fully adjusted logistic regression models, Asian Americans remained 30–50% more likely to have diabetes than their white counterparts. In addition, Asian Indians had the highest odds of prevalent type 2 diabetes, followed by Filipinos, other Asians, and Chinese.

CONCLUSIONS—Compared with their white counterparts, Asian Americans have a significantly higher risk for type 2 diabetes, despite having substantially lower BMI. Additional investigation of this disparity is warranted, with the aim of tailoring optimal diabetes prevention strategies to Asian Americans.

Diabetes Care 34:353–357, 2011

Asian Americans are a fast-growing subpopulation in the U.S., accounting for 1.4% of the total U.S. population in 1980 and tripling to 4.2% (11.9 million people) in 2000 (1,2). However, apart from focused studies of Japanese immigrants in Hawaii and the West Coast (3,4), few data are available regarding diabetes in Asian Americans nationwide (5,6). We therefore analyzed data from the NHIS to examine trends in the prevalence of type 2 diabetes and related conditions in Asian Americans compared with non-Hispanic whites.

RESEARCH DESIGN AND METHODS

Data source

The National Health Interview Survey (NHIS) is an ongoing annual survey designed to collect health-related information on the noninstitutionalized civilian population of the U.S. Conducted by the National Center for Health Statistics (NCHS), the survey uses a three-stage stratified cluster-probability sampling design. All data are based on respondent self-report. There are no laboratory assays or physical assessments. Details regarding study design and procedures are available elsewhere (7).

Study population

We used data collected from a sample of 359,156 adult participants, aged ≥18 years, who were interviewed between 1997 and 2008. The 12-year average response rate was 71.8% (range 62.6–80.4). We excluded individuals based on any one of the following criteria (individuals can be excluded for more than one reason): missing age at diabetes diagnosis (n = 1,013); diabetes diagnosed before the age of 25 years (n = 2,567); missing data on height or weight (n = 15,097); or outliers for BMI defined as the upper and lower 0.5% of BMI values (n = 3,351). Thus, the final sample comprised 11,056 Asian American (henceforth Asian) and 219,447 non-Hispanic white (henceforth white) adults.

Race and ethnicity

Race/ethnicity was categorized using a combination of variables: "race coded to single/multiple race group," based on self-reported primary race and "Hispanic origin" in 1997–2005 and the predefined "race/ethnicity recode" in 2006–2008. Asian subgroups consisted of Chinese, Filipinos, Asian Indians, and other Asians (i.e., Korean, Japanese, Vietnamese, and other Asian subgroups). In 1997 and 1998, the NHIS included Pacific Islanders (i.e., Hawaiian, Samoan, Guamanian, and other Pacific Islander subgroups) as a part of the "other Asians" category. However, we were unable to differentiate Pacific Islanders from other Asians based on the public-use dataset.

Prevalent diabetes

Individuals were classified as having diabetes if they gave a positive response to any of the following three questions: *1*) "Have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?"; *2*)"Are you now taking insulin?"; or *3*) "Are you now taking

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Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy

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REVIEW

Maintaining efficacy in the treatment of diabetic peripheral neuropathic pain: role of duloxetine

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Introduction: Neuropathy is one of the most frequent complications of diabetes. Of all the symptoms associated with diabetic neuropathy, pain has the largest impact on sleep and quality of life. In the past few years further medications have been added to the available therapies for neuropathic pain. One of these medications, duloxetine hydrochloride (duloxetine), is a balanced and potent selective serotonin and norepinephrine reuptake inhibitor.

Methods: Medline was searched from January 2005 to September 2009 using the key words duloxetine and peripheral neuropathy for clinical trials limited to human research published in English and duloxetine and pharmacology in the nervous system.

Results: Duloxetine has been shown to effectively reduce diabetic peripheral neuropathic pain compared to placebo at doses of 60 mg/day and 120 mg/day with minimal to moderate side effects. This effect is seen with minimal effects on glycemic control and without any clinically relevant effects on lipid control, or cardiovascular parameters. In addition, its efficacy and tolerability is comparable to other medications commonly used in the management of neuropathic pain. Furthermore, duloxetine performs favorably both in terms of quality of life and in cost utility analyses.

Discussion and conclusion: This article reviewed the issues related to management of diabetic peripheral neuropathic pain, the pharmacology and rationale for use of duloxetine, efficacy studies, and the safety and tolerability of treatment with duloxetine. Duloxetine is an acceptable initial or alternative treatment for patients with diabetic neuropathic pain.

Keywords: duloxetine, diabetic neuropathy, neuropathic pain

Frequency of diabetic neuropathy

The prevalence of diabetes mellitus in people age 20 years or greater in the United States has been estimated at 12.9%. Furthermore, the prevalence of impaired fasting glucose is 25.7% and of impaired glucose tolerance is 13.8%. This means that over 40% of individuals aged 20 years or older have either diabetes or pre-diabetes, and the prevalence is rising.[1] Peripheral neuropathy is one of the commonest complications of diabetes.[2] At least 1 in 4 patients with diabetes is affected by a distal symmetric peripheral neuropathy and neuropathic pain occurs in 7.5% to 24% of all patients with diabetes.[2,3] The yearly incidence of distal symmetric polyneuropathy in diabetics is approximately 2% and the lifetime incidence of neuropathy has been estimated to be 37% to 45% for patients with type 2 diabetes and 54% to 59% for patients with type 1 diabetes.[2,3] The growing prevalence of type 2 diabetes mellitus in the US and throughout the world will result in a larger number of individuals suffering from diabetic peripheral neuropathic pain.

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LETTER

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A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects

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Nuclear hormone receptors regulate diverse metabolic pathways and the orphan nuclear receptor LRH-1 (also known as NR5A2) regulates bile acid biosynthesis[1,2]. Structural studies have identified phospholipids as potential LRH-1 ligands[3–5], but their functional relevance is unclear. Here we show that an unusual phosphatidylcholine species with two saturated 12 carbon fatty acid acyl side chains (dilauroyl phosphatidylcholine (DLPC)) is an LRH-1 agonist ligand *in vitro*. DLPC treatment induces bile acid biosynthetic enzymes in mouse liver, increases bile acid levels, and lowers hepatic triglycerides and serum glucose. DLPC treatment also decreases hepatic steatosis and improves glucose homeostasis in two mouse models of insulin resistance. Both the antidiabetic and lipotropic effects are lost in liver-specific *Lrh-1* knockouts. These findings identify an LRH-1 dependent phosphatidylcholine signalling pathway that regulates bile acid metabolism and glucose homeostasis.

Increased fat accumulation in the liver—steatosis—is tightly correlated with insulin resistance and type 2 diabetes[6]. Modestly raised bile acid levels decrease steatosis[7]. Loss of the nuclear receptor LRH-1 decreases bile acid levels[1,2], indicating that an LRH-1 agonist could increase them and improve fatty liver. In screens of a number of different phosphatidylcholine (PC) and other phospholipid species for effects on human LRH-1 transactivation, dilauroyl PC (DLPC; C12:0/C12:0) and diundecanoyl PC (DUPC; C11:0/C11:0) showed strong stimulation (Fig. 1a). Comparable responses were not observed with closely related PCs differing in acyl chain length by only a single methylene group, or with any other C12:0/C12:0 phospholipid species (Supplementary Fig. 1a–c).

DLPC and DUPC, but not the bile acid chenodeoxycholic acid (CDCA) or the more conventional phospholipid dipalmitoyl PC (DPPC; C16:0/C16:0), also activated the synthetic LRH-1 reporter in several other cell lines, including CV-1 and HEK293T cells (data not shown), and specifically increased basal LRH-1 transactivation of the native mouse SHP promoter[8] by approximately twofold in HeLa cells (Supplementary Fig. 2a). DLPC and DUPC also induced a similar response with the OCT4 promoter, which was dependent on both LRH-1 cotransfection and an intact LRH-1 response element[9] (Supplementary Fig. 2a). DLPC and DUPC responsiveness was not altered in mutant LRH-1 derivatives previously shown to inactivate responses to LRH-1 phosphorylation[10] or sumoylation[11], but was strongly decreased by mutations shown to block phospholipid binding[4] (Supplementary Fig. 2d).

Mouse and human LRH-1 showed essentially equivalent responses to DLPC and DUPC, and both DLPC and DUPC also activate the close LRH-1 relative SF-1 (also known as NR5A1; Supplementary Fig. 2). The LRH-1 responses were dose dependent (Supplementary Fig. 2c). Neither DUPC nor DLPC showed significant activation of any of a number of additional nuclear receptors outside of the NR5A subgroup (Supplementary Fig. 2b). In particular, DLPC and DUPC failed to activate PPARα, which was recently reported to be specifically bound and activated by 1-palmitoyl-2-oleoyl (C16:0/C18:1) PC[12], and C16:0/C18:1 PC failed to affect LRH-1 transactivation (Supplementary Fig. 1a). DLPC rapidly induced expression of the LRH-1 target CYP8B1 in the C3A derivative of HepG2 cells (Supplementary Fig. 3a). This response as well as CDCA repression of CYP8B1 expression and transactivation of a synthetic LRH-1 reporter plasmid was specifically compromised in cells transfected with LRH-1 short interfering RNA (siRNA; Supplementary Fig. 3b, c).

We used the mammalian two-hybrid assay and a simple GST pull-down approach to initially test the predicted function of DLPC and DUPC as LRH-1 agonist ligands. In the mammalian two-hybrid analysis, interaction of a VP16–human LRH-1 ligand-binding-domain fusion with a second fusion of the Gal4 DNA-binding domain to the nuclear receptor interaction domain of the coactivator SRC-3 (also known as NCOA3) was unaffected by vehicle, CDCA or DPPC, but was stimulated by either DUPC or DLPC (Supplementary Fig. 4a). *In vitro*, SRC-3 protein did not bind to GST alone but showed a significant basal interaction with a GST–LRH-1-ligand-binding-domain fusion protein, as expected[4]. DLPC and DUPC further increased binding of the coactivator by approximately 3 fold, but vehicle, CDCA, or any of a number of other PC species, including DPPC, had little or no effect (Supplementary Fig. 4b). DLPC also unexpectedly but specifically decreased binding of an SRC-2 peptide to the LRH-1 ligand-binding domain with a half-maximum inhibitory concentration (IC_{50}) of approximately 500 nM, but DPPC had no effect (Supplementary Fig. 4c), and DLPC did not affect rosiglitazone binding to PPARγ (Supplementary Fig. 4d).

As a stringent test of specific binding, the purified bacterially expressed human LRH-1 ligand-binding domain was incubated with DLPC or DPPC at molar ratios of 1:1 or 1:5 (protein:PC), or with buffer alone, and the protein was then repurified to eliminate unbound lipids. Specifically bound lipids were extracted and compared to DLPC or DPPC by electrospray ionization mass spectrometry. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) species with 16–22 carbon acyl chain lengths occupy the ligand-binding pocket in the buffer-treated control, with the most abundant peak corresponding to 16:1/18:1 PG (Fig. 1b). DLPC completely replaced these *Escherichia coli* phospholipids, even at an added lipid to protein molar ratio of only 1:1, but DPPC showed no detectable displacement, even at a ratio of 1:5 (Fig. 1b). On the basis of these functional and *in vitro* biochemical results, as well as the extensive structural studies demonstrating phospholipid binding to NR5A receptors[3–5,13,14], we conclude that DLPC and DUPC act *in vitro* as LRH-1 agonists. The functional results indicate that they may also act directly as agonists *in vivo*, although it remains unclear how they might transit the cell membrane and cytosol and enter the nucleus.

PCs are normal dietary nutrients that are efficiently absorbed in the small intestine, and we used the simple route of oral gavage to deliver cholic acid (CA), DLPC, DUPC and DPPC to C5BL/6 mice. These treatments had no apparent toxic effects and did not alter normalized

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MicroRNA-29c Is a Signature MicroRNA under High Glucose Conditions That Targets Sprouty Homolog 1, and Its *in Vivo* Knockdown Prevents Progression of Diabetic Nephropathy*[S]

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Although several recent publications have suggested that microRNAs contribute to the pathogenesis of diabetic nephropathy, the role of miRNAs *in vivo* still remains poorly understood. Using an integrated *in vitro* and *in vivo* comparative miRNA expression array, we identified *miR-29c* as a signature miRNA in the diabetic environment. We validated our profiling array data by examining *miR-29c* expression in the kidney glomeruli obtained from *db*/*db* mice *in vivo* and in kidney microvascular endothelial cells and podocytes treated with high glucose *in vitro*. Functionally, we found that miR-29c induces cell apoptosis and increases extracellular matrix protein accumulation. Indeed, forced expression of miR-29c strongly induced podocyte apoptosis. Conversely, knockdown of miR-29c prevented high glucose-induced cell apoptosis. We also identified Sprouty homolog 1 (Spry1) as a direct target of miR-29c with a nearly perfect complementarity between *miR-29c* and the 3′-untranslated region (UTR) of mouse *Spry1*. Expression of miR-29c decreased the luciferase activity of *Spry1* when co-transfected with the mouse *Spry1* 3′-UTR reporter construct. Overexpression of miR-29c decreased the levels of Spry1 protein and promoted activation of Rho kinase. Importantly, knockdown of miR-29c by a specific antisense oligonucleotide significantly reduced albuminuria and kidney mesangial matrix accumulation in the *db*/*db* mice model *in vivo*. These findings identify miR-29c as a novel target in diabetic nephropathy and provide new insights into the role of miR-29c in a previously unrecognized signaling cascade involving Spry1 and Rho kinase activation.

MicroRNAs (miRNAs)[2] comprise a broad class of small noncoding RNAs that negatively regulate gene expression by base-pairing to partially complementary sites in the 3′-untranslated regions (UTR) of specific target mRNAs (1, 2). An emerging body of evidence suggests that miRNAs serve as important therapeutic targets in a wide range of complex human diseases, including cancer and cardiovascular diseases, by targeting multiple transcripts (3–6). Recent studies have also revealed the involvement of miRNAs in diabetic nephropathy (DN) (7–9). However, despite the growing evidence for the regulatory effects of miRNAs in DN, limited information is available on the consequences of modulating miRNAs expression *in vivo*.

We hypothesized that an unbiased global miRNA expression profiling might reveal novel miRNAs, which may play critical regulatory roles in the pathogenesis of DN. Accordingly, by using an integrated *in vitro* and *in vivo* comparative miRNA expression profiling, we identified up-regulated miR-29c as a signature miRNA in the diabetic environment.

Previously published work suggested that down-regulation of miR-29c resulted in cardiac fibrosis (10, 11). In contrast, herein we identified miR-29c as a signature miRNA in the diabetic milieu whose expression was increased in hyperglycemic conditions both *in vitro* and *in vivo*. Thus, our objective was to explore the role of increased miR-29c expression in DN.

We found that miR-29c targets Sprouty homolog 1 (Spry1) in hyperglycemic conditions. Spry1 is a cytoplasmic protein that plays a critical role in kidney development (12–14) and is primarily known to inhibit the Ras/MEK/ERK pathway (15, 16). Spry1 and its related proteins have also been implicated as negative regulators of RhoA and its downstream effector Rho kinase through the non-canonical Wnt signaling (17–19). Importantly, Rho kinase plays a key role in DN (20–23). Published studies from our laboratory and others have shown that pharmacological inhibition of Rho kinase in experimental models of diabetes results in a significant reduction in albuminuria and accumulation of glomerular matrix accumulation (22, 23). At the cellular level, Rho kinase has been implicated in cell proliferation, fibrosis, and apoptosis via multiple signaling pathways (24–26). However, the mechanisms that regulate Rho kinase activation in DN are not fully understood.

Here, we report the identification of miR-29c as a signature miRNA in DN. Together with functional studies, our results establish the role of miR-29c as a key miRNA governing kidney remodeling through a coordinated coupling of Spry1 with Rho kinase activation.

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S7 and Table S1.

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[2] The abbreviations used are: miRNA, microRNA; miR-29c, microRNA-29c; snRNA, small nuclear RNA; Spry1, Sprouty homolog 1; DN, diabetic nephropathy; ASO, antisense oligonucleotide; RT-qPCR, real-time quantitative polymerase chain reaction; MYPT1, myosin phosphatase target subunit 1; NG, normal glucose; HG, high glucose.

Resource

Cell

Analysis of the Human Endogenous Coregulator Complexome

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SUMMARY

Elucidation of endogenous cellular protein-protein interactions and their networks is most desirable for biological studies. Here we report our study of endogenous human coregulator protein complex networks obtained from integrative mass spectrometry-based analysis of 3290 affinity purifications. By preserving weak protein interactions during complex isolation and utilizing high levels of reciprocity in the large dataset, we identified many unreported protein associations, such as a transcriptional network formed by ZMYND8, ZNF687, and ZNF592. Furthermore, our work revealed a tiered interplay within networks that share common proteins, providing a conceptual organization of a cellular proteome composed of minimal endogenous modules (MEMOs), complex isoforms (uniCOREs), and regulatory complex-complex interaction networks (CCIs). This resource will effectively fill a void in linking correlative genomic studies with an understanding of transcriptional regulatory protein functions within the proteome for formulation and testing of future hypotheses.

INTRODUCTION

Protein-protein interactions constitute the molecular backbone of cell biology, where select proteins assemble into metastable complexes to form bioactive units (Alberts, 1998; Köcher and Superti-Furga, 2007). These complexes then dynamically associate with each other in context of larger networks to carry out diverse biological functions. Thus, understanding the basic mechanisms of cell homeostasis requires knowledge of both the composition of protein complexes and the interactions between them.

A systems biology view of protein interactions has begun to emerge from large-scale studies in model organisms such as yeast, worms, and fruit flies (Gavin et al., 2006; Giot et al., 2003; Ito et al., 2001; Krogan et al., 2006; Li et al., 2004; Uetz et al., 2000). These analyses were made possible due to the development of high-throughput (HT) methods for measuring protein-protein interactions by affinity purification of tagged protein baits followed by mass spectrometry (AP/MS) and yeast two-hybrid assays. Limitations in genetic manipulations hinder such studies in human cells. We thus developed a protocol for HT isolation and identification of endogenous protein complexes from human cell lines using primary antibody immunoprecipitation and mass spectrometry (IP/MS). We also addressed key limitations associated with such studies, which are cross-reactivity of primary antibodies and nonspecific binding, and proposed an approach for the deconvolution of HT-IP/MS data into discrete protein complexes (Malovannaya et al., 2010).

Our current work was inspired by—and for a large part included—the Nuclear Receptor Signaling Atlas (NURSA; http://www.nursa.org) consortium proteomics effort, whose goal is to systematically isolate and identify the human nuclear receptor (NR) coregulator complexome. NR coregulators are a diverse group of molecules that associate with sequence-specific transcription factors to collectively modulate target gene expression (Lonard and O'Malley, 2007; Lonard et al., 2007; McKenna et al., 1999; O'Malley et al., 2008; Weake and Workman, 2010). Initial biochemical isolations of the mammalian coactivators Mediator and BAF/P-BAF and HDAC corepressors revealed that many coregulators assemble into multisubunit protein complexes (Gu et al., 1999; Guenther et al., 2000; Wang et al., 1996; Xue et al., 1998), implying that a comprehensive picture of the protein interaction networks is needed to better understand the regulation of biological processes in the cell.

This study presents the most extensive interaction dataset for endogenous regulatory human proteins obtained to date. By preserving both stable and weak protein interactions during complex isolations, we unveiled a modular and hierarchical organization of protein complex networks that serve as a blueprint for a better understanding of mechanisms of mammalian cell regulation. Our approach can be used in other biological systems as well; with broad applicability in mind we therefore discuss the analysis schema we used for the definition and annotation of protein complexes. Knowing the composition of protein complexes and

nature medicine

Metabolite profiles and the risk of developing diabetes

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Emerging technologies allow the high-throughput profiling of metabolic status from a blood specimen (metabolomics). We investigated whether metabolite profiles could predict the development of diabetes. Among 2,422 normoglycemic individuals followed for 12 years, 201 developed diabetes. Amino acids, amines and other polar metabolites were profiled in baseline specimens by liquid chromatography–tandem mass spectrometry (LC-MS). Cases and controls were matched for age, body mass index and fasting glucose. Five branched-chain and aromatic amino acids had highly significant associations with future diabetes: isoleucine, leucine, valine, tyrosine and phenylalanine. A combination of three amino acids predicted future diabetes (with a more than fivefold higher risk for individuals in top quartile). The results were replicated in an independent, prospective cohort. These findings underscore the potential key role of amino acid metabolism early in the pathogenesis of diabetes and suggest that amino acid profiles could aid in diabetes risk assessment.

Metabolic diseases are often present for years before becoming clinically apparent. For instance, by the time relative insulin deficiency manifests as hyperglycemia and a diagnosis of type 2 diabetes is made, considerable pancreatic beta cell insufficiency has already occurred[1]. Current clinical and laboratory predictors such as body mass index or fasting glucose can be helpful in gauging diabetes risk[2], but they often reflect extant disease, are most useful when assayed in temporal proximity to the development of overt diabetes and may provide little additional insight regarding pathophysiologic mechanisms. Given the availability of effective interventions for delaying or preventing the onset of type 2 diabetes and the increasing burden of the condition worldwide, earlier identification of individuals at risk is particularly crucial[3–6].

Emerging technologies have made it more feasible to acquire high-throughput profiles of a whole organism's metabolic status (metabolite profiling, or metabolomics)[7–10]. These techniques, which allow assessment of large numbers of metabolites that are substrates and products in metabolic pathways, are particularly relevant for studying metabolic diseases such as diabetes. Furthermore, in addition to serving as potential biomarkers of disease[11], metabolites may have unanticipated roles as regulatory signals with hormone-like functions[12,13] or effectors of the disease process itself[14].

Recent cross-sectional studies have documented differences in blood metabolite profiles before and after glucose loading[15–17] and in obese compared with lean individuals[14]. These studies have noted differences in the abundance of C3 and C5 acylcarnitines, glutamine and glutamate, additional amino acids and other small molecules. These observations raise the possibility that alterations in plasma metabolite concentrations could presage the onset of overt diabetes and therefore aid in the identification of at-risk individuals by adding information over standard clinical markers. We performed metabolite profiling in participants from two large, longitudinal studies, with the goal of identifying early pathophysiological changes that might also serve as new predictors of future diabetes.

RESULTS

Metabolite profiling in the Framingham Offspring Study

We performed a nested case-control study in the Framingham Offspring Study. Among 2,422 eligible, nondiabetic subjects who underwent a routine examination between 1991 and 1995, 201 individuals developed new-onset diabetes during a 12-year follow-up period (cases). We performed metabolite profiling on the baseline samples from 189 of these individuals, for whom we found 189 propensity-matched control subjects from the same baseline examination who

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LETTER

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Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity

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The endoplasmic reticulum (ER) is the main site of protein and lipid synthesis, membrane biogenesis, xenobiotic detoxification and cellular calcium storage, and perturbation of ER homeostasis leads to stress and the activation of the unfolded protein response[1]. Chronic activation of ER stress has been shown to have an important role in the development of insulin resistance and diabetes in obesity[2]. However, the mechanisms that lead to chronic ER stress in a metabolic context in general, and in obesity in particular, are not understood. Here we comparatively examined the proteomic and lipidomic landscape of hepatic ER purified from lean and obese mice to explore the mechanisms of chronic ER stress in obesity. We found suppression of protein but stimulation of lipid synthesis in the obese ER without significant alterations in chaperone content. Alterations in ER fatty acid and lipid composition result in the inhibition of sarco/endoplasmic reticulum calcium ATPase (SERCA) activity and ER stress. Correcting the obesity-induced alteration of ER phospholipid composition or hepatic *Serca* overexpression *in vivo* both reduced chronic ER stress and improved glucose homeostasis. Hence, we established that abnormal lipid and calcium metabolism are important contributors to hepatic ER stress in obesity.

It has been generally accepted that a surplus of nutrients and energy stimulates synthetic pathways and may lead to client overloading in the ER. However, it has not been demonstrated whether increased *de novo* protein synthesis and client loading into the ER and/or a diminished productivity of the ER in protein degradation or folding leads to ER stress in obesity. Intriguingly, dephosphorylation of eukaryotic translation initiation factor 2α (eIF2α) in the liver of high-fat-diet-fed mice reduced the ER stress response[3], indicating that additional mechanisms other than translational upregulation may also contribute to ER dysfunction in obesity. To address these mechanistic questions, we first fractionated ER from lean and obese liver tissues (Supplementary Fig. 1a, b) and then extracted ER proteins for comparative proteomic analysis to examine the status of this organelle in obesity. We identified a total of 2,021 unique proteins (Supplementary Table 1). Among them, 120 proteins were differentially regulated in obese hepatic ER samples (Supplementary Fig. 1c and Supplementary Table 2a, b). We independently validated the differential regulation when possible by immunoblot analyses and verified the fidelity of the system (Supplementary Fig. 1d). Gene ontology analysis identified the enrichment of metabolic enzymes—especially ones involved in lipid metabolism—in the obese ER proteome, whereas protein synthesis and transport functions were overrepresented among downregulated ER proteins (Fig. 1a). Consistently, we found that ER-associated protein synthesis was downregulated in the obese liver as demonstrated by polysome profiling (data not shown), whereas the expression of genes involved in *de novo* lipogenesis (*Fas*, *Scd1*, *Ces1d*, *Dgat2* and *Dak*) and phospholipid synthesis (*Pcyt1a* and *Pemt*) were broadly upregulated (Fig. 1b, c). We also observed upregulation of protein degradation pathways but did not find a broad change in the quantity of ER chaperones (Supplementary Fig. 2 and Supplementary Table 2a). Taken together, these data revealed a fundamental shift in hepatic ER function in obesity from protein to lipid synthesis and metabolism.

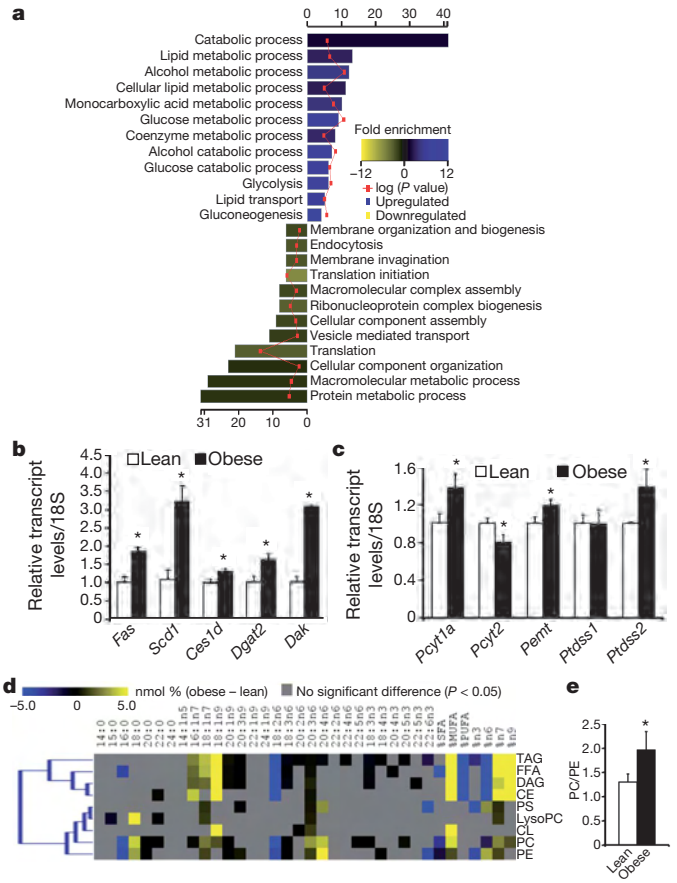

Figure 1 | Proteomic and lipidomic landscape of the lean and obese ER. **a**, Biological pathways associated with significantly regulated proteins in the obese ER proteome. Bar colours indicate the fold enrichment with significance values (negative log of *P* values) superimposed. **b**, **c**, Transcript levels of genes involved in lipid metabolism in the lean and obese mouse liver. **d**, Alterations of liver ER lipidome. Heatmap display of all significant ($P < 0.05$) alterations present between lean and obese ER lipidomes. The colour corresponds to differences in the relative abundance (nmol percentage) of each fatty acid among individual lipid groups detected in the lean and obese liver ER. **e**, The relative abundance of PC and PE in lean and obese liver ER samples. Values are mean ± s.e.m. ($n = 6$ for each group). $^*P < 0.05$, Student's *t*-test.

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mTOR phosphorylates IMP2 to promote IGF2 mRNA translation by internal ribosomal entry

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Variants in the *IMP2* (insulin-like growth factor 2 [IGF2] mRNA-binding protein 2) gene are implicated in susceptibility to type 2 diabetes. We describe the ability of mammalian target of rapamycin (mTOR) to regulate the cap-independent translation of IGF2 mRNA through phosphorylation of IMP2, an oncofetal RNA-binding protein. IMP2 is doubly phosphorylated in a rapamycin-inhibitable, amino acid-dependent manner in cells and by mTOR in vitro. Double phosphorylation promotes IMP2 binding to the IGF2 leader 3 mRNA 5′ untranslated region, and the translational initiation of this mRNA through eIF-4E- and 5′ cap-independent internal ribosomal entry. Unexpectedly, the interaction of IMP2 with mTOR complex 1 occurs through mTOR itself rather than through raptor. Whereas depletion of mTOR strongly inhibits IMP2 phosphorylation in cells, comparable depletion of raptor has no effect; moreover, the ability of mTOR to phosphorylate IMP2 in vitro is unaffected by the elimination of raptor. Dual phosphorylation of IMP2 at the mTOR sites is evident in the mouse embryo, likely coupling nutrient sufficiency to IGF2 expression and fetal growth. Doubly phosphorylated IMP2 is also widely expressed in adult tissues, including islets of Langerhans.

[*Keywords*: mTOR; IMP2; amino acid-dependent phosphorylation; IGF2; mRNA translation; internal ribosome entry; type 2 diabetes]

Supplemental material is available for this article.

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The insulin-like growth factor 2 (IGF2) is the major determinant of fetal growth in the mouse (Efstratiadis 1998), a function assumed after birth by the growth hormone/IGF1 system. Human and murine IGF2 are encoded by a set of mRNAs that differ only in their 5′ untranslated regions (UTRs) (Supplemental Fig. S1). In rapidly growing rhabdomyosarcoma (RD) cells, a human rhabdomyosarcoma cell line, the IGF2 mRNA designated leader 4 (L4) is constitutively translated, whereas the IGF2 mRNA designated leader 3 (L3) is translated in a rapamycin-inhibitable manner (Nielsen et al. 1995). The IGF2 mRNA-binding proteins (IMPs) were subsequently retrieved by their ability to bind differentially to the 1.2-kb L3 but not the 0.1-kb L4 5′ UTR (Nielsen et al. 1999). The IMPs comprise a family of three closely related 60- to 70-kDa RNA-binding proteins (IMP1–3), each containing two RRM domains followed by four KH domains (Nielsen et al. 2001; Yisraeli 2005). IMPs are oncofetal proteins expressed primarily during development but frequently re-expressed in and contributory to malignancy. IMPs have been independently identified repeatedly, usually as mediators of mRNA translational repression, localization, and/or stabilization. IMP1, for example, is orthologous to the chicken zipcode-binding protein (ZBP1), which binds to a 54-nucleotide (nt) "zipcode" sequence in the β-actin 3′ UTR, suppressing β-actin mRNA translation during its transport to the leading edge of the cell; there, Src tyrosine kinase phosphorylates ZBP1/IMP1 at a site between the second and third KH domains, promoting release of β-actin mRNA and disinhibition of its translation (Hüttelmaier et al. 2005). IMP3 is orthologous to *Xenopus* Vg1RBP/Vera, which mediates the polarized localization of Vg1 mRNA in oocytes; despite its similarity to IMP1, IMP3 does not bind the β-actin zipcode motif (Mori et al. 2001). IMP2 is most distant and lacks nonmammalian orthologs and little is

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nature medicine

Inhibition of Notch signaling ameliorates insulin resistance in a FoxO1-dependent manner

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Transcription factor FoxO1 promotes hepatic glucose production. Genetic inhibition of FoxO1 function prevents diabetes in experimental animal models, providing impetus to identify pharmacological approaches to modulate this function. Altered Notch signaling is evident in tumorigenesis, and Notch antagonists are in clinical testing for application in cancer. Here we report that FoxO1 and Notch coordinately regulate hepatic glucose metabolism. Combined haploinsufficiency of FoxO1 and Notch1 markedly raises insulin sensitivity in diet-induced insulin resistance, as does liver-specific knockout of the Notch transcriptional effector Rbp-Jκ. Conversely, Notch1 gain-of-function promotes insulin resistance in a FoxO1-dependent manner and induces glucose-6-phosphatase expression. Pharmacological blockade of Notch signaling with γ-secretase inhibitors raises insulin sensitivity after *in vivo* administration in lean mice and in obese, insulin-resistant mice. The data identify a heretofore unknown metabolic function of Notch and suggest that Notch inhibition is beneficial in diabetes treatment, in part by helping to offset excessive FoxO1-driven hepatic glucose production.

Type 2 diabetes is associated with obesity and insulin resistance[1]. The pathophysiology of the insulin-resistant state remains enigmatic, and currently available insulin sensitizers are only partially effective at improving glucose disposal in skeletal muscle and suppressing hepatic gluconeogenesis[2]. A more detailed knowledge of pathways that influence insulin resistance is necessary to identify new targets for the development of anti-diabetic drugs[3].

Forkhead box–containing transcription factors of the FoxO subfamily are key effectors of insulin action in metabolic processes, including hepatic glucose production (HGP)[4]. Hepatic FoxO1 promotes transcription of genes encoding glucose-6-phosphatase (*G6pc*) and phosphoenolpyruvate carboxykinase (*Pck1*), the rate-limiting enzymes in hepatic glycogenolysis and gluconeogenesis, respectively[5]. FoxO1 is phosphorylated by Akt, leading to its nuclear exclusion and degradation[6]. In insulin resistance, FoxO1 is constitutively active, leading to increased HGP and fasting hyperglycemia[7]. Despite the importance of FoxO1 in regulation of hepatic insulin sensitivity[8], it remains a poor candidate for a drug target owing to its lack of a ligand-binding domain and broad transcriptional signature.

Notch receptors mediate cell-fate decisions via interactions among neighboring cells; complexity arises from the presence of four transmembrane receptors (Notch1–Notch4), and five transmembrane ligands of the Jagged/Delta-like families[9]. Upon ligand-dependent activation, a series of cleavage events leads to release and nuclear entry of the Notch intracellular domain (NICD), binding and activation of transcription factor Rbp-Jκ and downstream expression of Notch target genes of the 'Hairy enhancer of split' (*Hes*) and *Hes*-related (*Hey*) families[10]. Mutations in the Notch pathway are etiologic in multiple developmental and neoplastic conditions[11], such as Alagille syndrome, a human disorder characterized by cholestasis and vascular anomalies[12,13]. In mice, nullizygosity of *Notch1*, *Jag1* and *Rbpj* is embryonic lethal, underscoring the developmental requirement for Notch signaling[9,14,15].

We have previously demonstrated that FoxO1 and Rbp-Jκ directly interact, leading to corepressor clearance from and coactivator recruitment to promoters of Notch target genes, which in turn allows differentiation of several cell types[16]. This provides a mechanism for the interaction between the PI 3-kinase–Akt–FoxO1 and Notch–Rbp-Jκ pathways to integrate growth with differentiation. We hypothesized that a similar interaction between these pathways exists in differentiated tissue and modulates FoxO1 metabolic functions. We used loss-of-function mutations in the two pathways, as well as adenovirus-mediated gain of function and pharmacological inhibition, to demonstrate that Notch can regulate HGP in a FoxO1-dependent manner.

RESULTS

Foxo1 and *Notch1* haploinsufficiency increase insulin sensitivity

To evaluate the physiologic relevance of Notch signaling in liver, we determined relative expression of the four Notch receptors. In wild-type (WT) mouse hepatocytes, predominantly *Notch1* and *Notch2* were expressed (data not shown). Notch1 activation, as reflected by cleavage at Val1744 and expression of canonical Notch targets, increased with fasting (**Fig. 1a**,**b**), in parallel with gluconeogenic

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HLA-E–restricted regulatory CD8+ T cells are involved in development and control of human autoimmune type 1 diabetes

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A key feature of the immune system is its ability to discriminate self from nonself. Breakdown in any of the mechanisms that maintain unresponsiveness to self (a state known as self-tolerance) contributes to the development of autoimmune conditions. Recent studies in mice show that CD8+ T cells specific for the unconventional MHC class I molecule Qa-1 bound to peptides derived from the signal sequence of Hsp60 (Hsp60sp) contribute to self/nonself discrimination. However, it is unclear whether they exist in humans and play a role in human autoimmune diseases. Here we have shown that CD8+ T cells specific for Hsp60sp bound to HLA-E (the human homolog of Qa-1) exist and play an important role in maintaining peripheral self-tolerance by discriminating self from nonself in humans. Furthermore, in the majority of type 1 diabetes (T1D) patients tested, there was a specific defect in CD8+ T cell recognition of HLA-E/Hsp60sp, which was associated with failure of self/nonself discrimination. However, the defect in the CD8+ T cells from most of the T1D patients tested could be corrected in vitro by exposure to autologous immature DCs loaded with the Hsp60sp peptide. These data suggest that HLA-E–restricted CD8+ T cells may play an important role in keeping self-reactive T cells in check. Thus, correction of this defect could be a potentially effective and safe approach in the therapy of T1D.

Introduction

The fundamental question of what is "self" and what is "foreign," as seen by the immune system, determines how the immune system discriminates self from nonself. In this regard, the pioneering work of Burnet and Medawar suggested that the definition of self versus nonself is arbitrary because foreign antigens presented during fetal life are thereafter considered self (1). Moreover, it is known that all T cells are self-referential in the sense that they are positively selected for survival on self-peptide(s) bound to MHC molecules during thymic positive selection (2) before thymic negative selection, in which thymocytes expressing TCR of high avidity to self-antigens are deleted (3–5).

We have previously proposed and tested an "avidity model" of peripheral T cell regulation that postulates that, like in the thymus, the immune system discriminates self from nonself during adaptive immunity in the periphery not by recognizing the structural differences between self versus foreign antigens, but rather by perceiving the avidity of T cell activation (6–9). It is generally accepted that thymic negative selection, in which, high-avidity self-reactive thymocytes are deleted, eliminates the imminent danger of pathogenic autoimmunity in the periphery and is the major mechanism of central self-tolerance (3–5). However, while releasing the "innocent" self-reactive T cells with low avidity, thymic negative selection also allows a large fraction of self-reactive T cells of intermediate avidity to be released into the periphery under normal circumstances (10–12), and functional activation of this population of cells has the potential to elicit pathogenic autoimmunity (12–15). The potential to develop autoimmune disease is therefore inherent in every individual and must be specifically dealt with by peripheral regulatory mechanisms (6–9). In this regard, we have demonstrated in murine studies that self/nonself discrimination is accomplished by thymic negative selection followed by peripheral T cell regulation in which Qa-1–restricted CD8+ T cells selectively downregulate intermediate-avidity T cells activated by any antigens (6–8). Since the peripheral self-reactive T cell repertoire is devoid of high-avidity self-reactive cells due to thymic negative selection, the selective downregulation of intermediate-avidity T cells simultaneously enables the suppression of autoimmunity and the preservation of the functional anti-infection immunity, which is dominated by high-avidity T cells.

The concept that perceiving the avidity of T cell activation can be translated into peripheral T cell regulation is the essence of the avidity model. The cellular mechanism that defines how perceiving the avidity of T cell activation is translated into peripheral T cell regulation and the molecular structures recognized by regulatory T cells that enable them to discriminate self from nonself in the periphery are the key issues in regulatory T cell biology. In this regard, we have recently demonstrated that the heat shock peptide Hsp60sp, coupled with the MHC class Ib molecule Qa-1, is a common surrogate target structure preferentially expressed on the intermediate-avidity T cells and is specifically recognized by a subset of Qa-1–restricted CD8+ T cells (7). Thus, by a unified and simple cognitive mechanism — specific recognition of a common target structure, preferentially expressed on the intermediate-avidity T cells — the Qa-1–restricted CD8+ T cells are able to selectively target and downregulate intermediate- but not high-avidity T cells to accomplish self/nonself discrimination in the periphery (8).

The translation of the murine Qa-1–restricted CD8+ T cell–mediated pathway to humans is based on evidence that the human homolog of Qa-1, HLA-E, can function as a restricting element for human regulatory CD8+ T cells (16). Here we show that humans have a cognitive mechanism similar to that discovered in mice, in

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Obesity 2

Health and economic burden of the projected obesity trends in the USA and the UK

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Rising prevalence of obesity is a worldwide health concern because excess weight gain within populations forecasts an increased burden from several diseases, most notably cardiovascular diseases, diabetes, and cancers. In this report, we used a simulation model to project the probable health and economic consequences in the next two decades from a continued rise in obesity in two ageing populations—the USA and the UK. These trends project 65 million more obese adults in the USA and 11 million more obese adults in the UK by 2030, consequently accruing an additional 6–8·5 million cases of diabetes, 5·7–7·3 million cases of heart disease and stroke, 492 000–669 000 additional cases of cancer, and 26–55 million quality-adjusted life years forgone for USA and UK combined. The combined medical costs associated with treatment of these preventable diseases are estimated to increase by $48–66 billion/year in the USA and by £1·9–2 billion/year in the UK by 2030. Hence, effective policies to promote healthier weight also have economic benefits.

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See **Editorial** page 741

See **Comment** pages 743, 744, and 746

This is the second in a **Series** of four papers about obesity

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Threat to population health

Increased prevalence of overweight and obesity is a worldwide health concern.[1] In a systemic analysis of epidemiological studies from 199 countries,[1] 1·46 billion adults worldwide were estimated to be overweight in 2008, and of these 502 million were obese. Despite signs of stabilisation in some populations,[2,3] the effects of consistently high prevalence of obesity on population health are far-reaching; societies are burdened by premature mortality, morbidity associated with many chronic disorders, and negative effects on health-related quality of life. The challenge to quantify the effect of these health burdens to inform public policies and health services are pressing. Furthermore, projected increases in these diseases in many ageing populations suggest a substantial cost burden to the health-care system in an era of ever-escalating medical expenditure. In a systematic review of the economic burden of obesity worldwide, Withrow and colleagues[4] concluded that obesity accounted for 0·7–2·8% of a country's total health-care costs, and that obese individuals had medical costs 30% higher than those with normal weight. The combination of rising obesity prevalence and increased spending on obese people has been estimated to account for 27% of the growth in US health-care expenditure between 1987 and 2001.[5] Total health-care costs attributable to obesity and overweight are projected to double every decade to account for 16–18% of total US health-care expenditure by 2030.[6]

Figure 1 shows obesity prevalence in adults and children in selected countries.[7] Since the 1970s, the USA and the UK have had striking increases in the proportion of their populations with a body-mass index (BMI) in overweight (BMI 25–29·9 kg/m²) and obese (BMI ≥30 kg/m²) ranges. If such trends were to continue unabated, the report's authors estimate that about three of four Americans and seven of ten British people will be overweight or obese by 2020.[7] Although population-wide secular trends seem much the same, obesity and overweight cluster differently according to socioeconomic status, educational attainment, and race and ethnic group (figure 2 and figure 3).

Health burden from rising obesity

The health burden from obesity is largely driven by an increased risk of type 2 diabetes, cardiovascular diseases, and several forms of cancer. For instance, every additional 5 kg/m² in BMI increases a man's risk of oesophageal cancer by 52% and for colon cancer by 24%, and in women, endometrial cancer by 59%, gall bladder cancer by 59%, and postmenopausal breast cancer by 12% (the association is strongest in women in the Asia–Pacific

Key messages

- Excess bodyweight is associated with negative effects on longevity, disability-free life-years, quality-of-life, and productivity. The obesity epidemic affects both high and middle-to-low income countries, posing a threat to population health and a substantial burden to many health systems.
- The burden of obesity includes an increased number of fatal and non-fatal diseases—including diabetes, coronary heart disease, stroke, cancer, and osteoarthritis—which impose substantial medical costs from treatment and productivity losses (absenteeism, presenteeism, and loss of productivity from premature deaths).
- The higher the proportion of the population that is overweight and obese, the greater the use of health services, resulting in higher treatment costs for the many obesity-related diseases than in a less obese population.
- The health and cost burden of overweight and obesity has a protracted time course. Epidemiological models such as the one we present enable us to link changes in obesity at the population level to disease burdens decades later, a crucial exercise for public policy.
- A systematic understanding of the potential morbidity and cost implications of specified hypothetical changes in body-mass index trajectories, driven by policy changes or otherwise, is crucial for formation of effective and cost-effective strategies, establishment of research and funding priorities, and creation of the political will to address the obesity epidemic.

Cell Metabolism
Article

Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism

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SUMMARY

The brain is the most cholesterol-rich organ in the body, most of which comes from in situ synthesis. Here we demonstrate that in insulin-deficient diabetic mice, there is a reduction in expression of the major transcriptional regulator of cholesterol metabolism, SREBP-2, and its downstream genes in the hypothalamus and other areas of the brain, leading to a reduction in brain cholesterol synthesis and synaptosomal cholesterol content. These changes are due, at least in part, to direct effects of insulin to regulate these genes in neurons and glial cells and can be corrected by intracerebroventricular injections of insulin. Knockdown of SREBP-2 in cultured neurons causes a decrease in markers of synapse formation and reduction of SREBP-2 in the hypothalamus of mice using shRNA results in increased feeding and weight gain. Thus, insulin and diabetes can alter brain cholesterol metabolism, and this may play an important role in the neurologic and metabolic dysfunction observed in diabetes and other disease states.

INTRODUCTION

Both type 1 (insulin-dependent) and type 2 (insulin-resistant) diabetes are associated with hyperglycemia; alterations in carbohydrate, lipid, and protein metabolism; and a variety of complications affecting tissues of the body. These complications extend to the central nervous system (CNS), where they range from acute alterations in mental status due to poor metabolic control to greater rates of decline in cognitive function with age (Biessels et al., 2008; Cukierman et al., 2005), higher prevalence of depression (Ali et al., 2006), and an increased risk of Alzheimer's disease (Craft and Watson, 2004).

The brain is the most cholesterol-rich organ, containing approximately 25% of the cholesterol present in the body. Almost all cholesterol present in the brain is formed by de novo synthesis, since the blood-brain barrier effectively prevents uptake from the circulation (Björkhem and Meaney, 2004; Dietschy and Turley, 2004). Mutations in genes regulating cholesterol metabolism result in several hereditary syndromes showing CNS manifestations, including Niemann-Pick Disease Type C and Smith-Lemli-Opitz syndrome (Korade and Kenworthy, 2008). The pathogenesis of Alzheimer's disease is also linked to brain cholesterol metabolism with genetic risk factors including variations in apolipoprotein E and other cholesterol-related genes (Puglielli et al., 2003; Shobab et al., 2005). Cholesterol metabolism in the brain plays an important role in myelin production (Dietschy and Turley, 2004) and has been implicated in regulation of many processes, including the synaptophysin/synaptobrevin interaction (Mitter et al., 2003) and geranylgeraniol production (Kotti et al., 2006).

A number of abnormalities have been reported in mouse models of diabetes, including alterations in learning, memory, synaptic plasticity, and glutamatergic neurotransmission (Biessels and Gispen, 2005). Some of these appear to be the result of direct effects of insulin, which is transported into the CNS across the blood-brain barrier by a receptor-mediated transport process (Banks et al., 1997), as well as access of insulin to brain areas where the blood-brain barrier is less tight. Mice with heterozygous knockout of the insulin receptor exhibit impairment in object recognition (Das et al., 2005). Intranasal insulin administration can improve cognitive function in diabetic (Francis et al., 2008) and nondiabetic mice (Marks et al., 2009) without significant alterations in blood glucose levels.

Despite the accumulated evidence indicating effects of diabetes and insulin on neuronal function, the molecular mechanisms underlying the cerebral complications of diabetes have been yet to be elucidated. In the present study we show that diabetes produces a global suppression of the enzymes of cholesterol synthesis and their master transcriptional regulator SREBP-2 in the brain, and this results in reduced cholesterol biosynthesis, reduced synaptosomal membrane cholesterol, and altered neuronal and physiological function.

RESULTS

Downregulation of the Cholesterol Biosynthesis Pathway in Hypothalami of Diabetic Mice

The hypothalamus is a major point of control of the endocrine system, appetite, and energy balance (Obici and Rossetti,

nature medicine

Hyperglycemia-induced cerebral hematoma expansion is mediated by plasma kallikrein

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Hyperglycemia is associated with greater hematoma expansion and poor clinical outcomes after intracerebral hemorrhage. We show that cerebral hematoma expansion triggered by intracerebral infusion of autologous blood is greater in diabetic rats and mice compared to nondiabetic controls and that this augmented expansion is ameliorated by plasma kallikrein (PK) inhibition or deficiency. Intracerebral injection of purified PK augmented hematoma expansion in both diabetic and acutely hyperglycemic rats, whereas injection of bradykinin, plasmin or tissue plasminogen activator did not elicit such a response. This response, which occurs rapidly, was prevented by co-injection of the glycoprotein VI agonist convulxin and was mimicked by glycoprotein VI inhibition or deficiency, implicating an effect of PK on inhibiting platelet aggregation. We show that PK inhibits collagen-induced platelet aggregation by binding collagen, a response enhanced by elevated glucose concentrations. The effect of hyperglycemia on hematoma expansion and PK-mediated inhibition of platelet aggregation could be mimicked by infusing mannitol. These findings suggest that hyperglycemia auguments cerebral hematoma expansion by PK-mediated osmotic-sensitive inhibition of hemostasis.

Intracerebral hemorrhage (ICH) accounts for 7–20% of all cases of stroke, with an overall fatality rate within 1 month of 42% (ref. 1). Both diabetes and admission hyperglycemia are independently associated with early and long-term mortality after ICH[2–5]. In addition, hyperglycemia is independently associated with symptomatic ICH in stroke patients treated with intravenous tissue plasminogen activator[6,7]. Hematoma volume is an independent determinant of both mortality and poor outcome after ICH[8,9]. Hyperglycemia is associated with greater hematoma volume and expansion[3,10–14]; however, the role of hyperglycemia in contributing to hematoma expansion and regulating cerebral hemostasis after vascular injury is not fully understood. Moreover, although more than 50% of individuals with stroke have hyperglycemia[15], the clinical benefit of glucose normalization in the setting of acute stroke is controversial[16,17]. Data on the clinical benefit of glucose lowering in ICH are limited, and the potential therapeutic window for glucose lowering is unknown[4,18,19]. Recent guidelines suggest a conservative approach to the management of hyperglycemia in individuals with spontaneous ICH until additional clinical information is available[20]. Here we have investigated the effect of diabetes and hyperglycemia on acute hematoma expansion and the mechanisms that may contribute to hematoma expansion after experimental ICH.

We first compared the response to intracerebral infusion of autologous whole blood in rats with 4 weeks of streptozotocin-induced diabetes with age-matched nondiabetic rats. This procedure allowed us to quantify the effect of diabetes on hemorrhagic response independently of other variables associated with the incidence and severity of ICH. Hematoma expansion to the subarachnoid space in diabetic rats infused with autologous blood was tenfold greater than that observed in nondiabetic rats (**Fig. 1a**,**b**). The contralateral cerebral hemisphere was injected with PBS and showed a smaller magnitude of hematoma expansion than did the hemisphere injected with blood, and there was a trend toward greater hematoma expansion in diabetic compared with nondiabetic rats (**Fig. 1a**,**b**). Infusion of autologous blood labeled with Evans blue dye showed that the blood remained localized to the site of injection. The hematoma observed within 30 min on the surface of the brain in diabetic rats was not labeled, indicating that the rapid appearance of blood in the subarachnoid space was probably derived from vessels ruptured during needle insertion (**Supplementary Fig. 1**). As in the streptozotocin-induced diabetic rats, hematoma expansion was greater in diabetic Akita mice (C57BL/6J-*Ins2*^{Akita}) subjected to autologous blood injection than in nondiabetic littermate controls (**Fig. 1c**).

Previously, we implicated PK in blood-brain barrier dysfunction after experimental ICH[21]. PK is activated from plasma prekallikrein (PPK) by coagulation factor XII through the contact activation system and has a central role in the intrinsic coagulation cascade, innate inflammation, vascular function and fibrinolysis[22]. We therefore investigated the potential role of PK in hematoma expansion in diabetic animals. Systemic administration of the small-molecule PK inhibitor ASP-440 (ref. 23) attenuated hematoma expansion in diabetic rats (**Fig. 1d**). ASP-440–treated diabetic rats receiving a sham injection of PBS in the contralateral hemisphere also showed a smaller magnitude of hematoma expansion than did vehicle-treated rats receiving the same injection (**Fig. 1d**). In addition, in diabetic rats, co-injection of blood with a neutralizing PK-specific antibody significantly attenuated

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nutrients act to more slowly alter a spectrum of signaling pathways and gene expression changes, ultimately leading to depolarization or hyperpolarization. Furthermore, POMC neurons are much less homogeneous than the NPY/AgRP neurons, with only a subset of cells depolarized by leptin (Williams et al., 2010). Data also suggest there may be both GABAergic and glutamatergic POMC cells (Hentges et al., 2009). Thus, electrical stimulation of POMC cells may produce a more mixed response than the pure inhibitory GABAergic drive from NPY/AgRP neurons or the potent anorexigenic response to α-MSH alone.

Nonetheless, optogenetics is a powerful new tool to apply to the analysis of the energy homeostasis circuitry, and the rapidly developing optogenetic armamentarium includes channels that can be used to hyperpolarize and inhibit neurons, channels that can be targeted to presynaptic nerve terminals, and light-activated molecules that couple to activation of G protein signaling pathways (Gradinaru et al., 2010). For illuminating the role of neurons that are too diffusely distributed to activate with a fiber-optic light stimulus, chemical-genetic tools, such as a G protein-coupled receptor capable of activating neurons in response to a pharmacologically inert, orally bioavailable drug, clozapine-N-oxide, have also been engineered (Alexander et al., 2009). Given the complexity of the circuitry involved in energy homeostasis, optogenetics and chemical-genetic tools may prove invaluable.

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Endothelium-Dependent Delivery of Insulin to Muscle Interstitium

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Insulin contributes to skeletal muscle glucose uptake by increasing blood flow and recruiting perfused capillaries. In this issue of *Cell Metabolism*, Kubota et al. (2011) show that deletion of IRS-2 in endothelial cells in mice causes impaired transcapillary insulin transport, decreased insulin-stimulated glucose uptake in muscle, and mild glucose intolerance.

Insulin signaling in vascular endothelium produces at least two types of discrete actions. First, insulin modifies endothelial homeostasis in arteries, thereby making the vascular wall less susceptible to atherosclerosis (Rask-Madsen and King, 2007). Second, insulin may regulate its own delivery to skeletal muscle and other tissues (Barrett et al., 2009; Chiu et al., 2008). Whether this mechanism contributes significantly to systemic insulin sensitivity is not clear in spite of extensive investigation. In this issue, Kubota et al. (2011) report that endothelial insulin signaling through insulin receptor substrate-2 (IRS-2), a docking protein relaying insulin receptor activation to intracellular signaling, contributes to transcapillary insulin transport in muscle and affects glucose tolerance in mice. These results suggest that endothelial cell function may be a therapeutic target for improving peripheral insulin sensitivity.

The rate of insulin delivery from the blood to the interstitial space is limited by transport across the capillary wall in tissues where endothelial cells form tight junctions. After binding to its receptors, insulin can be transported across cultured endothelial cell monolayers by transcytosis (King and Johnson, 1985), but little is known about intracellular insulin signaling in this process (Wang et al., 2008). It is also unclear whether transcytosis (Barrett et al., 2009) rather than passive diffusion at cell junctions (Chiu et al., 2008) is responsible for transendothelial transport of insulin in vivo, which is important because transcytosis is more likely to be a regulated process which can be modified for therapeutic gain. Insulin resistance developing during high-fat feeding

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Ancestry informative markers on chromosomes 2, 8 and 15 are associated with insulin-related traits in a racially diverse sample of children

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Abstract

Type-2 diabetes represents an increasing health burden. Its prevalence is rising among younger age groups and differs among racial/ethnic groups. Little is known about its genetic basis, including whether there is a genetic basis for racial/ethnic disparities. We examine a multiethnic sample of 253 healthy children to evaluate associations between insulin-related phenotypes and 142 ancestry informative markers (AIMs), while adjusting for sex, age, Tanner stage, genetic admixture, total body fat, height and socio-economic status. We also evaluate the effect of measurement errors in estimation of the individual ancestry proportions on the regression results. We find that European genetic admixture is positively associated with insulin sensitivity (S_I), and negatively associated with acute insulin response to glucose, fasting insulin, and homeostasis model assessment of insulin resistance. Our analysis reveals associations between individual AIMs on Chromosomes 2, 8, and 15 and these phenotypes. Most notably, marker rs3287 at chromosome 2p21 was found to be associated with S_I ($p=5.8 \times 10^{-5}$). This marker may be in admixture linkage disequilibrium with nearby loci (THADA and BCL11A) that have previously been reported to be associated with diabetes and diabetes-related phenotypes in several genome-wide association and linkage studies. Our results provide further evidence that variation in the 2p21 region containing THADA and BCL11A is associated with type-2 diabetes. Importantly, we have implicated this region in the early development of diabetes-related phenotypes, and in the genetic etiology of population differences in these phenotypes.

Keywords

insulin sensitivity; genetic admixture; type-2 diabetes; genetic association; ancestry informative marker

INTRODUCTION

Type-2 diabetes prevalence in the pediatric population is increasing, while age at onset is decreasing (1;2). Type-2 diabetes also disproportionately affects racial/ethnic minorities in

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Maternal Glucose Concentration During Pregnancy Predicts Fat and Lean Mass of Prepubertal Offspring

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OBJECTIVE—Intrauterine exposure to high maternal glucose is associated with excess weight gain during childhood, but it is not clear whether the excess weight represents increased fat or lean mass. The purpose of this study was to examine the relationship between maternal glucose concentrations during pregnancy and offspring body composition. A secondary goal was to examine whether the association between maternal glucose and children's body fat was independent of energy intake, energy expenditure, or physical activity.

RESEARCH DESIGN AND METHODS—Children aged 5–10 years and their biological mothers (n = 27) were recruited. Maternal glucose concentration 1 h after a 50-g oral glucose load, used to screen for gestational diabetes mellitus at 24–28 weeks gestation, was retrieved from medical records. Children underwent dual-energy X-ray absorptiometry to measure body composition, indirect calorimetry to measure resting energy expenditure (REE), accelerometry to measure physical activity, and three 24-h diet recalls to measure energy intake.

RESULTS—Maternal glucose concentration during pregnancy was positively associated with children's lean mass ($P < 0.05$) and adiposity (fat mass adjusted for lean mass; $P < 0.05$). The association between maternal glucose and children's adiposity was independent of children's REE, percent of time spent physically active, and energy intake ($P < 0.001$).

CONCLUSIONS—Intrauterine exposure to relatively high maternal glucose is associated with greater lean mass and adiposity among prepubertal offspring. Further research is needed to examine the mechanisms by which maternal glucose concentrations during pregnancy influence children's body composition.

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Children born to diabetic women (1–3) or those with relatively high glucose concentrations during pregnancy (4,5) have greater birth weight and are at greater risk for obesity than are those born to nondiabetic women. These conclusions are based on measures of BMI percentiles or skin fold measures of subcutaneous fat, and so it is not clear whether the larger body size among offspring of diabetic women also reflects greater lean mass in addition to greater fat mass. Excess childhood weight gain among offspring of diabetic mothers is believed to be a result of prenatal exposure to increased fuel from the mother, and glucose has been specifically implicated (6,7). It would be of interest, therefore, to examine the association between maternal glucose concentrations during pregnancy across a range incorporating both diabetic and nondiabetic extremes and children's fat and lean mass.

Despite the large body of literature supporting associations of maternal diabetes and glucose concentrations with offspring body weight, it is not known whether greater energy intake or reduced expenditure among offspring contributes to this effect. Evidence from animal models supports the hypothesis that regulation of appetite and energy balance may be impaired following prenatal exposure to high maternal glucose (rev. in 8), but in clinical studies, investigators have found no difference in energy expenditure, physical activity, or energy intake under controlled conditions among children with and without prenatal exposure to maternal diabetes (9,10). These studies did not consider maternal glucose concentration specifically, however, which may vary considerably in women with diabetes based on their degree of glycemic control.

The purpose of this study was to test the hypothesis that prepubertal children who were prenatally exposed to relatively high maternal glucose concentrations would have greater fat, but not lean, mass. A secondary hypothesis was to examine whether this association was independent of children's energy intake, expenditure, or physical activity. These hypotheses were tested in prepubertal children for whom mothers' medical records during pregnancy were available.

RESEARCH DESIGN AND METHODS

Children aged 5–10 years and their biological mothers were recruited. Mothers were eligible if they were ≥16 years old at delivery, and initiated prenatal care during the first trimester. Women who had developed gestational diabetes mellitus (GDM) during the target pregnancy were over-sampled relative to the 3–8% prevalence in the general population (11), in order to increase the range of gestational glucose concentrations represented. Children were eligible if they were singletons and were born at ≥37 weeks gestation. Children who were growth restricted in utero (<2,500 g at birth), had congenital defects, type 1 diabetes, or a current weight of <11 kg precluding adequate blood sampling, were excluded.

Procedure

Mother-child pairs attended two study related visits spaced ~10 days apart. During

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Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice

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Skeletal muscle development, nutrient uptake, and nutrient utilization is largely coordinated by growth hormone (GH) and its downstream effectors, in particular, IGF-1. However, it is not clear which effects of GH on skeletal muscle are direct and which are secondary to GH-induced IGF-1 expression. Thus, we generated mice lacking either GH receptor (GHR) or IGF-1 receptor (IGF-1R) specifically in skeletal muscle. Both exhibited impaired skeletal muscle development characterized by reductions in myofiber number and area as well as accompanying deficiencies in functional performance. Defective skeletal muscle development, in both GHR and IGF-1R mutants, was attributable to diminished myoblast fusion and associated with compromised nuclear factor of activated T cells import and activity. Strikingly, mice lacking GHR developed metabolic features that were not observed in the IGF-1R mutants, including marked peripheral adiposity, insulin resistance, and glucose intolerance. Insulin resistance in GHR-deficient myotubes derived from reduced IR protein abundance and increased inhibitory phosphorylation of IRS-1 on Ser 1101. These results identify distinct signaling pathways through which GHR regulates skeletal muscle development and modulates nutrient metabolism.

Introduction

Mammalian skeletal muscle has evolved to perform a diverse set of functions, including locomotion, breathing, protecting internal organs, and coordinating global energy expenditure. Skeletal muscle is formed and regenerated through a highly regulated process characterized by myoblast differentiation and fusion into multinucleated syncytia. During embryonic development, specification of mesodermal precursor cells into the myogenic lineage is controlled by signals from surrounding tissues and requires upregulation of several factors, including paired-box transcription factor 7 (pax-7) and basic helix-loop-helix transcriptional activators of the myogenic regulatory factor family, MyoD and Myf-5 (1). The proliferating precursor cells/myoblasts withdraw from the cell cycle and initiate muscle-specific gene expression (2, 3). Myoblasts then initially fuse to form nascent myotubes, with relatively few nuclei, through a highly ordered set of cellular events, including recognition, adhesion, alignment, and membrane union. Subsequent recruitment and fusion of additional myoblasts gives rise to multinucleated myotubes that ultimately mature to give rise to skeletal muscle fibers. The fusion process is controlled, in part, by the actions of calcium-sensitive transcription factors of the nuclear factor of activated T cells (NFAT) family (4, 5). During myoblast fusion, the nuclear translocation of NFATc2 transcriptionally activates IL-4, a cytokine essential for myoblast recruitment (6).

The growth hormone/IGF-1 (GH/IGF-1) axis represents an important physiological regulatory mechanism for coordinating postnatal skeletal muscle expansion and hypertrophy. Administration of GH to both animals and GH-deficient humans improves muscle strength and reduces body fat (7–9). Moreover, recent studies have shown that mice globally deficient in GH receptor (GHR) have reduced muscle mass with defective myofiber specification and growth (10). Such studies clearly demonstrate the importance of GH in skeletal muscle development but do not address the mechanisms responsible for these effects.

GH exerts growth-promoting and metabolic effects in target tissues (11) by binding to the transmembrane GHR and triggering enhanced GHR association with, and activation of, the cytoplasmic tyrosine kinase JAK2 (12, 13). Three major signaling systems activated in response to GH include STATs (most notably STAT5b), phosphoinositide 3-kinase (PI3K), and Erk (14, 15). STAT5b activation by GH results in transcriptional activation of GH target genes, including IGF-1 (16, 17). Many but not all of the anabolic effects of GH are exerted indirectly via this stimulation of IGF-1 from liver and peripheral tissues (18–27).

IGF-1 is a small polypeptide with homology to proinsulin, which is produced by many cell types. IGF-1 signals via the type-1 IGF-1 receptor (IGF-1R), a widely expressed cell surface heterotetramer, highly similar to the IR, which possesses intrinsic kinase activity in its cytoplasmic domains (28, 29). Activated IGF-1R engages the Erk and PI3K pathways via phosphorylation of SHC

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OBSTETRICS

Maternal insulin resistance and preeclampsia

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OBJECTIVE: The purpose of this study was to determine whether mid-trimester insulin resistance is associated with subsequent preeclampsia.

STUDY DESIGN: This was a secondary analysis of 10,154 nulliparous women who received vitamin C and E or placebo daily from 9-16 weeks gestation until delivery. Of these, 1187 women had fasting plasma glucose and insulin tested between 22 and 26 weeks gestation. Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index.

RESULTS: Obese women were twice as likely to have a HOMA-IR result of ≥75th percentile. Hispanic and African American women had a higher percentage at ≥75th percentile for HOMA-IR than white women (42.2%, 27.2%, and 16.9%, respectively; $P < .001$). A HOMA-IR result of ≥75th percentile was higher among the 85 nulliparous women who subsequently had preeclampsia, compared with women who remained normotensive (40.5% vs 24.8%; adjusted odds ratio, 1.9; 95% confidence interval, 1.1–3.2). Quantitative insulin sensitivity check index results were similar to the HOMA-IR results.

CONCLUSION: Midtrimester maternal insulin resistance is associated with subsequent preeclampsia.

Key words: insulin resistance, low-risk nulliparous woman, preeclampsia

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Prevalence and Predictors of Weight-Loss Maintenance in a Biracial Cohort

Results from the Coronary Artery Risk Development in Young Adults Study

Suzanne Phelan, PhD, Rena R. Wing, PhD, Catherine M. Loria, PhD, Yongin Kim, MS, Cora E. Lewis, MD

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Background: Few population-based studies have examined the behavioral and psychosocial predictors of long-term weight-loss maintenance.

Purpose: The goal of this study was to determine the prevalence and predictors of weight-loss maintenance in a biracial cohort of younger adults.

Methods: This study examined a population-based sample of overweight/obese African-American and white men and women who had ≥5% weight loss between 1995 and 2000. Subsequent changes in weight, physical activity, and behavioral and psychosocial factors were examined between 2000 and 2005. Analyses were conducted in 2008–2009.

Results: Among the 1869 overweight/obese individuals without major disease in 1995, a total of 536 (29%) lost ≥5% between 1995 and 2000. Among those who lost weight, 34% (n=180) maintained at least 75% of their weight loss between 2000 and 2005, whereas 66% subsequently regained. Higher odds of successful weight-loss maintenance were related to African-American race (OR=1.7, p=0.03); smoking (OR=3.4, p=0.0001); history of diabetes (OR=2.2, p=0.04); increases in moderate physical activity between 2000 and 2005 (OR=1.4, p=0.005); increases in emotional support over the same period (OR=1.6, p=0.01); and less sugar-sweetened soft drink consumption in 2005 (OR=0.8, p=0.006).

Conclusions: One third of overweight men and women who lost weight were able to maintain 75% or more of their weight loss over 5 years. Interventions to promote weight-loss maintenance may benefit from targeting increased physical activity and emotional support and decreased sugar-sweetened soft drink consumption.

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Introduction

Given the importance of obesity as a public health problem, it is surprising how little is known about the prevalence and predictors of long-term weight-loss maintenance. Weight-loss trials have shown that most patients regain weight after treatment termination,[1] but the average individual who continues to participate in a clinical trial maintains a weight loss of about 3% of initial body weight for up to 5 years after treatment.[2] Analyses[3–9] examining predictors of treatment outcomes have identified some behaviors that appear to improve success, including continued consumption of a low-calorie, low-fat diet; increased physical activity; and self-monitoring. Psychosocial correlates of better weight-loss maintenance also have been recognized,[8,10–16] including lower levels of depressive symptoms, stress, and disinhibition and higher levels of restraint and self-efficacy.

Much of the clinical trial literature, however, has been limited by short-term follow-up (2 years or less); small sample sizes; high drop-out rates; and lack of intent-to-

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treat analyses. Moreover, clinical trials have generally evaluated specific short-term treatment approaches in individuals attending weekly weight-loss programs. Individuals who seek assistance for weight loss tend to be heavier,[17] have more medical problems,[18,19] and have higher percentages of binge eating[20] than individuals in the general population. Because the individuals who attend clinical weight-loss treatments may be more difficult to treat, and clinical trials have generally evaluated specific short-term treatment methods (e.g., meal replacements, pharmacotherapy, very low–calorie diets),[1] the results from such programs may not represent the true prevalence or typical methods for weight-loss maintenance in the general population. Moreover, although cohort studies of larger samples ($n>5000$) of successful weight losers exist (e.g., the National Weight Control Registry[21]), these data are based on self-selected samples, and include primarily women, whites, and educated individuals; thus, the findings may not generalize to the population at large.

Only a few empirical studies have attempted to estimate the prevalence of long-term weight-loss maintenance in the general U.S. population. Prevalence estimates of successful maintenance after weight loss have ranged from 58.9% in the National Health and Nutrition Examination Survey (NHANES, 1999–2002)[22]; 47% in a random-digit-dial survey[23]; and 20% in the Nurses Health Study.[7] Overall, these studies suggest that sustaining weight loss may be possible for a substantial subset of the general population. However, existing population-based studies have been based on self-reported weights and included a limited array of behavioral and psychosocial measures. Clearly, to better understand the prevalence and predictors of long-term weight control, further research is needed in both men and women that includes more diverse samples, measured weights, multiple follow-ups, and comprehensive assessments.

The Coronary Artery Risk Development in Young Adults (CARDIA) Study enrolled more than 5000 African-American and white women and men, aged 18–30 years, in 1985–1986 and has followed the cohort for more than 20 years, recording serial measurements of weight and behavioral and psychosocial factors. Prior research in CARDIA has examined predictors of weight loss over 2 years of follow-up.[24] Weight loss generally was associated with greater baseline fatness, lower baseline physical fitness level, self-perception of being overweight, dieting, and previous weight loss and regain. Other CARDIA papers have examined predictors of weight increases over time. In these studies, limited physical activity,[25,26] greater fast-food consumption[27] and less dissatisfaction with body size[28] were identified as significant predictors of weight gain.

The purpose of the present study was to determine the prevalence of successful weight loss and maintenance in the CARDIA cohort and to identify the strongest demographic, behavioral, and psychosocial predictors of 5-year weight-loss maintenance. It was hypothesized that among individuals who had lost 5% or more of their body weight, those with higher levels of physical activity; better dietary intake (e.g., fewer sugar-sweetened beverages, less fast-food consumption); and less depressive symptoms would be most likely to maintain at least 75% of their weight loss over 5 years of follow-up.

Methods

Sample

The CARDIA study is a multicenter, longitudinal study of the development and determinants of cardiovascular disease over time among African-American and white adult men and women. The first CARDIA examination took place in 1985–1986 and included 5115 women and men. Sampling was designed to achieve balanced representation among white and African-American men and women; age groups (18–30 years); and education levels. Subsequent to baseline, the cohort was reexamined at Years 2, 5, 7, 10, 15, and 20 (spanning 1987–2005). All examinations were approved by IRBs at each institution. Details of the study design have been published elsewhere.[29]

To be included in the current study, participants had to have been overweight or obese (BMI $\geq$25) and without self-reported major disease in 1995. Of the 3950 participants who were assessed in 1995, a total of 2432 were overweight/obese; of these, 14 were eliminated because of pregnancy between 1995 and 2005 and 549 were eliminated because of a reported illness that potentially could have caused involuntary weight loss over the same time span, including one or more of the following: kidney failure ($n=14$); cirrhosis ($n=1$); cancers ($n=68$); hyperthyroidism ($n=32$); digestive diseases ($n=86$); tuberculosis in past year ($n=84$); HIV ($n=11$); and/or any reported medical problems interfering with exercise ($n=402$; predominantly reflecting recent injuries, surgeries, or chronic pain). The remaining 1869 made up the final sample in the current study.

The weight loss of these 1869 participants was examined between 1995 and 2000 and then weight-loss maintenance between 2000 and 2005 (note that in the current study, calendar years [i.e., 2000, 2005] are used rather than CARDIA assessment years [i.e., Year 15, Year 20] to refer to study time points).

Overall retention for the 1995, 2000, and 2005 examinations was 78.5%, 74%, and 72% of surviving participants, respectively (approximately 2.5% were deceased as of the 2000 examination and 3.4% in 2005).[30,31] Whites, nonsmokers, more educated participants, and slightly older participants were more likely to return for these exams than African Americans, smokers, those with less education, and younger participants.[32] There was no significant relationship with BMI and exam retention.

Measures

As the main purpose of the present study was to examine variables associated with weight-loss maintenance versus regain, assessments occurred after participants' initial weight loss (between 1995

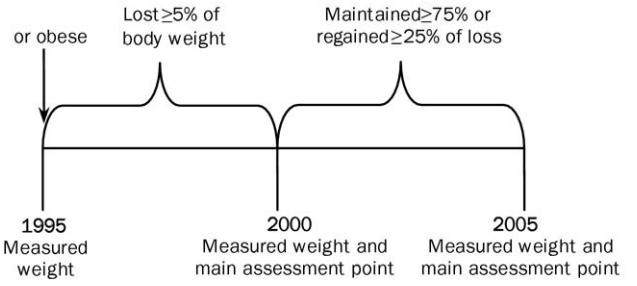

Figure 1. Scheme for assessing weight loss maintenance in overweight or obese participants who had lost ≥5% of their body weight between 1995 and 2000, and maintained ≥75% of the weight loss between 2000 and 2005. Weight regain was defined as regaining >25% of weight loss. All participants were without self-reported major disease or pregnancy in 1995.

and 2000) at the 2000 and 2005 examinations. Some variables (anger and coping) were assessed in the 2000 exam only, and others (i.e., diet history) in the 2005 examination only, as indicated in the sections that follow.

Outcome Definitions

Weight-loss maintainers were defined as participants who were overweight or obese in 1995 (and without pregnancy or major medical illnesses affecting weight); had lost ≥5% by 2000; and had maintained 75% of their weight loss by 2005. A 5% weight-loss criterion was chosen, as this has been shown in numerous studies to be associated with substantial health benefits[33] and has been recommended by the IOM as the weight-loss criterion for evaluating success of weight-loss programs[34]; this criterion has also been used in other epidemiologic research examining weight-loss maintenance.[7] Although successful weight control can involve some weight regain, successful weight loss further was defined as maintaining ≥75% of the weight loss for 5 years to identify a relatively weight-stable group of weight-loss maintainers.[13,35] Regainers were defined as individuals who were overweight or obese in 1995 (and without pregnancy or major medical illnesses affecting weight); had lost ≥5% by 2000; but had regained >25% of their weight loss by 2005 (Figure 1). Note that the terms *weight-loss maintainer* and *regainer* are used to denote these groups, but intentionality of weight changes should not be inferred by the use of these terms.

Weight, height, demographics. Weight and height were measured in light clothing and without shoes at the 1995, 2000, and 2005 examinations using calibrated equipment. BMI was calculated using standard formula. Demographic and medical information collected at the 2000 and 2005 examinations were used in analyses. At these time points, all participants were interviewed by trained personnel about their medical history and current use of cigarettes and alcoholic beverages. In 2005, participants also were asked whether they had ever had bariatric surgery. Voluntary versus involuntary weight loss and weight cycling between assessment points was not directly assessed.

Leisure time physical activity. The CARDIA physical activity questionnaire[36] was administered in both 2000 and 2005. Total physical activity was expressed in exercise units as a product of intensity × frequency × 100, to yield a total activity score.

Dietary intake. Diet variables were selected based on previous research in weight-loss maintenance[21,37] and included calorie intake, percentage of calories from fat, and fast-food and soft drink consumption using a diet history questionnaire administered in 2005.[31,38,39] Fast-food habits were assessed in 2000 and 2005.[31]

Psychosocial measures. Depressive symptomatology was assessed in both 2000 and 2005 using the 20-item Center for Epidemiologic Studies depression scale (CES-D).[40] The shortened version of the SF-36 was used to assess quality of life in the 2000 and 2005 examinations.[41] Social network was measured in 2000 and 2005.[42] Social support was measured also in 2000 and 2005 using eight items drawn from the MacArthur Network.[43] Anger was assessed in 2000 only, using the State–Trait Anger Expression Inventory-2.[44,45] Coping was assessed in 2000 only, using the Reactive Responding Measure.[46] Sleep disturbances were assessed in 2000 and 2005 using questions from the Sleep Heart Health Study[47] pertaining to excessive daytime sleepiness, trouble falling asleep, and frequent awakening.

Statistics

All analyses were performed using SAS version 9.2. Initial univariate *t* tests and chi-square analyses were conducted to compare groups on demographic and weight-related characteristics. Participants with missing weight data in the final assessment point (2000; $n=85$) were classified as weight regainers to provide a conservative estimate of the prevalence of successful weight-loss maintenance, but analyses that excluded these 85 individuals revealed similar findings. A three-step process was used to identify the most robust set of predictors of weight-loss maintenance versus weight regain. First, initial multivariate ANOVA for repeated measures was used to examine changes over time (between 2000 and 2005) in each variable and interactions with group (Maintainer versus Regainer), both with and without adjusting for demographic variables affecting weight (race, smoking status, age, gender, marital status, dieting history, 1995 BMI, and percentage weight loss since 1995).

Second, variables that were found to be significant or approached significance ($p<0.15$; see Table 2) in these initial adjusted models were entered into a stepwise analysis within predefined categories (i.e., demographic; smoking; physical activity; macronutrient [% of calories from carbohydrate, protein]; dietary components [fast food, diet soft drink, sugar-sweetened soft drinks]; psychosocial; and sleep). Third, variables that were significant ($p<0.05$) within each predefined category in the stepwise analyses were added individually to a sequential hierarchic model to see whether its inclusion improved the fit of the model using a significant likelihood ratio chi-square. The sequential order in the hierarchic model was based on previous research on variables affecting weight-loss maintenance[21,37] and was as follows: (1) demographic and smoking; (2) physical activity; (3) diet (either macronutrient or specific dietary components, such as fast food or soft drinks; each was analyzed in separate models); (4) psychosocial variables; and (5) sleep variables.

A sensitivity analysis also was conducted using an inverse weighting probability model in which the presence and absence in the final analysis was included as a dependent variable. However, accounting for missingness as an independent variable in the final model did not appreciably influence the findings; thus, only results from the completers' analyses are presented here.

Table 1. Comparison of those who maintained their weight loss and those who regained between 2000 and 2005 on demographic and weight-related variables in 2000

Variable	Weight-loss maintainer (*n*=180)	Weight regainer (*n*=356)	*p*-value
Demographic			
Age (years)	40.1±3.7	39.7±3.6	0.18
Female	47.5	46.0	0.75
White	36.3	41.7	0.22
African-American	63.7	58.2	
Married	47.2	48.2	0.84
High school educated or more	67.5	70.7	0.44
Weight/weight-loss information in 1995 and 2000 (M±SD)			
Weight (kg) in 1995	103.9±24.2	100.4±19.8	0.08
BMI in 1995	35.4±7.8	34.0±6.2	0.02
Weight (kg) at year 2000	92.3±19.0	90.6±17.3	0.29
BMI at year 2000	31.5±6.0	30.7±5.3	0.10
Weight loss (1995 wt [kg] – 2000 wt [kg])	11.6±9.0	9.9±6.0	0.007
Percentage weight loss ([1995 wt – 2000 wt]/1995 wt)	10.6±5.7	9.6±4.5	0.04

Note: Values are percentages unless otherwise mentioned. Weight regain = lost ≥5% between 1995 and 2000 and regained >5% between 2000 and 2005. Weight-loss maintenance = lost ≥5% between 1995 and 2000 and maintained ≥75% of that weight loss between 2000 and 2005. Weight and height in 1995, 2000, and 2005 were based on measured weights using calibrated equipment.

Results

Participants were 1869 nonpregnant overweight/obese individuals without major disease in 1995. They were, on average, aged 40.1±3.7 years, with 47% female, 39% white; 48% married; and 69% with a high school education or more. Of these 1869, a total of 536 (29%) lost at least 5% of their body weight between examinations in 1995 and 2000; of these, 180 (33.5%) maintained at least 75% of their weight loss between 2000 and 2005 and were classified as "weight-loss maintainers"; 356 (66.4%) had lost ≥5% but regained more than 25% of their weight loss during 2000–2005 and were classified as "weight regainers." The maintainers and regainers were compared (below) to identify the characteristics in year 2000 that best distinguished these two groups.

Baseline (Year 2000) and Changes from 1995 to 2000 As Predictors of Subsequent Regain

Demographic characteristics and weight changes. Maintainers and regainers differed on a number of weight-related characteristics (Table 1). Moreover, a significantly greater proportion of weight-loss maintainers than regainers self-reported a history of diabetes (7.5% vs 4.7%, respectively; $p=0.001$) but no significant differences were observed in reported history of high blood pressure (28% vs 21%, respectively; $p=0.19$).

Behavioral and psychosocial characteristics at year 2000. In general, the behavioral and psychosocial characteristics measured in the 2000 examination did not differ between those who subsequently gained or maintained their weight 5 years later (Table 2). However, a significantly greater proportion of weight-loss maintainers than regainers reported smoking in the 2000 examination (33% vs 23%, respectively; $p=0.0001$). Moreover, there was a trend for greater alcohol consumption in maintainers than regainers (7.4 vs 6.6 drinks/week, $p=0.09$; Table 2). Additionally, there were trends for maintainers to report engaging in slightly less physical activity initially and to report less prevalent awakenings at night (Table 2).

Change Between the 2000 and 2005 Examinations

Additional analyses compared changes between the 2000 and 2005 examinations for those who regained weight versus those who maintained their previous weight loss. During this time span, weight-loss maintainers continued to lose weight (4 kg loss) and further reduced their BMI from 31.5±6.0 to 30.4±6.5 whereas regainers increased to above baseline (8.8 kg gain) from a BMI of 30.7±5.3 to 33.6±6.4. In 2005, maintainers were lighter than regainers (88.5±18.7 vs 99.3±20.5 kg, $p=0.0001$) and were maintaining a weight loss of approximately 15% from 1995 compared with 1% weight regain above baseline for regainers.

Table 2. Mean behavioral and psychological characteristics at the 2000 and 2005 follow-up examinations

Characteristic	2000 Examination			2005 Examination		Significance		
	Maintainers	Regainers	*p*-value	Maintainers	Regainers	Group	Time	Group × time
Current smoker (%)	33.2	23.0	0.0001	23.6	15.8	0.0005	0.006	0.05
Physical activity								
Total	345.0±22.1	382.4±16.7	0.07	344.3±22.4	327.9±19.3	ns	ns	ns
High	203.2±16.9	238.4±12.8	0.10	199.1±17.2	205.8±14.8	ns	ns	ns
Moderate	141.8±8.4	143.9±6.3	0.14	145.2±8.5	122.1±7.3	ns	ns	0.08
Sleep (%)								
Daytime sleepiness	25	24	ns	30	31	ns	0.04	ns
Trouble falling asleep	23	19	ns	25	21	ns	ns	ns
Frequent awakenings	46	53	0.07	48	57	0.02	ns	ns
Psychosocial								
Quality of life: physical component	51.7±0.5	52.3±0.4	ns	50.3±0.5	49.9±0.5	ns	0.0001	ns
Quality of life: mental component	49.8±0.7	50.1±0.5	ns	50.9±0.7	51.1±0.5	ns	0.05	ns
Chronic burden	1.9±0.04	1.8±0.03	ns	1.7±0.05	1.7±0.04	ns	0.0001	ns
Social support: positive emotional	2.1±0.05	2.1±0.04	ns	2.0±0.05	2.1±0.04	ns	ns	0.10
Social support: negative emotional	2.0±0.1	2.1±0.03	ns	2.0±0.1	2.1±0.04	ns	ns	ns
Social network	7.3±0.2	7.5±0.1	ns	5.2±0.2	5.4±0.2	0.13	0.0001	ns
CES-D (Total)	10.0±0.6	10.0±0.4	ns	9.8±0.6	10.7±0.5	ns	ns	ns
Anger out	1.8±0.03	1.7±0.02	ns	—	—	0.14	—	—
Reactive responding: emotional	2.9±0.06	2.9±0.04	ns	—	—	ns	—	—
Reactive responding: goal	3.7±0.06	3.8±0.04	ns	—	—	0.13	—	—
Reactive responding: vigilance	2.8±0.05	2.8±0.04	ns	—	—	ns	—	—
Diet								
Total calories/day	—	—	—	2384±110.3	2580±101.3	ns	—	—
% kcal from fat/day	—	—	—	36.6±0.70	36.0±0.6	ns	—	—
% kcal from carbohydrates/day	—	—	—	45.1±0.81	46.8±0.70	0.10	—	—
% kcal from protein/day	—	—	—	16.1±0.30	15.3±0.30	0.08	—	—
Sugar-sweetened drinks (srv/day)	—	—	—	0.93±0.13	1.2±1.7	0.02	—	—
Diet beverages (srv/day)	—	—	—	1.4±0.20	0.99±0.18	0.08	—	—
Water (srv/day)	—	—	—	5.7±0.4	5.8±0.4	ns	—	—
Alcohol (srv/week)	7.7±0.9	6.6±0.6	0.09	7.2±0.9	7.2±0.8	ns	ns	ns
Fast food (srv/week)	3.4±0.04	3.3±0.03	ns	3.5±0.04	3.4±0.04	0.07	0.14	ns

Note: Unadjusted values are presented for ease in interpretation; *p*-values reflect analyses with adjustment for race, smoking status, age, gender, marital status, dieting history, and initial BMI and weight loss. *p*-values <0.15 are displayed in the table and denote variables that were entered into subsequent models.
CES-D, Center for Epidemiologic Studies–Depression scale; ns, nonsignificant ($p>0.15$); srv, servings

Examining variables assessed in both the 2000 and 2005 examinations, there was a significantly greater reduction in smoking prevalence among regainers than maintainers ($p=0.05$; Table 2). There was also a trend ($p<0.08$) for maintainers to slightly increase their physical activity and for regainers to decrease their activity. Group main effects also were found for sleep (Table 2). Examining psychosocial characteristics, several time effects were observed, including substantial declines in physical and mental quality of life scores (Table 2), but the maintainers and regainers had similar changes.

Table 2 also shows results of the diet history questionnaire administered in 2005. Maintainers consumed significantly ($p=0.02$) fewer daily servings of sugar-sweetened soft drinks than regainers and slightly more

Table 3. Odds of being in the weight-loss maintainers versus regain category

Factor	OR (95% CI)	*p*-value
African-American	1.7 (1.1, 3.0)	0.03
Female	0.9 (0.5, 1.4)	ns
Married (Nonmarried = ref)	0.9 (0.6, 1.6)	ns
BMI (year 1995)	0.9 (0.9, 1.0)	ns
Weight loss between 1995 and 2000	1.0 (0.9, 1.0)	ns
Smoker in 2000 and 2005 (Never-smokers = ref)	3.4 (1.9, 6.2)	0.0001
Dieting history	1.0 (0.9, 1.0)	ns
History of diabetes	2.2 (1.0, 5.1)	0.04
Increase in units of moderate activity (2000–2005)	1.4 (1.1, 1.7)	0.005
Soft drink consumption (servings/day; year 2005)	0.8 (0.7, .9)	0.006
Increases in emotional support (2000–2005)	1.6 (1.2, 2.7)	0.01

Note: Race, gender, marital status, dieting history, and history of diabetes, measured in 2000. Results based on sequential multiple regression in which demographic variables were entered first, followed by physical activity, dietary, and psychosocial variables.
ns, nonsignificant ($p>0.05$)

diet soft drinks, fewer calories from protein, and more fast food, but these latter trends were not significant. History of bariatric surgery was also assessed in 2005; four maintainers and no regainers reported ever having had bariatric surgery.

Multivariable Analyses

In models containing all variables that were significant or approached significance in univariate analyses, significant predictors of the odds of maintaining weight versus being a regainer included African-American race, history of diabetes, and current smoking at years 2000 and 2005, as well as increases in moderate physical activity between 2000 and 2005, increases in emotional support during the same time span, and less sugar-sweetened soft drink consumption in 2005 (Table 3). In analyses that included macronutrients (instead of foods) as the dietary block, similar findings were observed for changes in moderate activity (OR=1.3, 95% CI=1.1, 1.6, $p=0.007$) and emotional support (OR=1.6, 95% CI=1.1, 2.3, $p=0.01$); however, intake of macronutrients was not a significant predictor. Analyses excluding the four participants who reported a history of bariatric surgery revealed near identical findings.

Discussion

To our knowledge, the current study was the first to examine the prevalence of weight loss and maintenance in a diverse population-based cohort using prospectively measured weights. The first principal finding was that 29% (536 of 1869) of the overweight and obese population successfully lost a modest amount of weight (≥5%) over a 5-year time span, with only four of these 536 participants having reported bariatric surgery. In a similarly aged population of women, the Nurses Health Study found that fewer (13%) overweight and obese women had successfully lost 5% or more of their body weight over a 2-year period (determined using self-reported weights). Although encouraging that nearly one third of overweight and obese individuals were successful at weight loss, more effective strategies may be needed to increase the proportion of overweight and obese individuals in the population who lose weight.

Although it is commonly believed, based on clinical trial outcomes,[48,49] that very few individuals succeed at long-term weight-loss maintenance, 34% of the overweight individuals who had successfully lost weight in CARDIA were able to keep the weight off over 5 years. Using similar criteria but self-reported weights, the Nurses Health Study[7] found that approximately 20% of those who had lost 5% or more kept it off over 2 years. In NHANES (1999–2002), 58.9% of participants reported keeping 10% or more weight loss off (within 5%) for 1 year, also based on self-reported weight. The prevalence of *both* successful weight loss and maintenance among overweight individuals was 10% in the current study, 15% in the Nurses Health study, and 18% in a random-digit-dial survey.[23] As CARDIA is the only study that used measured weights, its estimates are potentially the most accurate. However, differences in the definitions used may also explain differences in the estimated prevalence. Nonetheless, these data and other national[8,50] and international[51] reports similarly suggest that successful weight-loss maintenance, although infrequent, may be more prevalent in the general population than commonly assumed.

Surprisingly, no significant differences were found in the prevalence of successful weight loss and maintenance across age and gender. In contrast, African Americans had higher odds of long-term weight-loss maintenance than whites. Another population-based study[8] similarly found greater percentages of self-reported successful weight-loss maintenance in African Americans than whites. However, the NHANES study found no significant differences in prevalence between African Americans and whites, and lower prevalence in Mexican Americans.[22] Some clinical weight-loss trials have shown

minorities to be somewhat less successful than nonminorities at weight loss[52,53] but as successful[54] or more successful[55] at weight-loss maintenance. Findings from the current cohort study, which may be more generalizable than clinical trial data, suggest that long-term weight-loss maintenance is similar in men and women and better in African-American than white populations.

Examining predictors and correlates of weight-loss maintenance, physical activity emerged as a significant variable, a finding that is consistent with findings from both clinical trial[56] and epidemiologic[7] studies. Lower sugar-sweetened soft drink consumption also was related to higher odds of successful weight-loss maintenance. Evidence is mixed on the role of sugar-sweetened beverages in the promotion of weight gain and obesity.[57–63] A recent study that compared successful weight losers and normal-weight controls indicated that weight-loss maintainers consumed little in the way of sugar-sweetened beverages.[64] The current study's findings are consistent with these latter data and further suggest that limiting intake of sugar-sweetened beverages is characteristic of long-term successful weight losers.

The study has some limitations. Even though, to be conservative, individuals were excluded who had diseases that could promote unintentional weight loss or inhibit physical activity, intentionality of weight loss was not directly assessed in 2000 and, thus, prevalence estimates of successful weight control could be inflated. Studies that have assessed and excluded unintentional weight losers have reported similar prevalences as those in the current study.[22,65] Nonetheless, the extent to which successful weight losers in the current study represent intentional versus unintentional weight losers remains unclear, so these results should be interpreted with caution. This is an observational study, so causality cannot be inferred. Moreover, although population-based, the current study was conducted with participants who have remained in CARDIA through 20 years of follow-up and who may differ in their motivation and/or weight change patterns than the population at large. Finally, the assessments done in the current study, although comprehensive, were not all administered at every examination and not every factor known or thought to be associated with successful weight control was measured (e.g., dietary restraint, disinhibition, self-efficacy, environmental factors). Also, because the measures were collected 5 years apart, the extent to which weight cycled in the interim years was unknown.

In summary, 29% of overweight and obese men and women successfully lost ≥5% of their body weight over a 5-year time span, and 34% of those who lost weight were able to maintain their weight losses over the next 5 years; African Americans were more likely than whites to be classified as a weight-loss maintainer. Public health interventions to promote weight-loss maintenance may benefit from targeting increased physical activity and emotional support, and decreased soft drink consumption.

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Targeted Disruption of the Idol Gene Alters Cellular Regulation of the Low-Density Lipoprotein Receptor by Sterols and Liver X Receptor Agonists▽§

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Previously, we identified the E3 ubiquitin ligase Idol (inducible degrader of the low-density lipoprotein [LDL] receptor [LDLR]) as a posttranscriptional regulator of the LDLR pathway. Idol stimulates LDLR degradation through ubiquitination of its C-terminal domain, thereby limiting cholesterol uptake. Here we report the generation and characterization of mouse embryonic stem cells homozygous for a null mutation in the Idol gene. Cells lacking Idol exhibit markedly elevated levels of the LDLR protein and increased rates of LDL uptake. Furthermore, despite an intact sterol responsive element-binding protein (SREBP) pathway, Idol-null cells exhibit an altered response to multiple regulators of sterol metabolism, including serum, oxysterols, and synthetic liver X receptor (LXR) agonists. The ability of oxysterols and lipoprotein-containing serum to suppress LDLR protein levels is reduced, and the time course of suppression is delayed, in cells lacking Idol. LXR ligands have no effect on LDLR levels in Idol-null cells, indicating that Idol is required for LXR-dependent inhibition of the LDLR pathway. In line with these results, the half-life of the LDLR protein is prolonged in the absence of Idol. Finally, the ability of statins and PCSK9 to alter LDLR levels is independent of, and additive with, the LXR-Idol pathway. These results demonstrate that the LXR-Idol pathway is an important contributor to feedback inhibition of the LDLR by sterols and a biological determinant of cellular LDL uptake.

Cholesterol plays key roles in biological systems, including developmental signaling, control of membrane fluidity, and formation of caveolae (3). However, free cholesterol can be harmful in excess, and for this reason its levels must be tightly regulated. Whole-body cholesterol homeostasis reflects a balance between endogenous synthesis, dietary uptake, and biliary excretion. Two major transcriptional regulatory pathways have evolved in mammals to coordinate responses to both elevated and reduced cellular cholesterol content: the sterol responsive element-binding proteins (SREBPs) and the liver X receptors (LXRs). These transcription factors regulate gene expression in a tissue-specific fashion to maintain both whole-body and cellular sterol homeostasis.

The response to low intracellular cholesterol content is mediated primarily by the transcription factor SREBP-2. The precursor protein resides in the endoplasmic reticulum (ER) and is transported to the Golgi apparatus under sterol-poor conditions, where it undergoes proteolytic processing. The mature SREBP protein translocates to the nucleus and switches on the transcription of sterol biosynthetic genes, including 3-hydroxy-3-methyl-glutaryl-coenzyme A (CoA) reductase (HMGCoAR) and 3-hydroxy-3-methyl-glutaryl-CoA synthase (HMGCoA synthase). In addition, SREBP promotes the expression of the low-density lipoprotein receptor (LDLR), thereby increasing LDL uptake and cholesterol delivery to cells (7). SREBPs also control the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein involved in the posttranscriptional regulation of the LDLR (15, 24). PCSK9 binds directly to the extracellular domain of the LDLR and alters its stability and trafficking, thereby increasing degradation in lysosomes (5, 15, 28). SREBP-mediated regulation of PCSK9 and its consequent downregulation of the LDLR have been proposed as a mechanism to prevent reuptake of newly secreted very low density lipoprotein (VLDL) particles by hepatocytes, thereby shunting them toward peripheral tissues (8).

On the other hand, the nuclear receptor superfamily members LXRα and LXRβ respond to excess cholesterol. Oxysterols, products of enzymatic or nonenzymatic cholesterol oxidation, are formed when cellular cholesterol rises and serve as ligands for LXRs (10). 24,25-Expoxycholesterol and 22(*R*)-hydroxycholesterol are particularly potent and efficacious LXR ligands (11). Activation of LXR by oxysterols induces the expression of genes involved in cholesterol efflux from cells, including ATP-binding cassette transporter G1 (ABCG1),

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AGING

Growth Hormone Receptor Deficiency Is Associated with a Major Reduction in Pro-Aging Signaling, Cancer, and Diabetes in Humans

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Mutations in growth signaling pathways extend life span, as well as protect against age-dependent DNA damage in yeast and decrease insulin resistance and cancer in mice. To test their effect in humans, we monitored for 22 years Ecuadorian individuals who carry mutations in the growth hormone receptor (*GHR*) gene that lead to severe GHR and IGF-1 (insulin-like growth factor–1) deficiencies. We combined this information with surveys to identify the cause and age of death for individuals in this community who died before this period. The individuals with GHR deficiency exhibited only one nonlethal malignancy and no cases of diabetes, in contrast to a prevalence of 17% for cancer and 5% for diabetes in control subjects. A possible explanation for the very low incidence of cancer was suggested by in vitro studies: Serum from subjects with GHR deficiency reduced DNA breaks but increased apoptosis in human mammary epithelial cells treated with hydrogen peroxide. Serum from GHR-deficient subjects also caused reduced expression of *RAS*, *PKA* (protein kinase A), and *TOR* (target of rapamycin) and up-regulation of *SOD2* (superoxide dismutase 2) in treated cells, changes that promote cellular protection and life-span extension in model organisms. We also observed reduced insulin concentrations (1.4 μU/ml versus 4.4 μU/ml in unaffected relatives) and a very low HOMA-IR (homeostatic model assessment–insulin resistance) index (0.34 versus 0.96 in unaffected relatives) in individuals with GHR deficiency, indicating higher insulin sensitivity, which could explain the absence of diabetes in these subjects. These results provide evidence for a role of evolutionarily conserved pathways in the control of aging and disease burden in humans.

INTRODUCTION

Reduced activity of growth hormone (GH) and insulin-like growth factor–1 (IGF-1) signaling proteins or of their orthologs in nonhuman organisms and the activation of stress resistance transcription factors and antioxidant enzymes contribute to extended life span and protection against age-dependent damage or diseases (*1*–*16*). Pathways that regulate growth and metabolism also promote aging and genomic instability, a correspondence that is conserved in simple eukaryotes and mammals (*7*). In yeast, life span–extending mutations in genes such as *SCH9*, the homolog of mammalian *S6K* (S6 kinase), protect against age-dependent genomic instability (*17*–*19*). Similarly, mutations in the insulin/IGF-1–like signaling pathway increase life span and reduce abnormal cellular proliferation in worms, and mice deficient in GH and IGF-1 are not only long-lived but also show delayed occurrence of age-dependent mutations and neoplastic disease (*20*–*23*). Among the frequently detected mutations in human cancers are those that activate two major signaling proteins downstream of the IGF-1 receptor (IGF-1R)—Ras and Akt—and those in the IGF-1R itself (*24*, *25*). This is in agreement with a potential role for the IGF-1 signaling pathway in promoting age-dependent mutations that lead to the activation of proto-oncogenes and for oncogenes in exacerbating the generation of additional mutations and changes required for cancer progression (*26*). It has been proposed that the growth-promoting and antiapoptotic functions of the IGF-1 pathway underlie its putative role in cancer development and progression (*27*). This link is supported by some population studies but not others, which instead indicate a modest association between high IGF-1 concentrations and increased risk of certain cancers (*27*, *28*).

GH may also promote insulin resistance. For example, age-dependent insulin resistance is reduced in GH- and GH receptor (GHR)–deficient mice (*29*–*32*), and GH replacement therapy can exacerbate insulin resistance in GH-deficient individuals, apparently because it causes a switch from glucose metabolism to lipolysis (*33*).

Here, we have monitored an Ecuadorian cohort with GHR deficiency (GHRD), which results in IGF-1 deficiency, for 22 years and investigated the effect of these deficiencies on cellular responses to stress and on markers of cancer and diabetes. We show that the fundamental link between pro-growth pathways, oxidative stress, age-dependent genomic instability, and cellular damage observed in yeast (*2*, *15*, *17*–*19*), worms, and mice (*5*, *6*, *20*–*23*, *34*) is conserved in humans.

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Cell

Adipocyte NCoR Knockout Decreases PPARγ Phosphorylation and Enhances PPARγ Activity and Insulin Sensitivity

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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.

Islet Transplantation in Type 1 Diabetic Patients Using Calcineurin Inhibitor-Free Immunosuppressive Protocols Based on T-Cell Adhesion or Costimulation Blockade

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Background. The applicability of islet transplantation as treatment for type 1 diabetes is limited by long-term graft dysfunction, immunosuppressive drug toxicity, need for multiple donors, and increased risk of allosensitization. We describe two immunosuppressive regimens based on the costimulation blocker belatacept (BELA) or the antileukocyte functional antigen-1 antibody efalizumab (EFA), which permit long-term islet allograft survival and address some of these concerns.
Methods. Ten patients with type 1 diabetes with hypoglycemic unawareness received intraportal allogeneic islet transplants. Immunosuppression consisted of antithymocyte globulin induction and maintenance with sirolimus or mycophenolate and BELA (n=5) or EFA (n=5).
Results. All five BELA-treated patients achieved independence after single transplants; one resumed partial insulin use 305 days after transplant but is now independent after a second transplant. All five patients treated with EFA achieved independence after one (3/5) or two (2/5) islet transplants and remained independent while on EFA (392–804 days). After EFA was discontinued because of withdrawal of the drug from the market, two patients resumed intermittent insulin use; the others remain independent. No patient in either group developed significant side effects related to the study drugs, and none have been sensitized to alloantigens. All have stable renal function.
Conclusions. These two novel immunosuppressive regimens are effective, well tolerated, and the first calcineurin inhibitor/steroid-sparing islet protocols resulting in long-term insulin independence. Although EFA is no longer available for clinical use, these early results demonstrate that a regimen using BELA may be an effective alternative to improve graft function and longevity while minimizing renal and β-cell toxicity.

Keywords: Islet transplantation, Belatacept, Efalizumab, Type 1 diabetes.

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Pancreatic islet transplantation offers a minimally invasive approach to restore normoglycemia in patients with type 1 diabetes while avoiding the hypoglycemic episodes observed with intensive insulin therapy and the surgical complications associated with pancreas transplantation (*1–6*). Beginning with the development of the Edmonton protocol, significant progress has been made in clinical islet transplantation; however, overall outcomes remain suboptimal in that most patients lose insulin independence several years after transplantation, multiple donors are needed to achieve independence, currently used immunosuppressive drugs are toxic, and patients are at risk for allosensitization (*5*, *7–12*).

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anism may partly compensate for the minor spliceosome deficiency resulting from mutations in *U4atac* snRNA.

This paper shows compelling evidence that mutations in the *U4atac* snRNA gene are responsible for the early postnatal sudden death and severe brain and bone malformations seen in TALS, through minor spliceosome deficiency. Decreased expression of a restricted number of U12 genes and subsequent effects on downstream metabolic pathways may explain the TALS phenotype.

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Supporting Online Material

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Eosinophils Sustain Adipose Alternatively Activated Macrophages Associated with Glucose Homeostasis

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Eosinophils are associated with helminth immunity and allergy, often in conjunction with alternatively activated macrophages (AAMs). Adipose tissue AAMs are necessary to maintain glucose homeostasis and are induced by the cytokine interleukin-4 (IL-4). Here, we show that eosinophils are the major IL-4–expressing cells in white adipose tissues of mice, and, in their absence, AAMs are greatly attenuated. Eosinophils migrate into adipose tissue by an integrin-dependent process and reconstitute AAMs through an IL-4– or IL-13–dependent process. Mice fed a high-fat diet develop increased body fat, impaired glucose tolerance, and insulin resistance in the absence of eosinophils, and helminth-induced adipose tissue eosinophilia enhances glucose tolerance. Our results suggest that eosinophils play an unexpected role in metabolic homeostasis through maintenance of adipose AAMs.

Adipose tissue macrophages have a central role in promoting chronic low-grade inflammation, which contributes to obesity, insulin resistance, and type 2 diabetes, which characterize the metabolic syndrome (*1*). Although adipose macrophages from obese animals have a classically activated inflammatory phenotype, adipose macrophages from healthy lean mice have an alternatively activated phenotype (*2*). Impeding the ability of macrophages to become alternatively activated by disrupting the nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ) renders mice susceptible to diet-induced obesity and glucose intolerance (*3*, *4*). Human PPARγ loss-of-function mutations are also associated with insulin resistance and type 2 diabetes (*5*). PPARγ is induced in macrophages by interleukin-4 (IL-4) or IL-13 and promotes arginase-1 expression, one of the signature genes in alternatively activated macrophages (AAMs) (*6*). In vitro studies with adipocyte cell lines suggest that adipocytes themselves can be sources of IL-4 and IL-13 (*7*), but analysis of adipose tissues in vivo are needed to ascertain more definitively the source of these cytokines.

To begin an unbiased analysis of IL-4–expressing cells in perigonadal white adipose tissue of mice fed a normal commercial diet, we used IL-4 reporter mice (4get mice) (*8*), which contain a green fluorescent protein (GFP) reporter downstream of an internal ribosomal entry site element after the endogenous *Il4* gene, which facilitates recognition of IL-4–competent cells in vivo as revealed by their fluorescence (*9*). To ensure minimal manipulation, we first analyzed cells spontaneously migrating out of minced adipose tissue after overnight incubation in medium. Although only small numbers of IL-4–expressing (GFP+) CD4+ T cells could be recovered, a large population of GFP+ eosinophils, which constitutively express GFP in 4get mice (*9*, *10*), were identified (fig. S1A). We next enzymatically digested perigonadal adipose tissue to prepare a stromal vascular fraction (SVF) and recovered all IL-4–expressing cells for analysis. Of the IL-4–competent cells recovered from perigonadal adipose tissue of mice fed a normal diet, 90% were eosinophils, with the remainder made up of small numbers of basophils, CD4+ T cells, and innate helper type 2 cells (Fig. 1A, eosinophil gating in fig. S1B). Similar to the relatively abundant adipose tissue macrophages, adipose tissue eosinophils were CD11b+ F4/80+ but were distinguished both by GFP expression in 4get mice and by expression of the sialic acid–binding immunoglobulin receptor, Siglec-F (fig. S1, B and C). Analysis of adipose tissues from 4get mice with a *Gata1* promoter mutation that lack eosinophils (ΔdblGATA mice) (*11*) confirmed that the isolated cells were eosinophils (fig. S1D). Using mice with a knock-in human *CD2* replacement gene at the *il4* start site to mark cells that have recently secreted IL-4 protein (*10*), we could show that the majority of IL-4–secreting cells in adipose tissue were eosinophils, although the number of IL-4–secreting cells was only a small proportion of the total GFP+ IL-4–competent cells (fig. S2). Eosinophils up-regulate the inhibitory Siglec-F receptor as they move from blood into tissues (*12*). Compared with blood eosinophils, adipose tissue eosinophils show increased Siglec-F expression, consistent with tissue residence (fig. S1E). Eosinophils account for 4 to 5% of the adipose SVF cells, more abundant than total adipose CD4+ T cells (fig. S1F) or eosinophils in spleen (0.33% ± 0.08%, mean % viable cells ± SEM) or blood (2.4% ± 0.4%, mean ± SEM). Examination of perigonadal adipose tissue by

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Report

Suppression of Ptf1a Activity Induces Acinar-to-Endocrine Conversion

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Summary

Pluripotent embryonic cells become progressively lineage restricted during development in a process that culminates in the differentiation of stable organ-specific cell types that perform specialized functions. Terminally differentiated pancreatic acinar cells do not have the innate capacity to contribute to the endocrine β cell lineage, which is destroyed in individuals with autoimmune diabetes [1]. Some cell types can be reprogrammed using a single factor [2, 3], whereas other cell types require continuous activity of transcriptional regulators to repress alternate cell fates [4–6]. Thus, we hypothesized that a transcriptional network continuously maintains the pancreatic acinar cell fate. We found that postembryonic antagonism of Ptf1a, a master regulator of pancreatic development [7] and acinar cell fate specification [8, 9], induced the expression of endocrine genes including *insulin* in the exocrine compartment. Using a genetic lineage tracing approach, we show that the induced *insulin*+ cells are derived from acinar cells. Cellular reprogramming occurred under homeostatic conditions, suggesting that the pancreatic microenvironment is sufficient to promote endocrine differentiation. Thus, severe experimental manipulations [10, 11] may not be required to potentiate pancreatic transdifferentiation. These data indicate that targeted postembryonic disruption of the acinar cell fate can restore the developmental plasticity that is lost during development.

Results and Discussion

The pancreas is derived from a dorsal and a ventral bud of endodermal tissue, which together generate endocrine and exocrine cell types [12]. In zebrafish, the acinar cells are exclusively derived from the ventral pancreatic bud [13]. To analyze the temporal differentiation of acinar cells, we examined pancreatic markers at multiple time points in *Tg(ptf1a:*eGFP*)* [14] zebrafish. The *Tg(ptf1a:eGFP)* bacterial artificial chromosome reporter marks the ventral pancreatic bud by 32 hours postfertilization (hpf) [8, 15]. At 36 hpf, *Tg(ptf1a:*eGFP*)*-expressing cells formed a homogeneous field and coexpressed the homeodomain transcription factors Prox1 and Nkx6.1 (Figures 1A–1B′; [8]). Coexpression of these pancreatic progenitor markers in the *ptf1a* expression domain suggests that the pancreatic primordium remains multipotent at 36 hpf. By 60 hpf, Nkx6.1 and *Tg(ptf1a:*eGFP*)* were expressed in mutually exclusive domains (Figures 1C and 1C′) and Prox1 was no longer detected in the pancreas (Figures 1D and 1D′). However, *Tg(ptf1a:*eGFP*)*-expressing cells were not fully differentiated by 60 hpf, because they did not coexpress the acinar cell marker Elastase (Figures 1E and 1E′). Zebrafish embryonic development (0–72 hpf) is fueled by the absorption of the maternally deposited yolk. After the embryonic-to-larval transition at 72 hpf, a functional digestive system becomes necessary to process external nutrient sources [16]. Consistent with this requirement, Elastase was cytoplasmically localized (Figures 1F and 1F′) and packaged into secretory granules on the luminal surface of *Tg(ptf1a:*eGFP*)* cells (Figure 1F′ inset) by 84 hpf. Our results are consistent with ultrastructural studies that have shown that acinar cell cytodifferentiation after 72 hpf coincides with the expression of acinar cell markers [17]. Because Ptf1a expression becomes restricted to acinar cells in the developing mouse pancreas [18], we predicted that coexpression of *Tg(ptf1a:*eGFP*)* and Elastase would coincide with cell fate commitment of the pancreatic progenitor pool. Consistent with this prediction, *Tg(ptf1a:*eGFP*)* expression was excluded from the Nkx6.1-positive intrapancreatic ducts (Figures 1G and 1G′) and the Islet-1-positive endocrine compartment (Figures 1H and 1H′) at 84 hpf. We conclude that the multipotent pancreatic primordium gives rise to terminally differentiated exocrine and endocrine cell types by 84 hpf.

During embryogenesis (0–72 hpf), the level of Ptf1a activity influences the fate of pancreatic cells: high levels of Ptf1a activity promote exocrine cell fates, whereas low levels of Ptf1a activity are compatible with endocrine differentiation [8]. Ptf1a activity requires assembly of the tripartite PTF1 complex, which consists of Ptf1a, an E protein, and RBPJ(L) and directly activates exocrine target gene expression [19]. In addition, the PTF1 complex autoregulates the *Ptf1a* promoter, resulting in sustained expression of high levels of Ptf1a in acinar cells [20]. To further address the function of PTF1, we generated an Engrailed-Ptf1a fusion protein, in which we fused the Engrailed transcriptional repressor domain to the amino terminus of the full-length Ptf1a protein. The Engrailed repressor domain recruits chromatin-modifying complexes to repress target gene transcription [21]. To explore the regulation of PTF1 targets, we generated a stable cell line that expresses luciferase under the control of a tandem array of PTF1 binding sites in human embryonic kidney 293 cells, which require exogenous Ptf1a to form a PTF1 complex [22] (Figure 2A). We found that Engrailed-Ptf1a repressed the transcriptional activity of wild-type Ptf1a in a dose-dependent manner (Figure 2B). Our results suggest that overexpression of Engrailed-Ptf1a antagonizes transcription initiation at Ptf1a binding sites and permits only low-level transcription of PTF1 target genes.

To test whether PTF1 activity is required to maintain acinar cell fate in vivo, we employed the *h*eat-inducible *o*verexpression of *t*ransgenes in a *cre*-restricted domain (HOTcre) system [15] to express the Engrailed-Ptf1a fusion protein specifically in acinar tissue (Figure 3A). We placed the Cre recombinase

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Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion

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The mechanism of thyroid hormone (TH) secretion from the thyroid gland into blood is unknown. Humans and mice deficient in monocarboxylate transporter 8 (MCT8) have low serum thyroxine (T_4) levels that cannot be fully explained by increased deiodination. Here, we have shown that Mct8 is localized at the basolateral membrane of thyrocytes and that the serum TH concentration is reduced in *Mct8*-KO mice early after being taken off a treatment that almost completely depleted the thyroid gland of TH. Thyroid glands in *Mct8*-KO mice contained more non-thyroglobulin-associated T_4 and triiodothyronine than did those in wild-type mice, independent of deiodination. In addition, depletion of thyroidal TH content was slower during iodine deficiency. After administration of ^{125}I, the rate of both its secretion from the thyroid gland and its appearance in the serum as trichloroacetic acid–precipitable radioactivity was greatly reduced in *Mct8*-KO mice. Similarly, the secretion of T_4 induced by injection of thyrotropin was reduced in *Mct8*-KO in which endogenous TSH and T_4 were suppressed by administration of triiodothyronine. To our knowledge, this study is the first to demonstrate that Mct8 is involved in the secretion of TH from the thyroid gland and contributes, in part, to the low serum T_4 level observed in MCT8-deficient patients.

Introduction

Over the past few decades, considerable progress has been made in our knowledge of the steps, genes, and mechanisms involved in thyroid hormone (TH) synthesis and its release from the thyroglobulin (Tg) backbone (Figure 1). Iodide, an essential element of the TH molecule, is actively transported by the Na^+/I^- symporter (NIS, encoded by the *SLC5A5* gene) at the basolateral membrane of the thyrocyte (1) and diffuses by an exchanger, known as pendrin (PDS, encoded by the *SLC26A4* gene), to the lumen at the apical membrane (2). At the extracellular apical membrane, thyroperoxidase (TPO) (3), with hydrogen peroxide (H_2O_2) generated by dual oxidase 2 (DUOX2) (4), oxidizes and binds covalently iodine to tyrosyl residues, producing monoiodotyrosine (MIT) and diiodotyrosine (DIT) within the Tg macromolecule. The same enzyme catalyzes the coupling of two iodotyrosine residues to produce the prohormone thyroxine (T_4) and smaller amounts of the active hormone triiodothyronine (T_3). After endocytosis, iodinated Tg is hydrolyzed in the lysosomes by cathepsins (5) and TH is released from the Tg backbone. The released MIT and DIT are deiodinated by a specific iodotyrosine deiodinase (IYD, or DEHAL1) (6), and the released iodine is recycled within the cell. However, the mechanism involved in the last step in the process, namely TH secretion, remains unknown.

The close correlation between the free TH concentration in serum and the level of its intracellular action has perpetuated the notion of passive hormone diffusion through the lipid bilayer (7). Over the years, potential membrane transporters have been identified (8, 9), among which is monocarboxylate transporter 8 (MCT8). Rat Mct8 was shown to function as a specific TH transmembrane transporter (10). Uptake of labeled T_4 and T_3 by Mct8 was potently inhibited by unlabeled T_4 and T_3, by the T_3 analogs 3,3′,5-triiodothyroacetic acid and *N*-bromoacetyl-3,3′,5-T_3, and by the organic anion bromosulfophthalein, but not by aromatic amino acids. The mechanisms by which rat and human MCT8 facilitate TH uptake are still unknown. It has been demonstrated that this transport is Na independent (10), while its dependence on pH needs to be investigated (11). The dogma of passive TH entry into cells was only recently abandoned with the demonstration that humans harboring *MCT8* gene mutations presented with debilitating psychomotor abnormality suggestive of TH deficiency in brain (12, 13). In addition, they manifested a characteristic though unusual combination of TH abnormalities consisting of high T_3 and low T_4 and reverse T_3 (rT_3), associated with normal or slightly elevated serum thyrotropin (TSH) levels (12, 13). The TH abnormalities have been faithfully reproduced in *Mct8*-KO mice (14, 15), which have provided much-needed insight into the mechanism responsible for the thyroid phenotype. They continue to be a useful tool in understanding the pathophysiology of Mct8 defects and in testing TH analogs as putative treatment agents (16–18). Initial studies on these mice demonstrated overall increased 5′ deiodination (14, 15); however, the postulated consumptive effect on T_4 through increased 5′ deiodination cannot fully explain the low serum T_4 levels observed in MCT8 deficiency.

Our preliminary finding that in *Mct8*-KO mice, serum TH concentrations are reduced early following the release of endogenous hormone suppression with methimazole and perchlorate, raised the question of a possible defect in thyroidal TH secretion in Mct8 deficiency. Moreover, the fact that Mct8 is localized at the basolateral membrane of thyrocytes suggests that Mct8 might play a role in TH secretion. The possible role of MCT8 in TH export was previously shown by in vitro transfection studies of human MCT8 in mammalian cell lines (19).

To further investigate the kinetics of TH secretion, we measured, in *Mct8*-KO and WT mice, the release of labeled iodothyronines from the thyroid gland following the administration of radioio-

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Formation of Pancreatic Islets Involves Coordinated Expansion of Small Islets and Fission of Large Interconnected Islet-like Structures

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ABSTRACT The islets of Langerhans, micro-organs for maintaining glucose homeostasis, range in size from small clusters of <10 cells to large islets consisting of several thousand endocrine cells. Islet size distributions among various species are similar and independent of body size, suggesting an intrinsic limit to islet size. Little is known about the mechanisms regulating islet size. We have carried out a comprehensive analysis of changes of islet size distribution in the intact mouse pancreas from birth to eight months, including mathematical modeling to quantify this dynamic biological process. Islet growth was size-dependent during development, with preferential expansion of smaller islets and fission of large interconnected islet-like structures occurring most actively at approximately three weeks of age at the time of weaning. The process of islet formation was complete by four weeks with little or no new islet formation thereafter, and all the β-cells had low proliferation potential in the adult, regardless of islet size. Similarly, in insulinoma-bearing mice, the early postnatal developmental process including fission followed the same time course with no new islet formation in adults. However, tumor progression led to uncontrolled islet growth with accelerated expansion of larger islets. Thus, islet formation and growth is a tightly regulated process involving preferential expansion of small islets and fission of large interconnected islet-like structures.

INTRODUCTION

Pancreatic islets are highly vascularized endocrine micro-organs composed of several hormone-secreting endocrine cells such as α (glucagon), β (insulin), and δ (somatostatin), PP (pancreatic polypeptide), and ε (ghrelin) cells that are embedded in the exocrine tissue and comprise 1–2% of the entire pancreas volume. Islet size varies from small cell clusters to large islets. In large animals, including humans, a proportionate increase in the pancreas size, islet number, and total islet mass compensates for an increased demand for insulin (1,2). However, as we have recently shown, there is no increase in the range of islet sizes in humans compared to those in mice and various other species (3,4). Indeed, upper and lower critical sizes for islet function have been reported: smaller islets (<1000 cells) can secrete more insulin per unit islet volume and have higher viability for islet transplantation in rats (5) and in humans (6), while some minimal clustering of cells is necessary for efficient insulin secretion (7–9). Collectively, these observations suggest that there are regulatory mechanisms that maintain optimal islet sizes in order to ensure their functional properties.

We reasoned that the developmental process of islet formation and subsequent islet growth holds key clues to understanding the mechanisms regulating functional islet size. To capture this dynamic process, we carried out a computer-assisted large-scale analysis of the entire distribution of islets in the intact mouse pancreas (10–12) and used this precise data for mathematical modeling of islet development. Our method identifies every islet within the intact pancreas with a specific identification number and provides multiple parameters such as islet area, circularity, and center coordinates: Circularity characterizes the shape of each islet, and center coordinates depict the spatial location of each islet in the whole pancreas. We utilized these parameters in the mathematical analysis of the fission of elongated interconnected islet-like structures and the subsequent spherical growth of islets during maturation. Here we develop an explicit mathematical model of the dynamic process of islet development based on the quantitative deduction of the recruitment rate of new islets and the size-dependent islet growth and fission rates. Acting in concert, these processes provide a tight regulatory mechanism for the islet size distribution.

MATERIALS AND METHODS

Mice

Mouse pancreata were excised from male/female (CD-1 background) transgenic mice in which pancreatic β-cells were endogenously tagged with green fluorescent protein (GFP) under the control of mouse insulin I promoter (MIP) (13,14). For generating insulinoma mice, MIP-GFP mice were further crossed with rat insulin II promoter (RIP)-Tag mice (15). The MIP-GFP and insulinoma mice are depicted as wild-type (WT) and RIP-Tag mice, respectively. All the procedures involving mice were approved by The University of Chicago Institutional Animal Care and Use Committee.

Preparation of specimens

Pancreata were removed intact with surrounding tissues such as spleen and duodenum, placed onto a standard glass slide flat to retain the orientation

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Conditional Gene Targeting in Mouse Pancreatic β-Cells
Analysis of Ectopic Cre Transgene Expression in the Brain

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OBJECTIVE—Conditional gene targeting has been extensively used for in vivo analysis of gene function in β-cell biology. The objective of this study was to examine whether mouse transgenic Cre lines, used to mediate β-cell– or pancreas-specific recombination, also drive Cre expression in the brain.

RESEARCH DESIGN AND METHODS—Transgenic Cre lines driven by *Ins1*, *Ins2*, and *Pdx1* promoters were bred to *R26R* reporter strains. Cre activity was assessed by β-galactosidase or yellow fluorescent protein expression in the pancreas and the brain. Endogenous *Pdx1* gene expression was monitored using $Pdx1^{tm1Cvw}$ lacZ knock-in mice. Cre expression in β-cells and co-localization of Cre activity with orexin-expressing and leptin-responsive neurons within the brain was assessed by immunohistochemistry.

RESULTS—All transgenic Cre lines examined that used the *Ins2* promoter to drive Cre expression showed widespread Cre activity in the brain, whereas Cre lines that used *Pdx1* promoter fragments showed more restricted Cre activity primarily within the hypothalamus. Immunohistochemical analysis of the hypothalamus from $Tg(Pdx1\text{-}cre)^{89.1Dam}$ mice revealed Cre activity in neurons expressing orexin and in neurons activated by leptin. $Tg(Ins1\text{-}Cre/ERT)^{1Lphi}$ mice were the only line that lacked Cre activity in the brain.

CONCLUSIONS—Cre-mediated gene manipulation using transgenic lines that express Cre under the control of the *Ins2* and *Pdx1* promoters are likely to alter gene expression in nutrient-sensing neurons. Therefore, data arising from the use of these transgenic Cre lines must be interpreted carefully to assess whether the resultant phenotype is solely attributable to alterations in the islet β-cells. ***Diabetes*** **59:3090–3098, 2010**

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See accompanying commentary, p. 2991.

In vivo analysis of gene function in the pancreas and β-cells has benefited from the development of mouse lines expressing Cre in all pancreatic compartments or restricted to the islet β-cells. The choice of promoter to drive recombinase expression is critical for controlling the location and timing of gene activity. In addition, inducible versions of Cre recombinase, e.g., CreER, allow temporal control to the manipulation of gene activity, which becomes important when analyzing gene function at specific embryonic and adult stages (1,2). Promoters of the *pancreas duodenal homeobox 1* (*Pdx1*) (3,4) and *insulin* (*Ins1* and *Ins2*) (5–8) genes have been well characterized to allow the use of regulatory sequences for directing Cre expression to specific pancreatic cell populations. Commonly used transgenic mouse lines that employ rat *Ins2* gene promoter sequences to drive Cre expression within the β-cell population include *Ins2-Cre/RIP-Cre* [Mouse Genome Informatics (MGI): $Tg(Ins2\text{-}cre)^{25Mgn}$ and $Tg(Ins2\text{-}cre)^{1Herr}$] (9–11) and *RIP-CreER* [MGI: $Tg(Ins2\text{-}cre/Esr1)^{1Dam}$] (12). *Pdx1* gene promoter sequences have proven useful for directing Cre expression throughout the early pancreatic epithelium (4,10,13,14) and to the endocrine cells of the pancreas (15). The *Pdx1* gene is expressed early in pancreas development throughout the endoderm of the dorsal and ventral buds, but expression becomes restricted during development such that high levels of *Pdx1* are maintained in the insulin-producing β-cells with lower levels in subpopulations of acinar cells (8,16). Examples of *Pdx1-Cre* transgenic lines include $Pdx1\text{-}Cre^{early}$ [MGI: $Tg(Pdx1\text{-}cre)^{89.1Dam}$] (13), $Pdx1\text{-}Cre^{late}$ [MGI: $Tg(Ipf1\text{-}cre/Esr1)^{1Dam/Mmcd}$] (10), *Pdx1-Cre* [MGI: $Tg(Ipf1\text{-}cre)^{1Tuv}$] (14), and *Pdx1-CreER* [MGI: $Tg(Pdx1\text{-}cre/ERT)^{1Mga}$] (15).

To assess the specificity of recombination and perform lineage tracing analysis, reporter lines such as the *ROSA26-stop-lacZ* [MGI: $Gt(ROSA)26Sor^{tm1Sho}$], also known as *R26R* (17), or the *ROSA26-stop-YFP* [MGI: $Gt(ROSA)26Sor^{tm1(EYFP)Cos}$] (18) mice have been developed. Upon Cre-mediated recombination, these reporter lines activate expression of a β-galactosidase (β-gal) or a yellow fluorescent protein (YFP) reporter under the control of the ubiquitously active *ROSA26* promoter, resulting

Cross-Sectional Associations Between Measures of Sleep and Markers of Glucose Metabolism Among Subjects With and Without Diabetes

The Coronary Artery Risk Development in Young Adults (CARDIA) Sleep Study

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OBJECTIVE—To examine whether sleep duration and quality are associated with fasting glucose, fasting insulin, or estimated insulin resistance in a community-based sample of early middle-aged adults.

RESEARCH DESIGN AND METHODS—This was an ancillary study to the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Habitual sleep duration and fragmentation were estimated from 6 days of wrist actigraphy collected in 2003–2005. Insomnia was defined as self-reported difficulty falling asleep or waking up in the night three or more times per week plus average sleep efficiency of <80% based on actigraphy. Fasting blood samples to measure glucose and insulin were collected after the sleep measures during the CARDIA clinical examination in 2005–2006. Insulin resistance was estimated using the homeostatic model assessment (HOMA) method. Analyses were cross-sectional and stratified by the presence of diabetes.

RESULTS—There was no association between sleep measures and fasting glucose, insulin, or HOMA in the 115 subjects without diabetes. Among the 40 subjects with diabetes, after adjustment for covariates, 10% higher sleep fragmentation was associated with a 9% higher fasting glucose level, a 30% higher fasting insulin level, and a 43% higher HOMA level. Insomnia was associated with a 23% higher fasting glucose level, a 48% higher fasting insulin level, and an 82% higher HOMA level.

CONCLUSIONS—The observed association between poor sleep quality and higher glucose, insulin, and estimated insulin resistance among subjects with diabetes warrants further examination of the effect of sleep disturbances on glucose control in type 2 diabetes.

Diabetes Care 34:1171–1176, 2011

The prevalence of type 2 diabetes nearly doubled in the U.S. between 1980 and 2006, and rates have been increasing rapidly throughout the world (1). Diabetes can be a debilitating disease associated with reduced quality-of-life, severe complications, shorter life expectancy, and increased economic burden (2,3). Much effort has been devoted to identifying factors associated with the increased risk of developing type 2 diabetes and improved prognosis of people with type 2 diabetes to improve the lives of millions of Americans. Disturbed sleep has recently been proposed as a novel risk factor.

Laboratory studies that manipulated bedtimes observed impaired glucose metabolism after sleep restriction compared with sleep extension (4–6). These laboratory studies lasted only 1 to 2 weeks and the results may not reflect the effects of habitual short sleep. Observational studies have examined the association between self-reported habitual sleep and diabetes risk. Many have found cross-sectional associations that indicated a higher prevalence of diabetes among short sleepers (7–9) and among those with poor subjective sleep quality (10,11). Several prospective studies found higher rates of incident diabetes associated with shorter sleep durations. For example, a meta-analysis reported a pooled risk ratio of 1.28 (95% CI 1.03–1.60) associated with sleep duration ≤6 h compared with 7–8 h per night (12). These observational studies all relied on self-reported sleep, which may not be very accurate (13). A small Italian study did use wrist actigraphy to compare the sleep of patients with type 2 diabetes with healthy control subjects and found higher sleep fragmentation in the patients with diabetes (14). Together, these studies suggest that glucose metabolism may be adversely affected by short sleep duration and poor sleep quality.

The goal of the current study was to examine whether sleep duration or quality measured using wrist actigraphy was associated with levels of fasting glucose, fasting insulin, or estimated insulin resistance in a community-based sample of early middle-aged adults.

RESEARCH DESIGN AND METHODS

—This study is ancillary to a large, ongoing cohort study, the Coronary Artery Risk Development in Young Adults (CARDIA) Study, which began in 1985–1986. CARDIA recruited black and white adults aged 18–30 years from four sites in the U.S., including Chicago, which is the site involved in our

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Total chemical synthesis of human proinsulin†‡

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A convergent synthetic strategy based on modern chemical ligation methods was used to make human proinsulin. The synthetic protein was characterized by LCMS, CD spectroscopy, and by 1D- and 2D-NMR spectroscopy. Synthetic human proinsulin had full biochemical activity in a receptor-binding assay.

The human proinsulin molecule is the key to efficient biosynthesis of human insulin. Proinsulin is biosynthesized as an 86 amino acid residue polypeptide chain, and after folding and formation of three native disulfide bonds is processed by prohormone convertases in the pancreatic β-cell to form insulin. Proinsulin is composed of the B and A peptides of insulin linked together by the 35-amino acid C domain.[1,2] Recent solution NMR structural studies of a monomeric proinsulin indicate that the C domain is largely unstructured.[3] The primary role of the C domain of intact proinsulin is to favor the formation of the correct disulfides, as found in mature insulin.[4] Several insulin gene mutations are thought to cause diabetes by impairment of proinsulin folding leading to unremitting endoreticular stress and beta-cell apoptosis.

The NMR structure of a monomeric analogue of human proinsulin was recently reported.[3] Although bovine proinsulin has been crystallized[5] and proinsulin was co-crystallized with insulin,[6,7] no X-ray structure of human proinsulin has been obtained. We anticipate that the use of a racemic protein mixture made up of L-proinsulin and D-proinsulin could lead to the formation of highly ordered centrosymmetric crystals, which can be used for X-ray crystal structure determination.[8] The mirror image protein D-proinsulin can only be prepared by total chemical synthesis. Here, we report the efficient total chemical synthesis of human proinsulin using modern chemical ligation methods.[9]

The polypeptide chain of proinsulin has 86 amino acids containing six cysteine residues (Fig. 1a), and the folded protein contains three intramolecular disulfide bonds. The presence of six Cys residues makes proinsulin a good target for total chemical synthesis by native chemical ligation, which involves the thioester-mediated amide-forming covalent condensation of unprotected synthetic peptides.[10,11] Our synthetic strategy is shown in Fig. 1b. The two peptide-thioesters, L-proinsulin(Thz[19]-Cys[71])-$^{\alpha}$COSR (R = –CH_2CH_2CO–Ala–COOH) and L-proinsulin(Phe[1]-Val[18])-$^{\alpha}$COSR, and the Cys-peptide L-proinsulin(Cys[72]-Asn[86]) building blocks were prepared by manual stepwise Boc-chemistry "*in situ* neutralization" solid phase peptide synthesis[12] (see ESI‡). The synthesis of the full length 86-amino acid polypeptide chain of the target L-proinsulin was achieved using two native chemical ligations, starting from the C-terminal segment.[13] Data for the ligations are shown in Fig. 2.

(a) FVNQHLCGSH[10] LVEALYLVCG[20] ERGFFYTPKT[30]
RREAEDLQVG[40] QVELGGGPGA[50] GSLQPLALEG[60]
SLQKRGIVEQ[70] CCTSICSLYQ[80] LENYCN[86]

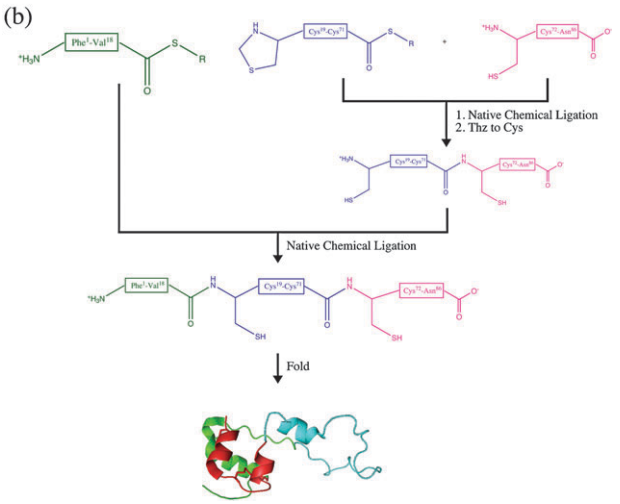

Fig. 1 (a) Amino acid sequence of human proinsulin. Color coding corresponds to the peptide segments used in synthesis. (b) Synthetic strategy used for the total chemical synthesis of human proinsulin by native chemical ligation. R = –CH_2CH_2CO–Ala–COOH.

L-proinsulin(Thz[19]-Cys[71])-$^{\alpha}$COSR (25 mg, 4.3 μmol) and L-proinsulin(Cys[72]-Asn[86]) (8.3 mg, 4.7 μmol) were dissolved to a concentration of 4.3 mM and 4.7 mM, respectively, in 6 M guanidine·HCl, buffered with 0.2 M sodium phosphate, pH 6.9, and containing 100 mM (4-carboxymethyl)thiophenol (MPAA) and 20 mM TCEP·HCl. The reaction mixture was purged with helium for 5 min and sealed. Upon complete reaction (~4.5 h), the N-terminal Thz- of the product was converted to Cys- by addition of 0.4 M methoxylamine·HCl and overnight reaction at pH 4.0. The crude product L-proinsulin(Cys[19]-Asn[86]) was separated from salts and low MW contaminants on a solid phase extraction C18 cartridge and lyophilized. The second ligation between L-proinsulin(Phe[1]-Val[18])-$^{\alpha}$COSR (5.1 mg, 2.3 μmol) and L-proinsulin(Cys[19]-Asn[86]) (17 mg, 2.3 μmol) was

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‡ Electronic supplementary information (ESI) available: Peptide synthesis; HPLC and LC-MS analysis; chemical synthesis of D-proinsulin; CD spectra of D- and L-proinsulin; 1D- and 2D-NMR spectra of D- and L-proinsulin. See DOI: 10.1039/c0cc03141k
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ARTICLE

Genome-wide association and meta-analysis in populations from Starr County, Texas, and Mexico City identify type 2 diabetes susceptibility loci and enrichment for expression quantitative trait loci in top signals

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Abstract
Aims/hypothesis We conducted genome-wide association studies (GWASs) and expression quantitative trait loci (eQTL) analyses to identify and characterise risk loci for type 2 diabetes in Mexican-Americans from Starr County, TX, USA.
Method Using 1.8 million directly interrogated and imputed genotypes in 837 unrelated type 2 diabetes cases and 436 normoglycaemic controls, we conducted Armitage trend tests. To improve power in this population with high disease rates, we also performed ordinal regression including an intermediate class with impaired fasting glucose and/or glucose tolerance. These analyses were followed by meta-analysis with a study of 967 type 2 diabetes cases and 343 normoglycaemic controls from Mexico City, Mexico.
Result The top signals (unadjusted p value $<1\times10^{-5}$) included 49 single nucleotide polymorphisms (SNPs) in eight gene regions (*PER3*, *PARD3B*, *EPHA4*, *TOMM7*, *PTPRD*, *HNT* [also known as *RREB1*], *LOC729993* and

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Reach-Out: A Family-Based Diabetes Prevention Program for African American Youth

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Previous Presentation: Results of this pilot study were presented in part at the North American Society for the Study of Obesity Annual Meeting, Fort Lauderdale, Florida, October 3, 2003 (*Obes Res.* 2003;11[suppl]:A141) and the Society for Pediatric Research Annual Meeting, San Francisco, May 2004 (*Ped Res*. 2004; suppl).

Objective: To pilot test and assess the feasibility of a culturally grounded approach to adolescent overweight and diabetes prevention.

Study Design: Reach-Out, a family-based nutrition and exercise program for overweight African American youth aged 9 to 12 years and their families, is led by lay health leaders and conducted in a community setting on Chicago's south side (Illinois). Age-appropriate interactive sessions focus on skills building, problem solving, and setting goals during 14 weekly sessions, with monthly meetings thereafter. Pre-post comparisons were made for 29 families (62 subjects) using physical (body mass index [BMI], blood pressure, waist circumference), biochemical (glucose, insulin, lipid levels) and behavioral data. Statistical analyses included mixed-effects linear models and logistic regression.

Results: Children's mean BMI z score fell from 2.46 at baseline to 2.38 at 14 weeks and 2.39 at 1 year (p = .02), while parents' BMI remained stable. Children reported increased walking (p = 0.07) and exhibited a corresponding rise in mean serum high-density lipoprotein cholesterol from 49.4 to 54.2 (p < .001). Qualitative assessment showed that participants enjoyed the program but felt the program could be improved by making the sessions even more interactive.

Conclusion: A community-based program for overweight minority youth and families can successfully address overweight, with the potential to decrease diabetes risk in youth.

Keywords: nutrition ■ exercise ■ intervention ■ obesity

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INTRODUCTION

Adolescent overweight prevalence is rising rapidly, with a parallel rise in type 2 diabetes.[1,2] African American and Latino youth are at highest risk.[3] Minorities experience high diabetes complication rates[4] and represent a growing proportion of the US population. Effective, culturally grounded approaches to adolescent diabetes prevention are urgently needed; however, translational research for minority youth presents significant challenges.[5]

We know from the Diabetes Prevention Program[6] that type 2 diabetes can be effectively prevented or delayed through behavioral lifestyle intervention. Risk factors for obesity cluster in families; parental obesity is the strongest predictor for childhood obesity that persists to adulthood.[7] Children's eating and physical activities are greatly influenced by parents, and family-based interventions have proven to work best for childhood obesity.[8] Lay health leaders have been used effectively in a variety of chronic disease prevention and management programs for adults in church[9-12] and community settings[13] in order to enhance cultural effectiveness. Lay-led diabetes prevention programs for African American families have not been tested.

The Reach-Out program represents a novel approach to diabetes prevention in African American youth. Using techniques of community-based participatory research,[14] we developed a family-based model implemented by lay health leaders in a community setting on Chicago's south side (Illinois). To assess feasibility, we conducted a pilot study of this behavioral intervention, with longitudinal data collection of physical, biochemical, and behavioral markers for both children and parents over one year. Here, we report on the conceptual development, implementation and pilot results of the Reach-Out Chicago Children's Diabetes Prevention Project,

Glycemic Control, Complications, and Death in Older Diabetic Patients

The Diabetes and Aging Study

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OBJECTIVE—To identify the range of glycemic levels associated with the lowest rates of complications and mortality in older diabetic patients.

RESEARCH DESIGN AND METHODS—We conducted a retrospective cohort study (2004–2008) of 71,092 patients with type 2 diabetes, aged ≥60 years, enrolled in Kaiser Permanente Northern California. We specified Cox proportional hazards models to evaluate the relationships between baseline glycated hemoglobin (A1C) and subsequent outcomes (nonfatal complications [acute metabolic, microvascular, and cardiovascular events] and mortality).

RESULTS—The cohort (aged 71.0 ± 7.4 years [means ± SD]) had a mean A1C of 7.0 ± 1.2%. The risk of any nonfatal complication rose monotonically for levels of A1C >6.0% (e.g., adjusted hazard ratio 1.09 [95% CI 1.02–1.16] for A1C 6.0–6.9% and 1.86 [1.63–2.13] for A1C ≥11.0%). Mortality had a U-shaped relationship with A1C. Compared with the risk with A1C <6.0%, mortality risk was lower for A1C levels between 6.0 and 9.0% (e.g., 0.83 [0.76–0.90] for A1C 7.0–7.9%) and higher at A1C ≥11.0% (1.31 [1.09–1.57]). Risk of any end point (complication or death) became significantly higher at A1C ≥8.0%. Patterns generally were consistent across age-groups (60–69, 70–79, and ≥80 years).

CONCLUSIONS—Observed relationships between A1C and combined end points support setting a target of A1C <8.0% for older patients, with the caution that A1Cs <6.0% were associated with increased mortality risk. Additional research is needed to evaluate the low A1C–mortality relationship, as well as protocols for individualizing diabetes care.

Diabetes Care 34:1329–1336, 2011

People aged >60 years comprise >40% of the type 2 diabetic population in the U.S., yet identifying the optimal glucose control level for older patients with diabetes remains a significant challenge. The widely accepted recommendation that all patients pursue a glycated hemoglobin (A1C) <7.0% is based largely on the results of the UK Prospective Diabetes Study (1), which actively excluded people aged >65 years (2,3).

More recent trials have generated controversy regarding the effects of pursuing very low glucose levels (A1C <6.5%) in older diabetic patients. In 2008, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial (4), the Action in Diabetes and Vascular Disease Trial (5), and the Veterans Affairs Diabetes Trial (6) provided some of the first data describing the impact of pursuing very intensive glucose lowering in the elderly. Two of the trials (5,6) found that intensive glucose lowering prevented the progression of nephropathy; however, none of the trials demonstrated a clear cardiovascular benefit. Further complicating this picture, the ACCORD trial found a higher rate of mortality in the intensive glucose-lowering arm (4).

As with clinical trials, observational studies in diabetes also have provided conflicting insights into the potential impacts of different levels of glycemic control. Numerous epidemiological studies have found a continuous relationship between A1C levels and microvascular and cardiovascular complications with no clear threshold (7). However, a recent observational study (8) of the relationship between A1C levels and mortality has reported an elevated risk of mortality at both the lower and upper ends of long-term glucose levels.

Although the pursuit of very low glucose levels may not always be appropriate, failing to address very high glucose levels may significantly increase the risk of acute metabolic events, chronic complications, and mortality. Medical organizations have confused matters by recommending different glycemic targets. Recommended glycemic targets range from an A1C <6.5% from the American Association of Clinical Endocrinologists (9), to an A1C <7.0% from the American Diabetes Association, to an A1C <8.0% from geriatric diabetes care guidelines for older patients with limited life expectancy (10). Unfortunately, there has been limited evidence for any of these targets of glycemic control for older patients, especially for the oldest older patients. We sought to identify the range of glycemic levels associated with the lowest rates of complications and mortality in a large, contemporary, multiethnic cohort of older diabetic patients.

RESEARCH DESIGN AND METHODS

—The Kaiser Permanente Northern California Diabetes Registry (also referred to here as the Registry) is a well-characterized population, maintained continuously since 1993, that has been the basis for extensive epidemiological research (11–13). Registry eligibility is based on multiple sources of data, including pharmacy records, laboratory

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Self-reported Racial Discrimination in Health Care and Diabetes Outcomes

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Background: Self-reported racial discrimination in healthcare has been associated with negative health outcomes, but little is known about its association with diabetes outcomes.

Methods: We used data from the Behavioral Risk Factor Surveillance System to investigate associations between self-reported healthcare discrimination and the following diabetes outcomes: (1) quality of care, (2) self-management and (3) complications.

Results: In unadjusted logistic regression models, significant associations were found between self-reported healthcare discrimination and most measures of quality of care [diabetes-related primary care visits odds ratio (OR), 0.38; 95% confidence interval (CI), 0.21-0.66), HbA1c testing (OR, 0.42; 95%CI, 0.21-0.82), and earlier eye examination interval (OR, 0.48; 95% CI, 0.24-0.93)] and health outcomes [foot disorders (OR, 2.32, 95%CI: 1.15, 4.68) and retinopathy (OR, 2.26; 95%CI, 1.24-4.12)], but not the number of provider foot examinations ($P = 0.48$) or diabetes self-management (self glucose monitoring, $P = 0.42$; self foot examinations, $P = 0.74$; diabetes class participation, $P = 0.37$). The effects of self-reported discrimination were attenuated or eliminated after controlling for sociodemographics, health status, and access to care.

Conclusions: Self-reported racial/ethnic discrimination in healthcare was associated with worse diabetes care and more diabetes complications, but not self-care behaviors, suggesting that factors beyond patients' own behaviors may be the main source of differential outcomes. The relationships between self-reported discrimination and diabetes outcomes were eliminated once adjusting for sociodemographics, health status, and access to care. Our findings suggest that other factors (ie, race, insurance, health status) may play equally or more important roles in determining diabetes health disparities, and that a comprehensive strategy is needed to effectively address health disparities.

Key Words: discrimination, healthcare delivery, diabetes, health disparities, race/ethnicity

(*Med Care* 2011;49: 618–625)

Understanding and addressing racial/ethnic health disparities is a national priority.[1–3] There is overwhelming evidence that racial/ethnic minorities are more likely to receive lower quality healthcare than Whites in a range of important clinical conditions that are not due to differences in clinical status or insurance, and that these differences translate into higher morbidity and mortality for racial/ethnic minorities.[3–8] Although recent data suggest that they are improving,[1] diabetes disparities also exist. For example, African-Americans have been less likely than whites to have eye examinations, HbA1c and low-density lipoprotein cholesterol monitoring, and influenza vaccinations.[9–12] The reasons for racial disparities in medical care are multifactorial, and healthcare discrimination may play a role. In a landmark study using standardized patients, Schulman et al[13] showed that patient race influenced physicians' recommendations for cardiac care, independent of clinical factors, diagnostic tests, and the physicians' assessed probability of coronary artery disease. Green et al[14] documented the association between implicit physician bias and disparities in treatment recommendations for myocardial infarctions.

In addition, self-reported racial/ethnic discrimination in healthcare is associated with important outcomes, such as less preventive healthcare,[15–17] prescription medication utilization and medical testing/treatment,[18] and patient satisfaction.[19] Few studies have investigated the impact of self-reported discrimination on patients with diabetes, a subpopulation potentially at increased risk for adverse outcomes from perceived discrimination because of frequent healthcare encounters.[17,20–22] Existing studies have used relatively small and/or geographically limited samples. Using data from the California Health Information Study, Trivedi and Ayanian[17] found that patients with diabetes reporting healthcare discrimination were less likely to

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The Cost-Effectiveness of Personalized Genetic Medicine

The case of genetic testing in neonatal diabetes

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OBJECTIVE—Neonatal diabetes mellitus is a rare form of diabetes diagnosed in infancy. Nearly half of patients with permanent neonatal diabetes have mutations in the genes for the ATP-sensitive potassium channel (*KCNJ11* and *ABCC8*) that allow switching from insulin to sulfonylurea therapy. Although treatment conversion has dramatic benefits, the cost-effectiveness of routine genetic testing is unknown.

RESEARCH DESIGN AND METHODS—We conducted a societal cost-utility analysis comparing a policy of routine genetic testing to no testing among children with permanent neonatal diabetes. We used a simulation model of type 1 diabetic complications, with the outcome of interest being the incremental cost-effectiveness ratio (ICER, $/quality-adjusted life-year [QALY] gained) over 30 years of follow-up.

RESULTS—In the base case, the testing policy dominated the no-testing policy. The testing policy was projected to bring about quality-of-life benefits that enlarged over time (0.32 QALYs at 10 years, 0.70 at 30 years) and produced savings in total costs that were present as early as 10 years ($12,528 at 10 years, $30,437 at 30 years). Sensitivity analyses indicated that the testing policy would remain cost-saving as long as the prevalence of the genetic defects remained >3% and would retain an ICER <$200,000/QALY at prevalences between 0.7 and 3%.

CONCLUSIONS—Genetic testing in neonatal diabetes improves quality of life and lowers costs. This paradigmatic case study highlights the potential economic impact of applying the concepts of personalized genetic medicine to other disorders in the future.

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Neonatal diabetes mellitus is a rare form of diabetes that is likely to have a monogenic cause, particularly when diagnosed before 6 months of age (1). Recent estimates from multiple national registries show the incidence is close to 1 in 100,000 live births (2). In 50% of cases, the condition spontaneously remits within a few months of age and is termed transient neonatal diabetes, whereas the remaining infants have permanent neonatal diabetes.

Of probands with permanent neonatal diabetes, 42% have activating heterozygous mutations in either of the two protein subunits, *KCNJ11* and *ABCC8*, of the ATP-sensitive potassium (KATP) channel, whereas 12% have mutations in the gene encoding insulin (*INS*) (3–6). In KATP-related neonatal diabetes, sulfonylurea binding allows KATP channel closure in patients whose channels would otherwise remain open and prevent insulin secretion from occurring. Reports indicate that treatment with oral sulfonylurea therapy in place of insulin has been successful in most of these patients, leading to immediate dramatic improvements in glucose control and quality of life (7,8).

These recent discoveries have raised questions regarding the extent to which routine testing for these mutations should be performed in any insulin-treated individual with presumed type 1 diabetes. Age at diagnosis of diabetes is a key determinant of a possible monogenic cause, where those diagnosed when aged younger than 6 months have a high likelihood of having a causal genetic variant. However, 1–2% of those diagnosed between age 6 and 12 months may also be monogenic (6,8,9). Some experts have even suggested that all newborns should perhaps undergo screening (10), given that routine newborn screening in all states includes such disorders as maple syrup urine disease, which has an incidence of 1 in 185,000 births.

In any consideration of genetic testing, the prevalence of genetic markers that could inform disease prediction, treatment, or outcomes must be weighed against the cost of testing. The promise of personalized genetic medicine is that highly individualized treatments may not only improve the health of patients but may also lower costs in some cases. There have been no rigorous cost-effectiveness analyses of genetic testing policies that lead to transformative changes in the selection of treatments (compare to studies of warfarin pharmacogenetics [11]). To test the hypothesis that genetic testing in some disorders can lead to dramatic health and cost benefits, we conducted a societal cost-utility analysis of a genetic testing policy for permanent neonatal diabetes.

RESEARCH DESIGN AND METHODS

—The conceptual organization of the analysis is displayed in Fig. 1. The analysis was conducted from the societal perspective with a 30-year time horizon, which was selected because the most extensive natural history data for type 1 diabetes spans only 30 years. Forecasts beyond 30 years are therefore

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EAK-7 controls development and lifespan by regulating nuclear DAF-16/FoxO activity

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SUMMARY

FoxO transcription factors control development and longevity in diverse species. Although FoxO regulation *via* changes in its subcellular localization is well established, little is known about how FoxO activity is regulated in the nucleus. Here we show that the conserved *C. elegans* protein EAK-7 acts in parallel to the serine/threonine kinase AKT-1 to inhibit the FoxO transcription factor DAF-16. Loss of EAK-7 activity promotes diapause and longevity in a DAF-16/FoxO-dependent manner. Whereas *akt-1* mutation activates DAF-16/FoxO by promoting its translocation from the cytoplasm to the nucleus, *eak-7* mutation increases nuclear DAF-16/FoxO activity without influencing DAF-16/FoxO subcellular localization. Thus, EAK-7 and AKT-1 inhibit DAF-16/FoxO activity *via* distinct mechanisms. Our results implicate EAK-7 as a FoxO regulator and highlight the biological impact of a new regulatory pathway that governs the activity of nuclear FoxO without altering its subcellular location.

INTRODUCTION

FoxO transcription factors (TFs) promote lifespan extension, stress resistance and metabolic homeostasis in diverse species (Accili and Arden, 2004; Arden, 2008; Calnan and Brunet, 2008; Gross et al., 2008; Partridge and Bruning, 2008). FoxO knockout mice develop tumors and exhibit abnormalities in glucose metabolism and bone mineral density (Ambrogini et al., 2010; Dong et al., 2008; Matsumoto et al., 2007; Paik et al., 2007; Rached et al., 2010), suggesting that dysregulation of FoxO TFs may contribute to the pathophysiology of common human diseases associated with aging such as cancer, type 2 diabetes, and osteoporosis. Intriguingly, FoxO3 polymorphisms are associated with extreme longevity in humans (Flachsbart et al., 2009; Li et al., 2009; Willcox et al., 2008). Thus, understanding how FoxO TFs are regulated has the potential to yield fundamental insights into both the pathophysiology of human disease as well as the physiology of normal aging.

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PLoS one

Multiple Roles for the Non-Coding RNA SRA in Regulation of Adipogenesis and Insulin Sensitivity

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Abstract

Peroxisome proliferator-activated receptor-γ (PPARγ) is a master transcriptional regulator of adipogenesis. Hence, the identification of PPARγ coactivators should help reveal mechanisms controlling gene expression in adipose tissue development and physiology. We show that the non-coding RNA, Steroid receptor RNA Activator (SRA), associates with PPARγ and coactivates PPARγ-dependent reporter gene expression. Overexpression of SRA in ST2 mesenchymal precursor cells promotes their differentiation into adipocytes. Conversely, knockdown of endogenous SRA inhibits 3T3-L1 preadipocyte differentiation. Microarray analysis reveals hundreds of SRA-responsive genes in adipocytes, including genes involved in the cell cycle, and insulin and TNFα signaling pathways. Some functions of SRA may involve mechanisms other than coactivation of PPARγ. SRA in adipocytes increases both glucose uptake and phosphorylation of Akt and FOXO1 in response to insulin. SRA promotes S-phase entry during mitotic clonal expansion, decreases expression of the cyclin-dependent kinase inhibitors p21Cip1 and p27Kip1, and increases phosphorylation of Cdk1/Cdc2. SRA also inhibits the expression of adipocyte-related inflammatory genes and TNFα-induced phosphorylation of c-Jun NH_2-terminal kinase. In conclusion, SRA enhances adipogenesis and adipocyte function through multiple pathways.

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Introduction

Obesity is a prevalent health hazard closely associated with a number of pathological disorders, including type 2 diabetes, cardiovascular disease, hypertension, cancer, and gallbladder disease. Adipocytes play a central role in energy balance, both as reservoirs of fuel and as endocrine cells, secreting factors (adipokines) that regulate whole body energy metabolism and glucose homeostasis [1]. Adipogenesis is a complex process that is highly regulated by positive and negative stimuli, including a variety of hormones and nutritional signals [2,3,4,5]. Adipocyte differentiation is commonly studied in immortalized cell lines such as 3T3-L1 preadipocytes [2,4,6] and the pluripotent bone marrow-derived mesenchymal cell line ST2 [7], both of which can be differentiated into mature adipocytes by standard hormone cocktails. During adipogenesis, fibroblast-like preadipocytes differentiate into lipid-laden and insulin-responsive adipocytes. This process occurs in several stages (growth arrest, mitotic clonal expansion and terminal differentiation) and is driven by the coordinated effects of a number of transcription factors and signaling molecules, including peroxisome proliferator-activated receptor gamma (PPARγ), the CCAAT/enhancer-binding proteins (C/EBPs) [4,8], Kruppel-like factors (KLFs) [9,10], Wingless proteins (Wnt) [11,12], GATA2 [13,14] and cell cycle proteins [15,16,17].

Transcription factors function in part by recruiting coregulators that epigenetically remodel chromatin and/or bridge the complexes in which they reside to the basal transcriptional machinery. Some coregulators important in adipogenesis have essential enzymatic activities, such as the SW1/SNF complex that controls ATP-dependent chromatin remodeling [18,19], and the histone acetyltransferase proteins CBP and p300 [20,21]. Others, such as the p160 family of coactivators, SRC-1, TIF2/SRC-2 and AIB1/SRC-3, function as scaffolds, although they also have some histone acetyltransferase activity [22,23,24]. Conversely, corepressors such as nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) recruit histone deacetylases to target promoters, and therefore are anti-adipogenic [25].

The steroid receptor RNA activator (SRA) is a unique coregulator that functions as a non-coding RNA [26], although

nature cell biology

Exocyst function is regulated by effector phosphorylation

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The exocyst complex tethers vesicles at sites of fusion through interactions with small GTPases. The G protein RalA resides on Glut4 vesicles, and binds to the exocyst after activation by insulin, but must then disengage to ensure continuous exocytosis. Here we report that, after recognition of the exocyst by activated RalA, disengagement occurs through phosphorylation of its effector Sec5, rather than RalA inactivation. Sec5 undergoes phosphorylation in the G-protein binding domain, allosterically reducing RalA interaction. The phosphorylation event is catalysed by protein kinase C and is reversed by an exocyst-associated phosphatase. Introduction of Sec5 bearing mutations of the phosphorylation site to either alanine or aspartate disrupts insulin-stimulated Glut4 exocytosis, as well as other trafficking processes in polarized epithelial cells and during development of zebrafish embryos. The exocyst thus serves as a 'gatekeeper' for exocytic vesicles through a circuit of engagement, disengagement and re-engagement with G proteins.

Exocytotic vesicles rely on the exocyst complex for efficient delivery to the plasma membrane[1–3]. Both the assembly and recognition of the exocyst require interactions with small GTPases that are spatially activated[4]; G proteins such as Rho3 and Cdc42 in yeast[4] or TC10 in mammalian cells[5] direct exocyst assembly at the target membrane, whereas vesicular G proteins such as Sec4 in yeast[6] or RalA in mammalian cells[7–9] mediate its recognition by the vesicle.

Ral GTPases interact with the exocyst subunits Exo84 and Sec5 (refs 8,9). While the function of Exo84 is not well understood, Sec5 assembles as part of an exocyst subcomplex that marks the site of exocytosis at the plasma membrane[10,11]. RalA binds to the exocyst after activation during cell growth and division[12,13], and functions in the delivery of receptors and transporters[14,15] to the cell surface, including the insulin-stimulated glucose transporter Glut4 (ref. 7), and also acts as a cargo receptor for the motor complex Myo1c–Calmodulin[7]. While exocyst recognition by RalA has received much attention, the mechanism underlying dissociation of the GTPase from the tethering complex before the fusion event remains unclear[1,16]. Although G proteins usually disengage from effectors through the acceleration of their intrinsic GTPase activity by GTPase-activator proteins (GAPs; refs 17,18), it is not known whether RalA–exocyst dissociation results from RalA inactivation, or alternatively from modifications in its exocyst partners. We report here that, on delivery of cargo vesicles to the exocyst following the activation of RalA, its partner Sec5 undergoes hormone-stimulated phosphorylation within the Ral-binding domain, an event directly catalysed by protein kinase C (PKC), resulting in the dissociation of the protein from active RalA. After disengaging from RalA, Sec5 undergoes dephosphorylation for another round of vesicle recognition. Thus, the RalA–exocyst interaction is dynamically regulated through a cycle of engagement, disengagement and re-engagement, crucial for the efficient delivery of exocytic vesicles.

RESULTS

Active RalA promotes exocyst function independently of GTP hydrolysis

The disengagement of RalA from the exocyst could involve either inactivation of the G protein, or modifications on its binding partners. To test whether RalA inactivation is required for exocyst-mediated vesicle delivery, we introduced GTPase-deficient RalA into 3T3-L1 adipocytes, in which insulin stimulates glucose transport through the exocyst-dependent exocytosis of Glut4 (refs 5,7,19). Instead of inhibiting glucose transport, as observed with loss of exocyst function[5,7,20], expression of constitutively active $RalA^{G23V}$ produced a twofold increase in basal glucose uptake, with an approximate 15% increase in insulin-stimulated levels (Fig. 1a). Similar effects were seen on expression of constitutively active $RalA^{G23V}$ after the endogenous protein

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Conditional Gene Targeting in Mouse Pancreatic β-Cells
Analysis of Ectopic Cre Transgene Expression in the Brain

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OBJECTIVE—Conditional gene targeting has been extensively used for in vivo analysis of gene function in β-cell biology. The objective of this study was to examine whether mouse transgenic Cre lines, used to mediate β-cell– or pancreas-specific recombination, also drive Cre expression in the brain.

RESEARCH DESIGN AND METHODS—Transgenic Cre lines driven by *Ins1*, *Ins2*, and *Pdx1* promoters were bred to *R26R* reporter strains. Cre activity was assessed by β-galactosidase or yellow fluorescent protein expression in the pancreas and the brain. Endogenous *Pdx1* gene expression was monitored using $Pdx1^{tm1Cvw}$ lacZ knock-in mice. Cre expression in β-cells and co-localization of Cre activity with orexin-expressing and leptin-responsive neurons within the brain was assessed by immunohistochemistry.

RESULTS—All transgenic Cre lines examined that used the *Ins2* promoter to drive Cre expression showed widespread Cre activity in the brain, whereas Cre lines that used *Pdx1* promoter fragments showed more restricted Cre activity primarily within the hypothalamus. Immunohistochemical analysis of the hypothalamus from $Tg(Pdx1\text{-}cre)^{89.1Dam}$ mice revealed Cre activity in neurons expressing orexin and in neurons activated by leptin. $Tg(Ins1\text{-}Cre/ERT)^{1Lphi}$ mice were the only line that lacked Cre activity in the brain.

CONCLUSIONS—Cre-mediated gene manipulation using transgenic lines that express Cre under the control of the *Ins2* and *Pdx1* promoters are likely to alter gene expression in nutrient-sensing neurons. Therefore, data arising from the use of these transgenic Cre lines must be interpreted carefully to assess whether the resultant phenotype is solely attributable to alterations in the islet β-cells. ***Diabetes*** **59:3090–3098, 2010**

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In vivo analysis of gene function in the pancreas and β-cells has benefited from the development of mouse lines expressing Cre in all pancreatic compartments or restricted to the islet β-cells. The choice of promoter to drive recombinase expression is critical for controlling the location and timing of gene activity. In addition, inducible versions of Cre recombinase, e.g., CreER, allow temporal control to the manipulation of gene activity, which becomes important when analyzing gene function at specific embryonic and adult stages (1,2). Promoters of the *pancreas duodenal homeobox 1* (*Pdx1*) (3,4) and *insulin* (*Ins1* and *Ins2*) (5–8) genes have been well characterized to allow the use of regulatory sequences for directing Cre expression to specific pancreatic cell populations. Commonly used transgenic mouse lines that employ rat *Ins2* gene promoter sequences to drive Cre expression within the β-cell population include *Ins2-Cre/RIP-Cre* [Mouse Genome Informatics (MGI): $Tg(Ins2\text{-}cre)^{25Mgn}$ and $Tg(Ins2\text{-}cre)^{1Herr}$] (9–11) and *RIP-CreER* [MGI: $Tg(Ins2\text{-}cre/Esr1)^{1Dam}$] (12). *Pdx1* gene promoter sequences have proven useful for directing Cre expression throughout the early pancreatic epithelium (4,10,13,14) and to the endocrine cells of the pancreas (15). The *Pdx1* gene is expressed early in pancreas development throughout the endoderm of the dorsal and ventral buds, but expression becomes restricted during development such that high levels of *Pdx1* are maintained in the insulin-producing β-cells with lower levels in subpopulations of acinar cells (8,16). Examples of *Pdx1-Cre* transgenic lines include $Pdx1\text{-}Cre^{early}$ [MGI: $Tg(Pdx1\text{-}cre)^{89.1Dam}$] (13), $Pdx1\text{-}Cre^{late}$ [MGI: $Tg(Ipf1\text{-}cre/Esr1)^{1Dam/Mmcd}$] (10), *Pdx1-Cre* [MGI: $Tg(Ipf1\text{-}cre)^{1Tuv}$] (14), and *Pdx1-CreER* [MGI: $Tg(Pdx1\text{-}cre/ERT)^{1Mga}$] (15).

To assess the specificity of recombination and perform lineage tracing analysis, reporter lines such as the *ROSA26-stop-lacZ* [MGI: $Gt(ROSA)26Sor^{tm1Sho}$], also known as *R26R* (17), or the *ROSA26-stop-YFP* [MGI: $Gt(ROSA)26Sor^{tm1(EYFP)Cos}$] (18) mice have been developed. Upon Cre-mediated recombination, these reporter lines activate expression of a β-galactosidase (β-gal) or a yellow fluorescent protein (YFP) reporter under the control of the ubiquitously active *ROSA26* promoter, resulting

Annals of Internal Medicine | ORIGINAL RESEARCH

Diabetes Control With Reciprocal Peer Support Versus Nurse Care Management

A Randomized Trial

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Background: Resource barriers complicate diabetes care management. Support from peers may help patients manage their diabetes.

Objective: To compare a reciprocal peer-support (RPS) program with nurse care management (NCM).

Design: Randomized, controlled trial. (ClinicalTrials.gov registration number: NCT00320112)

Setting: 2 U.S. Department of Veterans Affairs health care facilities.

Patients: 244 men with hemoglobin A_{1c} (HbA_{1c}) levels greater than 7.5% during the previous 6 months.

Measurements: The primary outcome was 6-month change in HbA_{1c} level. Secondary outcomes were changes in insulin therapy; blood pressure; and patient reports of medication adherence, diabetes-related support, and emotional distress.

Intervention: Patients in the RPS group attended an initial group session to set diabetes-related behavioral goals, receive peer communication skills training, and be paired with another age-matched peer patient. Peers were encouraged to talk weekly using a telephone platform that recorded call occurrence and provided reminders to promote peer contact. These patients could also participate in optional group sessions at 1, 3, and 6 months. Patients in the NCM group attended a 1.5-hour educational session and were assigned to a nurse care manager.

Results: Of the 244 patients enrolled, 216 (89%) completed the HbA_{1c} assessments and 231 (95%) completed the survey assessments at 6 months. Mean HbA_{1c} level decreased from 8.02% to 7.73% (change, −0.29%) in the RPS group and increased from 7.93% to 8.22% (change, 0.29%) in the NCM group. The difference in HbA_{1c} change between groups was 0.58% ($P = 0.004$). Among patients with a baseline HbA_{1c} level greater than 8.0%, those in the RPS group had a mean decrease of 0.88%, compared with a 0.07% decrease among those in the NCM group (between-group difference, 0.81%; $P < 0.001$). Eight patients in the RPS group started insulin therapy, compared with 1 patient in the NCM group ($P = 0.020$). Groups did not differ in blood pressure, self-reported medication adherence, or diabetes-specific distress, but the RPS group reported improvement in diabetes social support.

Limitation: The study included only male veterans and lasted only 6 months.

Conclusion: Reciprocal peer support holds promise as a method for diabetes care management.

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Many patients with diabetes would benefit from self-management assistance between clinic visits. In efficacy trials, nurse-led care management programs improve diabetes self-care and risk factor control (1–3). However, real-life practices face multiple barriers to delivering these services, especially in low-resource settings (4).

As recognized in such initiatives as the United Kingdom's Expert Patient Program (5), peer support among patients with the same chronic health problem is a promising approach to increasing the quality and quantity of support (6). Peer support could allow patients to share experiences and receive reinforcement that is not available from time-pressed clinicians, and it may especially benefit patients who are tackling challenging medical tasks, such as insulin management. Many patients with poor glycemic control require either initiation or intensification of insulin therapy but resist these because of concerns about the additional self-management burdens (7, 8), which results in neuropathic or microvascular complications (9).

Although few peer-support models have been evaluated in randomized, controlled trials (RCTs), the effective tested models combine peer support with a more structured program of education and assistance (10, 11). Face-to-face peer- and clinician-led group visits (12–14) and training sessions (15–17) improve some outcomes; however, many patients assigned to face-to-face peer support or group sessions do not attend them (14, 15). It is thus important to identify novel delivery mechanisms to extend the reach of evidence-based peer-support models (18).

To build on the potential benefits of face-to-face peer support while circumventing access barriers, we designed and piloted a novel intervention that supplemented optional periodic nurse-led group sessions with telephone-based peer support between paired diabetic patients (19). The model was intended to encourage both peers to receive

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Feasibility of group lifestyle intervention for diabetes prevention in Arab Americans☆

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ABSTRACT

Aims: To assess the feasibility and acceptability of a community-based, culturally-specific, Diabetes Prevention Program (DPP)-adapted, group lifestyle intervention in Arab-Americans.

Methods: Overweight (BMI $\geq$ 27 kg/m^2) Arab-Americans aged $\geq$30 years and without a history of diabetes were recruited to participate in a 24-week group lifestyle intervention. The DPP core-curriculum was culturally rewritten, translated into Arabic, and delivered in weekly sessions over a 12-week period. Follow-up was performed at week-24. The primary goals were to achieve $\geq$7% weight loss and $\geq$150 min/week of physical activity. An intent-to-treat analysis was performed.

Results: Of the 71 participants (mean age $\pm$ SD 47 $\pm$ 10 years, 38% males), 44% achieved $\geq$7% weight loss, 59% achieved $\geq$5% reduction in weight, and 78% reached the physical activity goal of $\geq$150-min/week. The mean $\pm$ SD weight loss was 5.2 $\pm$ 4.4 kg at week-24 ($p < 0.0001$), Marked reduction in body measurements, daily energy and fat intake were noted. Retention was high with 86% completing the intervention.

Conclusions: This trial demonstrates that a culturally-specific, DPP-adapted, group lifestyle intervention implemented in a community setting is feasible and effective in Arab-Americans.

1. Introduction

The largest concentration of Arabs in the United States is in the Detroit Metropolitan area estimated at approximately 392,000 individuals according to the Arab American Institute Foundation [1]. The Arab-American community is primarily composed of recent immigrants [2]. A number of cultural elements distinguish this population including a deep religious orientation, reliance on the extended family, defined gender roles and strong gender taboos, use of the Arabic language, lack of acculturation, and adherence to traditional beliefs, and practices.

Diabetes is a growing clinical and public health problem in the Arab-American community. We have previously shown

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LESS IS MORE

Variation in the Net Benefit of Aggressive Cardiovascular Risk Factor Control Across the US Population of Patients With Diabetes Mellitus

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Background: Lowering low-density lipoprotein cholesterol (LDL-C) and blood pressure (BP) in patients with diabetes mellitus (DM) can significantly reduce the risk of cardiovascular disease (CVD). However, to our knowledge, previous studies have not assessed variability in both the benefit and harm from pursuing LDL-C and BP target levels.

Methods: Our sample comprised individuals 30 to 75 years old with DM participating in the National Health and Nutrition Examination Survey III. We used Monte Carlo methods to simulate a treat-to-target strategy, in which patients underwent treatment intensification with the goal of achieving LDL-C and BP target levels of 100 mg/dL and 130/80 mm Hg, respectively. Patients received up to 5 titrations of statin therapy and 8 titrations of antihypertensive therapy. Treatment adverse effects and polypharmacy risks and burdens were incorporated using disutilities. Health outcomes were simulated using a Markov model.

Results: Treating to targets resulted in gains of 1.50 (for LDL-C) and 1.35 (for BP) quality-adjusted life-years (QALYs) of lifetime treatment-related benefit, which declined to 1.42 and 1.16 QALYs after accounting for treatment-related harms. Most of the total benefit was limited to the first few steps of medication intensification or to tight control for a limited group of very high-risk patients. However, because of treatment-related disutility, intensifying beyond the first step (LDL-C) or third step (BP) resulted in either limited benefit or net harm for patients with below-average risk.

Conclusion: The benefits and harms from aggressive risk factor modification vary widely across the US population of individuals with DM, depending on a patient's underlying CVD risk, suggesting that a personalized approach could maximize a patient's net benefit from treatment.

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NEARLY ALL DIABETES mellitus (DM) practice guidelines recommend aggressive treatment of low-density lipoprotein cholesterol (LDL-C) and blood pressure (BP) to lower a patient's risk of developing cardiovascular disease (CVD) or preventing its sequelae.[1,2] These recommendations, which are based on the average results of trials evaluating the relative benefits of intensive risk factor control,[3-6] are not tailored to an individual's underlying CVD risk. While this approach is often advocated in patients without DM,[2] there is an implicit assumption that all patients with DM are at equally high risk, requiring all patients to be treated aggressively. However, the benefit of intensifying treatment to attain low risk factor targets, or "treating to targets," could vary greatly across the population of individuals with DM depending on the distribution of CVD risk in the population.

While clinical trials have demonstrated that intensive risk factor control can provide significant benefits on average for persons with DM, most of these trials included many patients with very high CVD risk and limited treatment to statins and up to 3 to 4 blood pressure medications.[7,8] Two

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recent substudies of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial have confirmed that intensive BP control[9] and intensive treatment with combination lipid-lowering therapies[10] offered no survival advantage overall, and perhaps caused harm, but may still be beneficial to very high risk groups. But for at least 3 reasons, results from these most recent trials provide limited guidance for a typical clinical decision-making context. First, because many of the studies of tight risk factor control enrolled patients with a range

Exercising control over molecular reactivity by confinement has great potential for both understanding and measuring complex chemical reactions (*40*, *41*). Regioselective photoreactions between individual molecules by molecular design and self-assembly with in situ monitoring are powerful demonstrations of chemical control via the local environment (*7*, *42*, *43*). The extraordinary resolution of the STM enables monitoring molecular motions and reactions. Controlling the chemical environment and monitoring such selective reactions between individual molecules will be important elements in directing chemistry with this approach.

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Supporting Online Material

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A Circadian Rhythm Orchestrated by Histone Deacetylase 3 Controls Hepatic Lipid Metabolism

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Disruption of the circadian clock exacerbates metabolic diseases, including obesity and diabetes. We show that histone deacetylase 3 (HDAC3) recruitment to the genome displays a circadian rhythm in mouse liver. Histone acetylation is inversely related to HDAC3 binding, and this rhythm is lost when HDAC3 is absent. Although amounts of HDAC3 are constant, its genomic recruitment in liver corresponds to the expression pattern of the circadian nuclear receptor Rev-erbα. Rev-erbα colocalizes with HDAC3 near genes regulating lipid metabolism, and deletion of HDAC3 or Rev-erbα in mouse liver causes hepatic steatosis. Thus, genomic recruitment of HDAC3 by Rev-erbα directs a circadian rhythm of histone acetylation and gene expression required for normal hepatic lipid homeostasis.

In mammals, metabolic processes in peripheral organs display robust circadian rhythms, coordinated with the daily cycles of light and nutrient availability (*1*, *2*). Circadian misalignment causes metabolic dysfunction, and people engaged in night-shift work suffer from higher incidences of obesity, diabetes, and metabolic syndrome (*3*–*5*). The molecular basis of this is unknown, but genetic disruption of circadian clock components in mice leads to altered glucose and lipid metabolism (*6*–*10*).

Gene expression profiles in multiple metabolic organs have revealed a circadian control of the transcriptome, which might be mediated by regulation of histone acetylation (*11*–*13*) that alters the structure of the epigenome. Regulation of histone acetylation is complex, involving multiple histone acetyltransferases (HATs) and histone deacetylases (HDACs) (*14*). Histone deacetylase 3 (HDAC3) functions in the regulation of circadian rhythm and glucose metabolism (*15*). We report diurnal recruitment of HDAC3 to the mouse liver genome detected by chromatin immunoprecipitation with an HDAC3-specific antibody (fig. S1) and massively parallel DNA sequencing (ChIP-seq).

At ZT10 [where ZT is Zeitgeber time (light on at ZT0 and off at ZT12)], in the light period when mice are inactive, HDAC3 bound to over 14,000 sites in adult mouse liver (the HDAC3 ZT10 cistrome) (fig. S2A); a majority of these binding sites were distant from transcription start sites (TSS) or present in introns (fig. S2, B and C). However, at ZT22, in the dark period when mice are active and feeding, the HDAC3 signal was dramatically reduced at ZT10 sites, with only 120 specific peaks (Fig. 1A and figs. S2, A and D, and S3). HDAC3 recruitment oscillated in a 24-hour cycle (Fig. 1B), and this rhythm was retained in constant darkness (Fig. 1C), suggesting that it was controlled by the circadian clock. The liver clock is entrained by food intake (*16*), and, indeed, the pattern of HDAC3 enrichment was reversed when food was provided only during the light period (Fig. 1D), further supporting the conclusion that the rhythm of HDAC3 genomic recruitment was controlled by the circadian clock.

Despite its known role in histone deacetylation and transcriptional repression, HDAC3

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Adiponectin suppresses gluconeogenic gene expression in mouse hepatocytes independent of LKB1-AMPK signaling

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The adipocyte-derived hormone adiponectin signals from the fat storage depot to regulate metabolism in peripheral tissues. Inversely correlated with body fat levels, adiponectin reduction in obese individuals may play a causal role in the symptoms of metabolic syndrome. Adiponectin lowers serum glucose through suppression of hepatic glucose production, an effect attributed to activation of AMPK. Here, we investigated the signaling pathways that mediate the effects of adiponectin by studying mice with inducible hepatic deletion of LKB1, an upstream regulator of AMPK. We found that loss of LKB1 in the liver partially impaired the ability of adiponectin to lower serum glucose, though other actions of the hormone were preserved, including reduction of gluconeogenic gene expression and hepatic glucose production as assessed by euglycemic hyperinsulinemic clamp. Furthermore, in primary mouse hepatocytes, the absence of LKB1, AMPK, or the transcriptional coactivator CRTC2 did not prevent adiponectin from inhibiting glucose output or reducing gluconeogenic gene expression. These results reveal that whereas some of the hormone's actions in vivo may be LKB1 dependent, substantial LKB1-, AMPK-, and CRTC2-independent signaling pathways also mediate effects of adiponectin.

Introduction

The discovery of adipose tissue as an endocrine organ that can secrete adipokine hormones has provided new insights into the role of this tissue in the homeostasis of organismal nutrient metabolism. The identification and characterization of one such adipokine, adiponectin, revealed an intriguing interaction between adipocytes and nutrient metabolism in peripheral tissues (1, 2). The importance of adiponectin is heightened by the clear association of decreased adiponectin levels in obese individuals with an elevated risk for insulin resistance and cardiovascular disease (3, 4) and the identification of adiponectin missense mutations that associate with increased risk for insulin resistance and coronary artery disease (5, 6). While the precise mechanisms by which adiponectin may antagonize adverse outcomes are unclear, administration of adiponectin is known to increase fatty acid oxidation in metabolic tissues, decrease hepatic glucose production, increase glucose uptake in muscle cell culture, and alter food intake and energy expenditure through central actions (5, 7–11). For these reasons, adiponectin-mimetic therapies have become an attractive potential treatment for the metabolic syndrome.

Multiple studies have found that adiponectin lowers blood glucose acutely through a reduction in hepatic glucose output with little or no effect on glucose disposal (9, 10, 12). Adiponectin also reduces hepatic and serum triglyceride levels and protects from both alcoholic and nonalcoholic hepatic steatosis (7, 13). Reduction of adiponectin in mice leads to impaired glucose tolerance and elevated hepatic glucose production, further corroborating the role for adiponectin in normal hepatic metabolism (14). The role has been further established in studies showing that the reversal of decreased serum adiponectin levels in *ob/ob* mice through transgenic expression can dramatically improve their metabolic profile (15). The glucose-lowering functions of adiponectin have been attributed to the hepatic activation of AMPK, providing a mechanistic link to a signal transduction pathway already established as an antagonist of hepatic glucose output and lipogenesis (16).

AMPK is an evolutionarily conserved protein kinase that serves as a primary cellular monitor of energy charge; conditions that deplete energy and thus elevate the AMP/ATP ratio activate the kinase and result in a decrease in anabolic and increase in catabolic pathways (17). AMPK is a heterotrimeric enzyme, consisting of regulatory β and γ subunits and a catalytic α subunit that requires phosphorylation by an upstream AMPK kinase (AMPKK) to achieve full activity. Once activated, AMPK phosphorylates target proteins such as acetyl-CoA carboxylase (ACC) and CREB-regulated transcription coactivator (CRTC2), also known as transducer of regulated cAMP response element–binding protein 2 (TORC2), restoring the energy status through multiple mechanisms including increased fatty acid oxidation and glucose uptake in muscle, and decreased hepatic lipogenesis and gluconeogenesis (16–19). The principle AMPKKs are the kinases STK11/LKB1 and CaMKKβ, which phosphorylate AMPK in its activation loop at threonine 172 (20–23). In many cells, LKB1 is the dominant AMPKK, as its loss decreases basal and stimulated AMPK phosphorylation and activity (24–26). For example, deletion of LKB1 in liver elevates serum glucose levels and impairs glucose tolerance, which is associated with a significant loss of basal and metformin-stimulated AMPK phosphorylation (26). These data establish LKB1 as an important regulator of hepatic glucose production.

AMPK mediates some hepatic actions of adiponectin as evidenced by loss of adiponectin-induced glucose lowering in mice expressing a dominant negative AMPK protein in liver (27). More-

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Three-amino-acid-loop-extension homeodomain factor Meis3 regulates cell survival via PDK1

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Three-amino-acid-loop-extension (TALE) homeodomain proteins including Meis and Pbx families are generally recognized for their roles in growth and differentiation during vertebrate embryogenesis and tumorigenesis. Whereas genetic studies indicate that Pbx1 regulates the development and function of insulin-producing pancreatic β-cells, the role of Meis family members in β-cells is still unknown. Here we show that Meis3 is abundantly expressed in pancreatic islets and β-cells and that it regulates β-cell survival. We further identify the 3-phosphoinositide–dependent protein kinase 1 (PDK1), a well-known kinase involved in the PI3K–Akt signaling pathway, as a direct Meis3 target, which mediates its role in β-cell survival. This regulatory module appears to function broadly as we also identify Meis3 regulation of cell survival and PDK1 expression in ovarian carcinoma cells, suggesting a unique function for Meis3 beyond the traditional roles for TALE homeodomain factors during embryogenesis.

diabetes | transcription | pancreas | apoptosis

Three-amino-acid-loop-extension (TALE) homeodomain proteins are recognized as transcription factors responsible for regulating growth and differentiation during vertebrate embryogenesis. In general, TALE homeodomain factors act via the formation of heterodimeric and -trimeric DNA-binding complexes with one another and/or Hox proteins to activate specific gene expression (1–5). The TALE homeodomain superclass is composed of Pbx (Pbx1, Pbx2, Pbx3, and Pbx4) and Meis (Prep1, Prep2, Meis1, Meis2, and Meis3) family members in mammals (6). The founding members of both families were identified through their association with tumorigenesis. The mammalian Meis (myeloid ecotropic viral insertion site) genes were first identified in a murine leukemia model (7). Subsequent studies report that Meis proteins are extensively expressed in a large set of ovarian carcinomas and neuroblastoma (8, 9). Further, Meis1 and Meis2 are expressed in 10 different "cancer modules" within gene expression profiles of 22 diverse tumor types, raising the expectation that more data on Meis members in oncogenesis will emerge (10). However, apart from its role as a Hox cofactor, the function and underlying molecular mechanisms of Meis genes in cancer cells are still poorly understood.

Genetic studies indicate the role of TALE homeodomain protein Pbx1 in the development and function of pancreatic islets, which contain the insulin secreting β-cells required for maintaining normal glucose homeostasis. *Pbx1*$^{-/-}$ embryos exhibit pancreatic hypoplasia and marked defects in exocrine and endocrine cell differentiation before death at embryonic day (E) 15 or E16, whereas *Pbx1*$^{+/-}$ adults have pancreatic islet malformations, impaired glucose tolerance, and hypoinsulinemia. Analysis of transheterozygous *Pbx1*$^{+/-}$*Pdx1*$^{+/-}$ mice revealed in vivo genetic interactions between Pbx1 and Pdx1 that are essential for postnatal pancreatic function. Consequently, these mice develop age-dependent overt diabetes mellitus (11). Similarly, a Pbx interaction-defective Pdx1 transgene is unable to rescue normal glucose homeostasis in *Pdx1* null mice (12). Although Meis/Pbx/Pdx1 complexes regulate gene expression in cultured ductal and acinar cells (2, 4), no transcriptional targets of Pbx or Meis have been identified in the β-cell, and whether Meis family members are involved in regulating β-cell development and/or function is still unknown.

Here, we show that Meis3 is abundantly expressed in pancreatic islets and cultured β-cells and is required for β-cell survival. We identify the 3-phosphoinositide–dependent protein kinase 1 (PDK1), a well-known kinase involved in the PI3K–Akt signaling pathway, as a direct Meis3 target that mediates its role in β-cell survival. A similar regulatory module operates in ovarian carcinoma cells, despite low levels of Meis3 expression and markedly higher expression of other Meis family members, Meis1 and Meis2. These results define a unique regulatory role for Meis3 in cell survival.

Results

Meis3 Is the Predominant Meis Family Member Expressed in Pancreatic β-cells and Meis3 Deficiency Induces Cell Apoptosis in Min6 β-Cells. We initially evaluated the expression levels of mouse Meis family members in primary pancreatic islets. Expression of all three Meis genes was detectable, but the level of Meis3 expression was threefold higher than that of Meis1 or Meis2 (Fig. 1*A*). A similar profile of Meis3 enrichment was observed in cultured Min6 β-cells (Fig. 1*B*). Meis3 protein was easily detectable in the nuclei of adult pancreatic islets, in both insulin-expressing β-cells as well as non–β-cells (Fig. 1*C*). Meis1 protein was also nuclear in β- and non–β-cells of the islet, whereas Meis2 protein appeared to be expressed in the perinuclear region of β-cells only (Fig. S1).

To explore the functional role of Meis3 in pancreatic β-cells, siRNA-mediated silencing of Meis3 was performed in Min6 β-cells. Q-PCR analysis showed an 80% reduction of Meis3 transcript compared with nontargeting siRNA control (Fig. 1*D*) and a marked reduction in Meis3 protein was detected by Western blot analysis (Fig. 1*E*). In contrast, Meis1 and Meis2 transcripts were relatively unaffected (Fig. 1*F*), indicating the specificity of Meis3 silencing and the absence of compensatory up-regulation of other Meis family members. We observed increased numbers of floating cells in siMeis3-transfected groups, suggesting that Meis3 depletion results in cell death. Indeed, increased caspase-3 cleavage was observed in Meis3-deficient Min6 cells, accompanied by reduced procaspase-3 (Fig. 1*G*). Further, increased numbers of apoptotic cells were observed by TUNEL staining (Fig. 1*H*) and expression of the antiapoptotic gene Bcl-2 was reduced in Meis3-deficient cells (Fig. 1*I*). Thus, these findings indicate that Meis3 is abundantly expressed in mature pancreatic β-cells and plays a role in β-cell survival.

Meis3 Targets Pdpk1 and Regulates the PDK1/Akt Signaling Pathway in Pancreatic β-Cells. To identify genes regulated by Meis3, we performed Agilent cDNA microarray analysis comparing RNA samples isolated from Meis3-deficient or control Min6 cells. Of

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High-Density Lipoprotein Suppresses the Type I Interferon Response, a Family of Potent Antiviral Immunoregulators, in Macrophages Challenged With Lipopolysaccharide

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Background—High-density lipoprotein (HDL) protects the artery wall by removing cholesterol from lipid-laden macrophages. However, recent evidence suggests that HDL might also inhibit atherogenesis by combating inflammation.

Methods and Results—To identify potential antiinflammatory mechanisms, we challenged macrophages with lipopolysaccharide, an inflammatory microbial ligand for Toll-like receptor 4. HDL inhibited the expression of 30% (277 of 911) of the genes normally induced by lipopolysaccharide, microarray analysis revealed. One of its major targets was the type I interferon response pathway, a family of potent viral immunoregulators controlled by Toll-like receptor 4 and the TRAM/TRIF signaling pathway. Unexpectedly, the ability of HDL to inhibit gene expression was independent of macrophage cholesterol stores. Immunofluorescent studies suggested that HDL promoted TRAM translocation to intracellular compartments, which impaired subsequent signaling by Toll-like receptor 4 and TRIF. To examine the potential in vivo relevance of the pathway, we used mice deficient in apolipoprotein A-I, the major protein of HDL. After infection with *Salmonella typhimurium*, a Gram-negative bacterium that expresses lipopolysaccharide, apolipoprotein A-I–deficient mice had 6-fold higher plasma levels of interferon-β, a key regulator of the type I interferon response, than did wild-type mice.

Conclusions—HDL inhibits a subset of lipopolysaccharide-stimulated macrophage genes that regulate the type I interferon response, and its action is independent of sterol metabolism. These findings raise the possibility that regulation of macrophage genes by HDL might link innate immunity and cardioprotection. **(*Circulation*. 2010;122:1919-1927.)**

Key Words: chemokines ■ cytokines ■ interferon regulatory factor 7 ■ lipid cell membrane ■ myeloid differentiation factor 88

High-density lipoprotein (HDL) protects against vascular disease by removing cholesterol from artery wall macrophages through reverse cholesterol transport.[1–4] There is mounting evidence, however, that it has additional antiatherosclerotic effects.[2,5–8] One such activity may be modulation of the inflammatory response of the innate immune system.[9–14]

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Two cholesterol-transporting proteins, ABCA1 and ABCG1, might link HDL to both cholesterol removal and regulation of inflammation.[10–12] Both membrane-associated ATP-binding cassette transporters are found in macrophages. ABCA1 promotes cholesterol efflux to lipid-poor apolipoprotein (apo) A-I, the major HDL protein, and ABCG1 induces cholesterol efflux to intact HDL particles.[10] Macrophages isolated from mice with genetically engineered deficiencies in ABCA1 and/or ABCG1 overexpress well-known inflammatory genes such as tumor necrosis factor-α (TNF-α), interleukin (IL) -1β, and IL-8 when the cells are stimulated with bacterial lipopolysaccharide (LPS).[11] In both macrophages and endothelial cells, the antiinflammatory effects of HDL have been proposed to reflect changes in membrane cholesterol and lipid rafts, with downstream effects on signal transduction and the activities of membrane-associated proteins.[11,12,15]

The response of the innate immune system to infection involves membrane-associated Toll-like receptors (TLRs), which can sense a diverse range of conserved microbial structures.[16] TLR signal transduction begins when 1 or more adaptor proteins are recruited to the intracellular TIR (Toll/interleukin-1 receptor) domain of the receptor. These adaptors include myeloid differentiation primary response protein

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Reduced Cytochrome *c* Is an Essential Regulator of Sustained Insulin Secretion by Pancreatic Islets*

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Influx of calcium is an essential but insufficient signal in sustained nutrient-stimulated insulin secretion, and increased metabolic rate of the beta cell is also required. The aim of the study was to test the hypothesis that the reduced state of cytochrome *c* is a metabolic co-factor necessary for insulin secretion, over and above its participation in the ATP-generating function of electron transport/oxidative phosphorylation. We found that nutrient stimulation of insulin secretion by isolated rat islets was strongly correlated with reduced cytochrome *c*, and agents that acutely and specifically reduced cytochrome *c* led to increased insulin secretion, even in the face of decreased oxygen consumption and calcium influx. In contrast, neither sites 1 nor 4 of the electron transport chain were both necessary and essential for the stimulation of insulin secretion to occur. Importantly, stimulation of islets with glucose, α-ketoisocaproate, or glyceraldehyde resulted in the appearance of cytochrome *c* in the cytosol, suggesting a pathway for the regulation of exocytotic machinery by reduction of cytochrome *c*. The data suggest that the metabolic factor essential for sustained calcium-stimulated insulin secretion to occur is linked to reduction and translocation of cytochrome *c*.

It is well established that a major determinant in the development of Type 2 diabetes is failure of pancreatic beta cells to compensate for increased demand for insulin secretion (1, 2). It follows that there is a critical need to understand the fundamental mechanisms regulating insulin secretion. An essential factor for sustained insulin secretory response to glucose is an increase in calcium (Ca^{2+}) influx via L-type Ca^{2+} channels (3–6). This increased Ca^{2+} influx occurs when the metabolism of glucose generates ATP, leading to the closure of ATP-sensitive potassium (K_{ATP}) channels and the resultant opening of voltage-dependent L-type Ca^{2+} channels (7). However, it is recognized that increased Ca^{2+} in the absence of the elevated metabolic rate does not elicit second-phase insulin secretion (8, 9). For example, at low glucose, agents that increased Ca^{2+} influx such as potassium (K^+) (10), tolbutamide, or arginine (11) elicited only a rapid transient response in insulin secretion that quickly waned. Thus, there are additional co-factors generated when substrates are metabolized that in concert with Ca^{2+} are essential for a physiologic response of insulin secretion.

These factors have been long sought after as reviewed (12–14) and a number of candidates have been investigated including products of pyruvate cycling (NADPH and α-ketoglutarate) (15), anaplerosis (16), as well as K_{ATP} channel-independent affects of ATP/ADP (17) and long chain acyl-CoA (12). Since we first began measuring reduced cytochrome *c* in rat islets (18), we noticed that sustained insulin secretion never occurred unless accompanied by an increase in this parameter. An increase in cytochrome *c* reduction occurred in response to changes in metabolic substrates that can independently increase insulin secretion such as glucose and α-ketoisocaproate (KIC),[2] but not with potentiators of nutrient-stimulated insulin secretion such as GLP-1, arginine, and acetylcholine that do not have an affect on ISR in the absence of nutrients. Notably, cytochrome *c* reduction did not change in response to agents that altered the oxygen consumption rate (OCR) due to changes in workload (18, 19), an intuitively requisite attribute for a control element that would faithfully transduce the glucose signal. Thus, we reasoned that cytochrome *c* reduction behaves like an ideal signal transduction mechanism supporting the function of the beta cell as a glucose sensor that would be independent of metabolic need by intracellular processes. Cytochrome *c* is the only protein in the electron transport chain (ETC) that is mobile, and is a known regulatory protein representing a committed step in apoptosis by binding to APAF-1 (20). In addition, its reductive state is linked to the generation of reactive oxygen species (21, 22) and mitochondrial pH (23), factors thought to stimulate ISR. Based on these data we hypothesized that the reduction of cytochrome *c* is a metabolic co-factor necessary for Ca^{2+}-mediated insulin secretion, over and above its participation in the ATP-generating function of ETC/oxidative phosphorylation.

In addition to testing this hypothesis, in this study, we endeavored to elucidate where in the stimulus-secretion coupling pathway reduced cytochrome *c* might act. Our predictions of how the essential metabolic factor could facilitate Ca^{2+}-stimulated insulin secretion were based on a conceptual

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[2] The abbreviations used are: KIC, α-ketoisocaproate; CMCP, Ca^{2+}/metabolic coupling process; ETC, electron transport chain; ISR, insulin secretion rate; K^+, potassium; K_{ATP}, ATP-sensitive potassium channel; KCN, potassium cyanide; OCR, oxygen consumption rate; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; RFU, relative fluorescence unit.

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Cell Injury, Repair, Aging, and Apoptosis

β-Cell Loss and β-Cell Apoptosis in Human Type 2 Diabetes Are Related to Islet Amyloid Deposition

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Amyloid deposition and reduced β-cell mass are pathological hallmarks of the pancreatic islet in type 2 diabetes; however, whether the extent of amyloid deposition is associated with decreased β-cell mass is debated. We investigated the possible relationship and, for the first time, determined whether increased islet amyloid and/or decreased β-cell area quantified on histological sections is correlated with increased β-cell apoptosis. Formalin-fixed, paraffin-embedded human pancreas sections from subjects with (n = 29) and without (n = 39) diabetes were obtained at autopsy (64 ± 2 and 70 ± 4 islets/subject, respectively). Amyloid and β cells were visualized by thioflavin S and insulin immunolabeling. Apoptotic β cells were detected by colabeling for insulin and by TUNEL. Diabetes was associated with increased amyloid deposition, decreased β-cell area, and increased β-cell apoptosis, as expected. There was a strong inverse correlation between β-cell area and amyloid deposition ($r = -0.42$, $P < 0.001$). β-Cell area was selectively reduced in individual amyloid-containing islets from diabetic subjects, compared with control subjects, but amyloid-free islets had β-cell area equivalent to islets from control subjects. Increased amyloid deposition was associated with β-cell apoptosis ($r = 0.56$, $P < 0.01$). Thus, islet amyloid is associated with decreased β-cell area and increased β-cell apoptosis, suggesting that islet amyloid deposition contributes to the decreased β-cell mass that characterizes type 2 diabetes. ***(Am J Pathol 2011, 178:2632–2640; DOI: 10.1016/j.ajpath.2011.02.036)***

Type 2 diabetes is characterized by insulin resistance and β-cell failure,[1] the latter resulting from reductions in β-cell function[2,3] and/or β-cell mass.[4–6] Together, these contribute to impaired insulin release and the inability to maintain euglycemia without glucose-lowering therapy.

A pathological hallmark of the pancreatic islet in type 2 diabetes is islet amyloid deposition. These deposits occur in the majority of patients with diabetes,[5,7–10] but have also been reported in a small proportion of subjects who are apparently nondiabetic (but may have undiagnosed abnormalities in glucose metabolism), and especially in those who are older.[7,11] The formation of islet amyloid occurs by aggregation of islet amyloid polypeptide (IAPP, or amylin),[12,13] which is normally cosecreted with insulin by the β cell.[14]

In vitro studies have demonstrated that the process of IAPP aggregation is cytotoxic, resulting in β-cell apoptosis.[15,16] *In vivo* studies of spontaneous islet amyloid deposition in nonhuman primates and in domestic cats,[17–20] as well as in transgenic rodent models of islet amyloid formation,[21–23] have shown that the accumulation of islet amyloid formation precedes fasting hyperglycemia and is associated with decreased β-cell function and β-cell loss.

Human studies investigating the relationship between β-cell mass and islet amyloid are more limited. Several

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RESEARCH COMMUNICATION

Context-specific α-to-β-cell reprogramming by forced Pdx1 expression

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Using single transcription factors to reprogram cells could produce important insights into the epigenetic mechanisms that direct normal differentiation, or counter inappropriate plasticity, or even provide new ways of manipulating normal ontogeny in vitro to control lineage diversification and differentiation. We enforced *Pdx1* expression from the Neurogenin-3-expressing endocrine commitment point onward and found during the embryonic period a minor increased β-cell allocation with accompanying reduced α-cell numbers. More surprisingly, almost all remaining Pdx1-containing glucagon/Arx-producing cells underwent a fairly rapid conversion at postnatal stages, through glucagon–insulin double positivity, to a state indistinguishable from normal β cells, resulting in complete α-cell absence. This α-to-β conversion was not caused by activating Pdx1 in the later glucagon-expressing state. Our findings reveal that Pdx1 can work single-handedly as a potent context-dependent autonomous reprogramming agent, and suggest a postnatal differentiation evaluation stage involved in normal endocrine maturation.

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A major hurdle for cell replacement-based diabetes therapy is the difficulty of supplying vast numbers of functioning insulin-producing β cells. One method could be through the reprogramming of alternative cell types. While this process might be easier with closely lineage-related cells, even substantially different cells may be susceptible (e.g., Zhou et al. 2008).

Recent studies reveal significant plasticity between pancreatic α and β cells under certain induced conditions, implying a potential route to β cells through α cells. In a near-total β-cell destruction and regeneration model in adult mice, a proportion of new β cells were produced from α cells via a bihormonal glucagon$^+$insulin$^+$ (Gcg$^+$Ins$^+$) transitional state (Thorel et al. 2010). The interconversion presumably occurs in response to a combination of the physiological need to replenish β cells and regeneration-induced stress, raising questions as to the local or systemic signals triggered by such lesions. Direct superimposition of a pro-β-lineage condition was reported when *Pax4* expression was forced in pancreatic or endocrine progenitors or in embryonic α cells to redirect endocrine differentiation or coax pre-existing α cells into β cells. The converted cells seemed similar to normal β cells and temporarily improved glycemia under induced diabetes, although the effect was superseded by uncontrolled α-cell neogenesis and fatality caused by extreme hyperglycemia (Collombat et al. 2009). These studies on the ability of a single lineage-allocating transcription factor to sustain complete cell fate conversion suggest that similar analyses for other transcription factors could be insightful. Determining which factors induce specific types of lineage reprogramming, as well as the repertoire of cellular competence states amenable to fate switching, could lead to pharmacological intervention to activate such factors in vivo, or to improved differentiation of embryonic stem cells to β cells.

Clues to the fate-instructing capacity of *Pdx1* as a β-cell selector are inferred from its enriched expression in embryonic and mature β cells. Ectopic *Pdx1* alone can induce incomplete reprogramming of liver or pancreatic acinar cells (e.g., Ferber et al. 2000; Heller et al. 2001). A synergistic effect between Pdx1, Neurog3, and MafA was observed when acinar cells were converted into β-like cells (Zhou et al. 2008), which inefficiently ameliorated hyperglycemia caused by loss of endogenous β cells, perhaps because the reprogrammed cells did not assemble into islet-like clusters. Rather than triggering a redirection into endocrine cells, forced *Pdx1* expression in *Ptf1a*-expressing cells caused late stage acinar-to-ductal hyperplasia (Miyatsuka et al. 2006). While these studies suggest that *Pdx1* alone is contextually sufficient to induce partial *trans*-differentiation or *trans*-determination, little is known about how different competence states affect the response to this single factor.

Here, we report on the previously unknown sufficiency for *Pdx1* as a potent regulator of endocrine lineage allocation and maintenance of the mature state. With Pdx1 expression enforced from the Neurog3$^+$ endocrine progenitor state onward, two periods of dominant lineage redirection occurred: (1) during early organogenesis, a minor reproducible reduction in cells directed to the α fate, and (2) a surprising peri/postnatal redirection of Pdx1-expressing α cells, with rapid reprogramming into Ins$^+$ cells that are indistinguishable from normal β cells. The delayed conversion occurred despite α cells having expressed exogenous Pdx1 from their endocrine commitment point onward, suggesting the possibility of a cryptic chromatin-priming effect. In contrast, exogenous Pdx1 in Gcg$^+$ embryonic or adult α cells suppressed Gcg expression but did not induce α/β fate switching. Our findings reveal differential α-to-β plasticity between endocrine progenitors and hormone-secreting cells in response to *Pdx1*. We speculate on the epigenetic ramifications of these differential lineage-switching findings.

[*Keywords*: pancreas; endocrine progenitors; α and β cells; reprogramming; Pdx1]
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Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs

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In rodents, acute brain insulin action reduces blood glucose levels by suppressing the expression of enzymes in the hepatic gluconeogenic pathway, thereby reducing gluconeogenesis and endogenous glucose production (EGP). Whether a similar mechanism is functional in large animals, including humans, is unknown. Here, we demonstrated that in canines, physiologic brain hyperinsulinemia brought about by infusion of insulin into the head arteries (during a pancreatic clamp to maintain basal hepatic insulin and glucagon levels) activated hypothalamic Akt, altered STAT3 signaling in the liver, and suppressed hepatic gluconeogenic gene expression without altering EGP or gluconeogenesis. Rather, brain hyperinsulinemia slowly caused a modest reduction in net hepatic glucose output (NHGO) that was attributable to increased net hepatic glucose uptake and glycogen synthesis. This was associated with decreased levels of glycogen synthase kinase 3β (GSK3β) protein and mRNA and with decreased glycogen synthase phosphorylation, changes that were blocked by hypothalamic PI3K inhibition. Therefore, we conclude that the canine brain senses physiologic elevations in plasma insulin, and that this in turn regulates genetic events in the liver. In the context of basal insulin and glucagon levels at the liver, this input augments hepatic glucose uptake and glycogen synthesis, reducing NHGO without altering EGP.

Introduction

The ability of insulin to suppress hepatic glucose production (HGP) in vivo has been well defined (1), but the role of the CNS in this suppression remains controversial. The brain is an insulin-sensitive organ, and brain insulin action has been shown to regulate whole-body glucose metabolism, in part, by altering pancreatic insulin and glucagon secretion (2–4). Obici et al., on the other hand, demonstrated that i.c.v. infusion of insulin could reduce glucose production by approximately 30% in the rodent, even when insulin was clamped at a basal level, by infusing it into a peripheral vein while infusing somatostatin to inhibit endogenous secretion (5). It should be noted that this clamp protocol would have eliminated the approximately 3-fold insulin gradient that normally exists between the portal vein and arterial blood and created insulin deficiency at the liver. An elevation in HGP secondary to hepatic hypoinsulinemia was prevented by the lack of glucagon replacement during the clamp. Thus, the effect of brain insulin action was observed when the liver was deficient in 2 of its primary glucoregulatory signals. Subsequent studies, many of which used a similar peripheral insulin clamp design, suggested that the mechanism by which insulin (and other factors) acts within the hypothalamus to suppress HGP requires modification of vagal input to the liver (6–8), phosphorylation of hepatic STAT3 (9), and suppression of gluconeogenic gene expression (8–13). Although gluconeogenic gene expression was typically used as a surrogate for gluconeogenesis, one group was able to show that centrally mediated inhibition of HGP was caused by a decrease in gluconeogenesis, although it required a number of hours to be seen (6, 10–12), consistent with regulation at the genetic level. Based on the collective observations in rodents, it has been concluded that insulin signaling in the brain is a physiologically important component of the acute effect of insulin on HGP in vivo (14). Furthermore, aberrant hypothalamic insulin signaling has been suggested to contribute to the disruption of glucose homeostasis associated with the diabetic state, and thus may be a target with therapeutic potential (14–16).

Data from studies in humans and large animals, however, suggest that insulin signaling in the hypothalamus may not play a role in the control of HGP in such species. The regulation of HGP by insulin is maintained in human recipients of liver transplants and in dogs subjected to hepatic denervation (17, 18). Moreover, HGP in dogs is exquisitely sensitive to small changes in portal vein insulin levels, even when the arterial plasma insulin level (and thus the brain insulin level) remains basal (19). In fact, insulin's direct hepatic effect has been established as being dominant (relative to all of its actions in nonhepatic tissues) in regulating HGP (20). It is possible that the suppression of HGP by i.c.v. insulin in rodents was only apparent because of the context in which it was studied (i.e., supraphysiological brain insulin levels, nonphysiologic access to CNS insulin receptors, and/or hepatic hypoinsulinemia and glucagonopenia). Thus, our aim was to determine whether the

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Bridging the Digital Divide in Diabetes: Family Support and Implications for Health Literacy

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Abstract

Background: Patient web portals (PWPs) offer patients remote access to their medical record and communication with providers. Adults with health literacy limitations are less likely to access and use health information technology (HIT), including PWPs. In diabetes, PWP use has been associated with patient satisfaction, patient–provider communication, and glycemic control.
Methods: Using mixed methods, we explored the relationships between health literacy, numeracy, and computer literacy and the usage of a PWP and HIT. Participants ($N = 61$ adults with type 2 diabetes) attended focus groups and completed surveys, including measures of health literacy, numeracy, and computer anxiety (an indicator of computer literacy) and frequency of PWP and HIT use.
Results: Computer literacy was positively associated with health literacy ($r = 0.41$, $P < 0.001$) and numeracy ($r = 0.35$, $P < 0.001$), but health literacy was not associated with numeracy. Participants with limited health literacy (23%), numeracy (43%), or computer literacy (25%) were no less likely to access PWPs or HIT, but lower health literacy was associated with less frequent use of a computer to research diabetes medications or treatments. In focus groups, participants spontaneously commented on family support when accessing and using PWPs or HIT for diabetes management.
Conclusions: Participants reported family members facilitated access and usage of HIT, taught them usage skills, and acted as online delegates. Participant statements suggest family members may bridge the HIT "digital divide" in diabetes by helping adults access a PWP or HIT for diabetes management.

Introduction

Patient web portals (PWPs) are web-based applications linked to an electronic health record. PWP functions often include the ability to view test results, message providers, and manage medical appointments and bills.[1,2] In a recent review evaluating the impact of PWPs on diabetes outcomes,[3] patient usage of a PWP was associated with patient–provider communication, patient satisfaction, diabetes self-care activities, glycemic control, and a reduction in emergency room visits and hospital admissions.

Although an estimated 79% of Americans used the Internet in 2010,[4] there continues to be a "digital divide" in using the internet for health-related reasons by age,[5–8] race/ethnicity,[5] education,[6–8] income,[6] and health literacy.[7–10] Among diabetes patients who have Internet access, those with health literacy limitations are less likely to use a PWP compared with those with adequate health literacy.[9] By definition, health literacy is the ability to understand and act on medical information,[11] numeracy is "the ability to understand and use numbers in daily life,"[12] and computer literacy is "fluency with information technology"[13] or the "ability to seek, find, understand, and appraise information from electronic sources and apply the knowledge gained."[14]

Only one study to our knowledge has explored the role of health literacy and usage of a PWP among individuals with diabetes.[9] Numeracy and computer literacy were not measured in that study, nor was qualitative information gathered to understand the details of patients' usage or non-usage of the PWP. Thus, we used a mixed methods approach to explore the role of patient health literacy, numeracy, and computer literacy on usage of a different PWP and other forms of health information technology (HIT) (e.g., health information and diabetes self-management websites, mobile health applications).

Subjects and Methods

Recruitment and study participants

Focus groups were conducted as part of a larger project studying PWP and HIT use for diabetes management. From

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ORIGINAL ARTICLE

Diabetes and Co-morbid Depression Among Racially Diverse, Low-Income Adults

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Abstract
Background Research suggests individuals with diabetes are twice as likely as those without diabetes to be clinically depressed. Still unknown is the relationship between diabetes and depression in socioeconomically disadvantaged populations.
Purpose We examined the relationship between diabetes and depressive symptoms in a large, racially diverse, low-income cohort in the southeastern USA.
Methods A total of 69,068 adults were recruited from community health centers in 12 southeastern states. A fully adjusted polytomous logistic regression model tested the relationship between demographics, lifestyle behaviors, antidepressant use, body mass index, diabetes diagnosis, diabetes duration, diabetes medication compliance, and depressive symptoms using the Centers for Epidemiological Studies Depression scale.
Results Diabetes was present in 21.7% of sample. While a diabetes diagnosis was associated with having severe depressive symptoms (AOR, 1.24; 95% CI, 1.14–1.34), demographics, lifestyle behaviors, body mass index and antidepressant use were more strongly associated with severe depressive symptoms than a diabetes diagnosis.
Conclusions Having diabetes was associated with the presence and severity of depressive symptoms in a large, low-income sample of racially diverse adults. However, the relationship between diabetes and depressive symptoms was weaker than in other studies with higher socioeconomic groups.

Keywords Depression · Diabetes · Disparities · Income · Education

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Introduction

According to the World Health Organization, depression continues to be highly prevalent, accounts for more disability worldwide than any other psychiatric disorder, and is projected to be the second most prevalent medical condition by 2020 [1]. Prospective, retrospective and meta-analytic studies highlight significantly higher rates of depression among persons with diabetes relative to the general population [2, 3]. The most commonly cited

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Development and Validation of a Spanish Diabetes-Specific Numeracy Measure: DNT-15 Latino

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Abstract

Background: Although deficits in health literacy and numeracy have been described among Latinos, the impact of low numeracy on diabetes outcomes has not been studied. Study objectives were (1) to establish the reliability and validity of a 15-item Spanish, diabetes-specific numeracy measure (Diabetes Numeracy Test [DNT]-15 Latino) and (2) to examine the relationship between diabetes-specific numeracy and diabetes-related outcomes among a sample of Latino adults with diabetes.

Methods: Data collection included patient demographics, health literacy, general numeracy, diabetes-specific numeracy, acculturation, self-efficacy, self-care behaviors, and most recent glycosylated hemoglobin (HbA1c).

Results: Participants ($n = 144$) were on average 47.8 years old (SD = 12.1). The majority were female (62%), uninsured (81%), and of Mexican nationality (78%) and reported low levels of acculturation (96%). The DNT-15 Latino had high internal reliability (Kruder–Richardson 20 = 0.78). The DNT-15 Latino demonstrated construct validity, correlating with measures of health literacy ($\rho = 0.291$), general numeracy ($\rho = 0.500$), education ($\rho = 0.361$), and income ($\rho = 0.270$) ($P < 0.001$ for each). The DNT-15 Latino was significantly associated with acculturation but unrelated to self-efficacy, self-care behaviors, insulin use, and HbA1c.

Conclusions: The DNT-15 Latino is a reliable and valid measure of diabetes-specific numeracy for Latino patients with diabetes; however, additional studies are needed to further explore the association between diabetes-specific numeracy and acculturation and their impact on diabetes-related outcomes for Latinos.

Introduction

According to the Agency for Healthcare Research and Quality, Latinos are a priority group in need of improved healthcare access and quality.[1] Latinos with diabetes often receive care that is suboptimal.[2] For example, compared with non-Latino whites, Latinos have a higher prevalence of type 2 diabetes (11.8% vs. 7.1%),[3] are less likely to receive timely recommended services (such as eye exams, foot exams, and glycosylated hemoglobin [HbA1c] measurement), and are more likely to experience hospital admission for lower-extremity amputations secondary to diabetes-related complications.[2] Caring for patients with diabetes is often a challenge in that self-care plays a major role in successful treatment (e.g., monitoring blood glucose levels, following specific dietary and exercise programs, and administering medications).

Health literacy is "the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions"[4] and has been associated with the ability to perform diabetes self-care activities.[5–8] In a study of 400 English- and Spanish-speaking patients with diabetes, lower health literacy was associated with poorer glycemic control.[5] In 2003, the U.S. Department of Education conducted a study in which 66% of Latinos were found to have basic or below basic health literacy skills.[9] This high prevalence was based on a subsample of Latinos with *some* English proficiency; the prevalence may be even higher in the millions of Latinos residing in the United States who do not speak English.[10]

Quantitative literacy, also referred to as numeracy, is the ability to use and understand numbers in daily life.[8] Examples of specific numeracy skills relevant to diabetes care include the ability to accurately calculate and adjust insulin doses, count carbohydrates, calculate portion sizes from food labels, and understand number hierarchy when testing blood sugar. In general, low numeracy skills have been associated with difficulty understanding health information, less disease-specific knowledge, and difficulty performing self-management tasks,

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Health Literacy Explains Racial Disparities in Diabetes Medication Adherence

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Although low health literacy and suboptimal medication adherence are more prevalent in racial/ethnic minority groups than Whites, little is known about the relationship between these factors in adults with diabetes, and whether health literacy or numeracy might explain racial/ethnic disparities in diabetes medication adherence. Previous work in HIV suggests health literacy mediates racial differences in adherence to antiretroviral treatment, but no study to date has explored numeracy as a mediator of the relationship between race/ethnicity and medication adherence. This study tested whether health literacy and/or numeracy were related to diabetes medication adherence, and whether either factor explained racial differences in adherence. Using path analytic models, we explored the predicted pathways between racial status, health literacy, diabetes-related numeracy, general numeracy, and adherence to diabetes medications. After adjustment for covariates, African American race was associated with poor medication adherence ($r = -0.10$, $p < .05$). Health literacy was associated with adherence ($r = .12$, $p < .02$), but diabetes-related numeracy and general numeracy were not related to adherence. Furthermore, health literacy reduced the effect of race on adherence to nonsignificance, such that African American race was no longer directly associated with lower medication adherence ($r = -0.09$, $p = .14$). Diabetes medication adherence promotion interventions should address patient health literacy limitations.

In diabetes, suboptimal adherence to pharmacotherapy is associated with poor glycemic control (Rhee et al., 2005), and increased risk of hospitalization and mortality (Ho et al., 2006). African Americans are less adherent to their diabetes medications than Whites (Trinacty et al., 2009), and, as a result, have worse glycemic control than Whites (Heisler et al., 2007). However, the factors that explain racial disparities in diabetes medication adherence are unknown. One potential explanatory factor is

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Targeting cyclophilin D and the mitochondrial permeability transition enhances β-cell survival and prevents diabetes in Pdx1 deficiency

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Mutations of the pancreatic duodenal homeobox gene-1, *Pdx1*, cause heritable diabetes in humans and mice. A central abnormality with Pdx1 deficiency is increased death of β-cells, leading to decreased β-cell mass. We show that lentiviral suppression of Pdx1 increases death of mouse insulinoma MIN6 β-cells associated with dissipation of the mitochondrial inner membrane electrochemical gradient, $\Delta\psi_m$. Preventing mitochondrial permeability transition pore opening with the cyclophilin D inhibitor cyclosporin A restored $\Delta\psi_m$ and rescued cell viability. Reduced β-cell mass, markers of β-cell apoptosis, necrosis, and decreased proliferation are present in *Pdx1* haploinsufficient mice. Genetic ablation of the *Ppif* gene, encoding cyclophilin D, restored β-cell mass and decreased TUNEL and complement complex labeling without affecting β-cell proliferation. In adult mice maintained on a high-fat diet, *Ppif* ablation normalized fasting glucose and glucose and insulin responses to acute glucose challenge. Thus, cyclophilin D and the mitochondrial permeability transition are critical regulators of β-cell death caused by Pdx1 insufficiency.

cell necrosis | apoptosis | insulin

Loss of insulin-producing β-cells contributes to type 1 and type 2 diabetes (1, 2). Normal postnatal β-cell homeostasis is maintained largely by self-replication, not differentiation from progenitor cells (3). β-Cell proliferation is also essential for compensatory islet hyperplasia under various conditions of metabolic stress, and therefore for prevention of diabetes (4). Proliferation is opposed by programmed β-cell elimination; β-cell mass in the adult pancreas is dynamically regulated by constant adjustment of the balance between these two processes (5).

The transcription factor, pancreatic duodenal homeobox gene-1 (*Pdx1*) regulates β-cell proliferation and other events in insulin secretion during normal pancreatic development and in response to insulin-resistant states (4, 6). In humans, loss-of-function mutations of this factor are a cause of familial early-onset diabetes (7). In mice, germ-line *Pdx1* haploinsufficiency and β-cell–specific *Pdx1* ablation produce glucose intolerance associated with decreased β-cell mass, a proportional increase in islet α-cells, and markers of β-cell apoptosis (4, 8–10). The observation that insufficiency of this β-cell proliferative factor not only impairs normal islet development and reactive islet hyperplasia, but also induces apoptosis in its target cells, suggests a mechanistic connection between Pdx1 regulation of β-cell cycling and programmed elimination.

There are three distinct mechanisms of programmed cell death: apoptosis, autophagy, and necrosis. Although the stimuli for, and signaling of, these cell death pathways are distinct, they share a critical involvement of mitochondria (11). Mitochondrial dysfunction resulting in decreased levels of ATP is a potent stimulus for autophagy. Permeabilization of the mitochondrial outer membranes releases cytochrome *c* that activates the apoptosis caspase cascade. Finally, opening of the mitochondrial permeability transition pore (MPTP) is the irreversible step in programmed necrosis. The latter process plays an important role in the pathogenesis of Alzheimer's disease and amyotrophic lateral sclerosis (12, 13).

Mitochondria are implicated in the pathogenesis of diabetes mellitus (14). Recently, a connection between Pdx1 insufficiency and mitochondrial dysfunction was described, implicating dysregulation of the mitochondrial transcription factor TFAM in abnormal insulin secretion (15). However, a cause–effect relationship between mitochondria and programmed β-cell death in diabetes has not been identified. Here, we combined studies in insulinoma cells with in vivo genetic complementation to examine the role of cell death by MPTP-dependent programmed necrosis in Pdx1 insufficiency. Our results reveal an essential role for MPTP opening in β-cell death and show that pharmacological or genetic inhibition of the mitochondrial permeability transition can protect against programmed β-cell necrosis and prevent diabetes in Pdx1 insufficiency.

Results

Cyclosporin A Protects MIN6 Cells Against Death Induced by Pdx1 Insufficiency. Suppression or genetic ablation of Pdx1 in vitro and in vivo has previously revealed that Pdx1 insufficiency impairs islet growth both by inhibiting β-cell proliferation and stimulating β-cell programmed death (4, 9, 16). In our studies of Pdx1 deficient β-cell death, we observed markers for both apoptosis (cleaved caspase-3) and autophagy (LC3 punctae, autophagic vesicles). However, inhibition of autophagy only temporarily diminished β-cell death (16). Because caspase activation and autophagy after loss of ATP production might be secondary consequences of primary mitochondrial disruption caused by permeability transition (17), we postulated that the primary cause of Pdx1-deficient β-cell death could be programmed necrosis, rather than apoptosis or autophagy. Accordingly, we used a lentivirus–shRNA system to suppress Pdx1 in mouse MIN6 insulinoma cells (18) and measured cell viability, TUNEL positivity, and mitochondrial inner membrane potential as a function of Pdx1 level. As previously described (16), expression of Pdx1 protein was decreased by ≈70% by shRNA in MIN6 cells (Fig. 1*A*). Compared with scrambled control shRNA, Pdx1 shRNA markedly increased cell death measured as propidium iodide (PI) staining (Fig. 1*B*) and TUNEL labeling (Fig. 1*C*). Strikingly, Pdx1 suppression also dissipated the inner mitochondrial membrane potential, $\Delta\psi_m$, measured as loss of fluorescence for the voltage-sensitive mitochondrial dye rhodamine 123 (Fig. 1*D*).

Author contributions: K.F. and Y.C. performed research; K.F., K.S.P., and G.W.D. analyzed data; Y.C. contributed new reagents/analytic tools; K.S.P. and G.W.D. designed research; and K.F., K.S.P., and G.W.D. wrote the paper.

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RNA sensor–induced type I IFN prevents diabetes caused by a β cell–tropic virus in mice

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Viral infections have been linked to the onset of type I diabetes (T1D), with viruses postulated to induce disease directly by causing β cell injury and subsequent release of autoantigens and indirectly via the host type I interferon (IFN-I) response triggered by the virus. Consistent with this, resistance to T1D is associated with polymorphisms that impair the function of melanoma differentiation associated gene-5 (*MDA5*), a sensor of viral RNA that elicits IFN-I responses. In animal models, triggering of another viral sensor, TLR3, has been implicated in diabetes. Here, we found that MDA5 and TLR3 are both required to prevent diabetes in mice infected with encephalomyocarditis virus strain D (EMCV-D), which has tropism for the insulin-producing β cells of the pancreas. Infection of *Tlr3*$^{-/-}$ mice caused diabetes due to impaired IFN-I responses and virus-induced β cell damage rather than T cell–mediated autoimmunity. Mice lacking just 1 copy of Mda5 developed transient hyperglycemia when infected with EMCV-D, whereas homozygous *Mda5*$^{-/-}$ mice developed severe cardiac pathology. TLR3 and MDA5 controlled EMCV-D infection and diabetes by acting in hematopoietic and stromal cells, respectively, inducing IFN-I responses at kinetically distinct time points. We therefore conclude that optimal functioning of viral sensors and prompt IFN-I responses are required to prevent diabetes when caused by a virus that infects and damages the β cells of the pancreas.

Introduction

Innate responses to viruses depend on pathogen recognition sensors that detect viral products and trigger the secretion of type I interferons (IFN-I), i.e., IFN-β and IFN-α (1–4). Two types of sensors detect double-stranded (ds) RNA generated during infection with RNA viruses: endosomal TLR-3 and cytoplasmic retinoic acid-inducible gene-I–like (RIG-I–like) receptors (RLR). TLR3 senses the dsRNAs that reach the endosomal compartment after phagocytosis of virally infected cells or apoptotic debris. RLRs include 2 IFN-inducible helicases, melanoma differentiation-associated gene 5 (MDA5) and RIG-I, which detect dsRNA intermediates that are generated in the cytoplasm during viral replication. MDA5 specializes in the detection of picornaviruses, whereas RIG-I senses many other RNA viruses (5). These viral specificities depend on the ability of MDA5 and RIG-I to recognize dsRNA molecules of different lengths, structures, and 5′ caps (6–9).

TLR3 transmits its intracellular signal through the adaptor protein Toll/IL-1 receptor domain–containing adaptor-inducing IFN-β (TRIF), which activates the transcription factor IFN regulatory factor-3 (IRF-3) and induces IFN-β production (1–4). RIG-I and MDA5 signal through another adaptor, IFN-β promoter stimulator-1 (IPS-1), which activates IRF-3 and IRF-7, inducing both IFN-β and IFN-α production. A third RLR, laboratory of genetics and physiology-2 (LGP2), detects dsRNA but lacks signaling domains and is thought to regulate MDA5 and RIG-I (10–12). IFN-I induces an antiviral state in uninfected cells and promote apoptosis of infected cells, limiting viral spread (13). Moreover, IFN-I has independent immunomodulatory effects and helps to orchestrate NK, T, and B cell responses, which facilitate viral clearance (13–15).

Type I diabetes (T1D) is an autoimmune disease that is primarily caused by selective destruction of islet β cells of the endocrine pancreas by autoreactive T cells (16, 17). Predisposing genetic factors, particularly MHC class II polymorphisms, play a predominant role in the pathogenesis of T1D. However, clinical (18–22) and experimental (23–25) studies have suggested that viral infections also may contribute to T1D, particularly infections by members of the enterovirus family of RNA viruses. These viruses may induce T1D by directly causing β cell damage and subsequent release of autoantigens that trigger autoreactive T cells (26–29). Paradoxically, the host IFN-I response can have detrimental effects when it activates preexisting autoreactive T cells that escaped thymic selection (30–33). Accordingly, a synthetic analog of viral dsRNA, poly(I:C), precipitates diabetes in mouse and rat models, acting in part through TLR3 (34–40). Consistent with this, genetic studies have recently shown that resistance to T1D is associated with polymorphisms in *MDA5* that diminish the IFN-I response to dsRNA (41–44). Collectively, these studies pose a conundrum: by eliciting IFN-I response, dsRNA sensors may limit the cytopathic effect of β cell–tropic viruses, but they can also precipitate T1D by promoting IFN-I–induced autoimmunity.

Herein, we evaluated the contributions of MDA5 and TLR3 to the host response to virus infection and development of diabetes using encephalomyocarditis virus strain D (EMCV-D). EMCV-D has preferential tropism for pancreatic β cells and can induce diabetes in selective mouse strains, such as DBA/2 (45, 46), as well

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Recurrent Moderate Hypoglycemia Ameliorates Brain Damage and Cognitive Dysfunction Induced by Severe Hypoglycemia

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OBJECTIVE—Although intensive glycemic control achieved with insulin therapy increases the incidence of both moderate and severe hypoglycemia, clinical reports of cognitive impairment due to severe hypoglycemia have been highly variable. It was hypothesized that recurrent moderate hypoglycemia preconditions the brain and protects against damage caused by severe hypoglycemia.

RESEARCH DESIGN AND METHODS—Nine-week-old male Sprague-Dawley rats were subjected to either 3 consecutive days of recurrent moderate (25–40 mg/dl) hypoglycemia (RH) or saline injections. On the fourth day, rats were subjected to a hyperinsulinemic (0.2 units · kg^{-1} · min^{-1}) severe hypoglycemic (~11 mg/dl) clamp for 60 or 90 min. Neuronal damage was subsequently assessed by hematoxylin-eosin and Fluoro-Jade B staining. The functional significance of severe hypoglycemia–induced brain damage was evaluated by motor and cognitive testing.

RESULTS—Severe hypoglycemia induced brain damage and striking deficits in spatial learning and memory. Rats subjected to recurrent moderate hypoglycemia had 62–74% less brain cell death and were protected from most of these cognitive disturbances.

CONCLUSIONS—Antecedent recurrent moderate hypoglycemia preconditioned the brain and markedly limited both the extent of severe hypoglycemia–induced neuronal damage and associated cognitive impairment. In conclusion, changes brought about by recurrent moderate hypoglycemia can be viewed, paradoxically, as providing a beneficial adaptive response in that there is mitigation against severe hypoglycemia–induced brain damage and cognitive dysfunction. ***Diabetes*** **59:1055–1062, 2010**

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Hypoglycemia is the major obstacle in achieving tight glycemic control in people with diabetes (1). Intensive insulin therapy increases the risk of iatrogenic hypoglycemia (2). Episodes of both moderate and severe hypoglycemia have long-term clinical consequences. Recurrent moderate hypoglycemia induces a maladaptive response that limits symptoms of hypoglycemia (hypoglycemia unawareness), limits the counterregulatory response to subsequent hypoglycemia (hypoglycemia-associated autonomic failure), and thus jeopardizes patient safety (1). By depriving the brain of glucose, more severe hypoglycemia causes brain damage in animal studies and leads to long-term impairments in learning and memory (3,4). However, studies examining the effect of severe hypoglycemia in humans are conflicting. Severe hypoglycemia has been shown to alter brain structure (5–7) and cause significant cognitive damage in many (5,7–12) but not all (13–16) studies. Reasons for the discrepancy between human and animal studies are unknown, but a major contributing factor may be the extent of glycemia control (including recurrent hypoglycemia) prior to the episode of severe hypoglycemia.

In other models of brain damage, such as ischemic stroke, brief, mild episodes of antecedent brain ischemia has been shown to cause a beneficial adaptation that protects the brain against a subsequent episode of more severe ischemia (a phenomena known as ischemic preconditioning) (17). In a similar fashion, antecedent, recurrent episodes of moderate hypoglycemia were hypothesized to protect the brain against damage caused by a subsequent episode of more severe hypoglycemia.

To investigate this hypothesis, recurrent moderately hypoglycemic (25–40 mg/dl) rats (RH rats) and control saline-injected rats (CON rats) were subjected to hyperinsulinemic, severe hypoglycemic clamps (10–15 mg/dl). One group of rats was killed 1 week after severe hypoglycemia to quantify brain damage, while a second group of rats was evaluated by behavioral and cognitive tests 6–8 weeks after the severe hypoglycemia. The results demonstrated that recurrent antecedent moderate hypoglycemia preconditioned the brain and protected it against neurological damage and cognitive defects induced by an episode of severe hypoglycemia.

RESEARCH DESIGN AND METHODS

Nine-week-old male Sprague-Dawley rats (Charles River Laboratories) were individually housed in a temperature- and light-controlled environment maintaining the animal's diurnal cycle (12 h light and 12 h dark) with an ad libitum standard rat chow diet. All studies were done in accordance with the Animal Studies Committee at the Washington University School of Medicine.

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● *Original Contribution*

BAYESIAN PARAMETER ESTIMATION FOR CHARACTERIZING THE CYCLIC VARIATION OF ECHOCARDIOGRAPHIC BACKSCATTER TO ASSESS THE HEARTS OF ASYMPTOMATIC TYPE 2 DIABETES MELLITUS SUBJECTS

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Abstract—Previous studies have shown that effective quantification of the cyclic variation of myocardial ultrasonic backscatter over the heart cycle might provide a non-invasive technique for identifying the early onset of cardiac abnormalities. These studies have demonstrated the potential for measurements of the magnitude and time delay of cyclic variation for identifying early onset of disease. The goal of this study was to extend this approach by extracting additional parameters characterizing the cyclic variation in an effort to better assess subtle changes in myocardial properties in asymptomatic subjects with type 2 diabetes. Echocardiographic images were obtained on a total of 43 age-matched normal control subjects and 100 type 2 diabetics. Cyclic variation data were generated by measuring the average level of ultrasonic backscatter over the heart cycle within a region of interest placed in the posterior wall of the left ventricle. Cyclic variation waveforms were modeled as piecewise linear functions, and quantified using a novel Bayesian parameter estimation method. Magnitude, rise time and slew rate parameters were extracted from models of the data. The ability of each of these parameters to distinguish between normal and type 2 diabetic subjects, and between subjects grouped by glycated hemoglobin (HbA1c) was compared. Results suggest a significant improvement in using measurements of the rise time and slew rate parameters of cyclic variation to differentiate ($P < 0.001$) the hearts of patients segregated based on widely employed indices of diabetic control compared to differentiation based on the magnitude of cyclic variation. (E-mail: james.g.miller@wustl.edu) **© 2011 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Diabetes mellitus, Cardiomyopathy, Ultrasonics, Echocardiography, Tissue characterization, Bayesian probability theory.**

INTRODUCTION

Type 2 diabetes mellitus is a known risk factor for coronary artery disease and subsequent heart failure. In addition, an increasing body of evidence indicates that diabetes can lead to heart disease independent of atherosclerosis, a condition known as "diabetic cardiomyopathy" (Fang et al. 2004; Hamby et al. 1974; Kannel et al. 1974; Witteles and Fowler 2008; Rijzewijk et al. 2008). The mechanisms underlying the development of diabetic cardiomyopathy are not fully understood, but several studies suggest that lipid metabolic abnormalities may play a role in lipid accumulation in nonadipose tissue, including myocardium, and that the accumulation of lipids in myocardium contributes to cell dysfunction, cell death and, subsequently, cardiomyopathy (Kusminski et al. 2009; Augustus et al. 2003; Carley and Severson 2005; Peterson et al. 2004; Stremmel 1988; Borradaile and Schaffer 2005; Chiu et al. 2001; Finck et al. 2003; Nielsen et al. 2002; Rijzewijk et al. 2008; Zhou et al. 2000).

Ultrasonic backscatter from myocardium has long been known to vary systematically over the cardiac cycle (Madaras et al. 1983; Mottley et al. 1984; Barzilai et al. 1984; Wickline et al. 1985; Mobley et al. 1995; Naito et al. 1996; Bello et al. 1998; Hu et al. 2003; Holland et al. 2004, 2007, 2009; Gibson et al. 2009). Quantification of this cyclic variation of myocardial backscatter has provided a tool for noninvasive ultrasonic tissue characterization in a range of pathologies, including diabetes (Gibson et al. 2009; Holland et al. 2007; Wagner et al. 1995; Bello et al. 1995; Pérez et al. 1992). Traditionally, cyclic variation has been quantified by using the magnitude and time

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Accelerating Evidence Reviews and Broadening Evidence Standards to Identify Effective, Promising, and Emerging Policy and Environmental Strategies for Prevention of Childhood Obesity

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Keywords

intervention, children/youth, nutrition, physical activity

Abstract

The childhood obesity epidemic has stimulated the emergence of many policy and environmental strategies to increase healthy eating and active living, with relatively few research recommendations identifying the most effective and generalizable strategies. Yet, local, state, and national decision makers have an urgent need to take action, particularly with respect to lower-income and racial and ethnic populations at greatest risk. With the surge of promising and emerging policy and environmental strategies, this review provides a framework, criteria, and process modeled from existing expert classification systems to assess the strength of evidence for these strategies. Likewise, this review highlights evidence gaps and ways to increase the types and amount of evidence available to inform policy and environmental strategies. These priorities include documenting independent and interdependent effects, determining applicability to different populations and settings, assessing implementation fidelity and feasibility, identifying cumulative benefits and costs, ascertaining impacts on health equity, and tracking sustainability.

Sex and Type 2 Diabetes: Obesity-Independent Effects on Left Ventricular Substrate Metabolism and Relaxation in Humans

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Patients with type 2 diabetes (T2DM), particularly women, are at risk for heart failure. Myocardial substrate metabolism derangements contribute to cardiac dysfunction in diabetic animal models. The purpose of this study was to determine the effects of diabetes and sex on myocardial metabolism and diastolic function in humans, separate from those of obesity. Thirty-six diabetic subjects (22 women) and 36 nondiabetic, BMI-matched subjects (21 women) underwent positron emission tomography (myocardial metabolism) and echocardiography (structure, function). Myocardial blood flow and oxygen consumption (MVO_2) were higher in women than men ($P = 0.003$ and <0.0001, respectively). Plasma fatty acid (FA) levels were higher in diabetics (vs. obese, $P < 0.003$) and sex and diabetes status interacted in its prediction ($P = 0.03$). Myocardial FA utilization, oxidation, and esterification were higher and percent FA oxidation lower in diabetics (vs. obese, $P = 0.0004$, $P = 0.007$, $P = 0.002$, $P = 0.02$). FA utilization and esterification were higher and percent FA oxidation lower in women (vs. men, $P = 0.03$, $P = 0.01$, $P = 0.03$). Diabetes and sex did not affect myocardial glucose utilization, but myocardial glucose uptake/plasma insulin was lower in the diabetics ($P = 0.04$). Left ventricular relaxation was lower in diabetics ($P < 0.0001$) and in men ($P = 0.001$), and diabetes and sex interacted in its prediction ($P = 0.03$). Sex, T2DM, or their interaction affect myocardial blood flow, MVO_2, FA metabolism, and relaxation separate from obesity's effects. Sexually dimorphic myocardial metabolic and relaxation responses to diabetes may play a role in the known cardiovascular differences between men and women with diabetes.

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INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) in the United States has increased dramatically over the past 30 years, paralleled by increases in the prevalence of obesity (1–3). Both entities are associated with significant cardiovascular morbidity and mortality, with cardiovascular disease as the leading cause of death in diabetics (4,5). Both obesity and T2DM are predictors of left ventricular (LV) dysfunction, independent of associated comorbidities such as coronary artery disease and hypertension (3). Diabetes also increases the mortality risk in patients with LV dysfunction. However, T2DM does not affect men and women in exactly the same way. Women with T2DM have both a higher risk of developing heart failure (4) and a higher risk of dying from heart failure than men with diabetes (6). However, the reasons for sex differences in diabetic cardiovascular disease remain poorly understood.

Results of studies in animal models of T2DM suggest that derangements in myocardial substrate metabolism contribute to the development of cardiac dysfunction and heart failure in diabetes. Specifically, excessive fatty acid (FA) uptake, oxidation and/or storage contribute directly to cardiac dysfunction in these models (7–9). In addition, excessive dependence on FA metabolism in the diabetic heart may impair the heart's ability to cope with superimposed conditions that require a switch toward glucose metabolism (such as ischemia). Few previous studies evaluated myocardial substrate metabolism rates in patients with T2DM but without significant coronary artery disease (10,11). Even fewer measured myocardial fatty acid metabolism (12), and none have evaluated the combined effect

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Circulating glucose levels modulate neural control of desire for high-calorie foods in humans

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Obesity is a worldwide epidemic resulting in part from the ubiquity of high-calorie foods and food images. Whether obese and nonobese individuals regulate their desire to consume high-calorie foods differently is not clear. We set out to investigate the hypothesis that circulating levels of glucose, the primary fuel source for the brain, influence brain regions that regulate the motivation to consume high-calorie foods. Using functional MRI (fMRI) combined with a stepped hyperinsulinemic euglycemic-hypoglycemic clamp and behavioral measures of interest in food, we have shown here that mild hypoglycemia preferentially activates limbic-striatal brain regions in response to food cues to produce a greater desire for high-calorie foods. In contrast, euglycemia preferentially activated the medial prefrontal cortex and resulted in less interest in food stimuli. Indeed, higher circulating glucose levels predicted greater medial prefrontal cortex activation, and this response was absent in obese subjects. These findings demonstrate that circulating glucose modulates neural stimulatory and inhibitory control over food motivation and suggest that this glucose-linked restraining influence is lost in obesity. Strategies that temper postprandial reductions in glucose levels might reduce the risk of overeating, particularly in environments inundated with visual cues of high-calorie foods.

Introduction

Glucose is an important regulatory signal and the primary fuel source for the brain (1). Specialized glucose-sensing neurons located in the hypothalamus, hindbrain, and forebrain are important in the control of glucose homeostasis and feeding behavior (1, 2). Transient declines in blood glucose increase hunger and therefore mobilize an individual toward food consumption (3, 4), particularly high-sugar (5) and high-fat foods (6). Further, hypoglycemia provokes a physiological stress response to mobilize the individual toward seeking food and restoring glucose levels (6). While the role of hindbrain and hypothalamic neuronal responses in hypoglycemia and maintaining energy homeostasis is well characterized (1, 2, 7), the specific neural mechanisms mediating the motivational drive for food under mild hypoglycemic conditions are not known. We hypothesized that a reduction in circulating glucose, to levels commonly observed several hours after glucose ingestion in healthy individuals (8), would activate brain reward and motivation pathways, including the striatum and insula, while concomitantly increasing desire for high-calorie foods.

To test this hypothesis, we performed functional MRI (fMRI) studies in 14 healthy (9 nonobese and 5 obese) subjects 2 hours after ingestion of a standardized lunch. Subjects viewed high-calorie food, low-calorie food, and non-food images while lying in the scanner during a stepped hyperinsulinemic euglycemic-hypoglycemic clamp. To control for potential session order effects, 7 additional subjects viewed the same pictures during a hyperinsulinemic euglycemic clamp under identical conditions (Figure 1A). Behavioral ratings of wanting and liking were presented after each food and non-food image (Figure 1B), and hunger ratings were assessed at the beginning and end of each phase. This approach allowed us to identify how a standardized reduction in circulating glucose, independent of changes in circulating insulin, interacts with external food cues to modulate the neural circuitry that controls feeding behavior.

Results

Metabolic changes

Plasma glucose was maintained at 88 ± 2 mg/dl during the euglycemic phase and reduced to 67 ± 1 mg/dl during the hypoglycemic phase of the study. Glucose levels were maintained at 92 ± 4 mg/dl throughout the euglycemic control study (Figure 2A). Plasma insulin levels were equivalent during both study sessions (Figure 2B). Plasma cortisol levels were significantly higher during the hypoglycemic versus the euglycemic phase ($P = 0.003$) but were not different throughout the euglycemic control study (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI57873DS1). The mild hypoglycemic stimulus did not significantly alter plasma epinephrine, glucagon, leptin, or ghrelin levels. Growth hormone levels rose (hypoglycemic: 11.5 ± 1; euglycemic: 5.9 ± 2 ng/dl, $P < 0.001$) and C-peptide levels declined (hypoglycemic: 0.50 ± 0.07; euglycemic: 0.87 ± 0.1 ng/ml, $P < 0.001$) during the hypoglycemic versus the euglycemic phase, and they remained unchanged during the euglycemic control study.

Authorship note: Kathleen A. Page and Dongju Seo contributed equally to this work and serve as joint first authors. Rajita Sinha and Robert S. Sherwin contributed equally and serve as joint senior authors.

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Cell Metabolism
Article

Deletion of the Mammalian *INDY* Homolog Mimics Aspects of Dietary Restriction and Protects against Adiposity and Insulin Resistance in Mice

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SUMMARY

Reduced expression of the *Indy* (*I'm Not Dead, Yet*) gene in *D. melanogaster* and its homolog in *C. elegans* prolongs life span and in *D. melanogaster* augments mitochondrial biogenesis in a manner akin to caloric restriction. However, the cellular mechanism by which *Indy* does this is unknown. Here, we report on the knockout mouse model of the mammalian *Indy* (*mIndy*) homolog, *SLC13A5.* Deletion of *mIndy* in mice (*mINDY*$^{-/-}$ mice) reduces hepatocellular ATP/ADP ratio, activates hepatic AMPK, induces PGC-1α, inhibits ACC-2, and reduces SREBP-1c levels. This signaling network promotes hepatic mitochondrial biogenesis, lipid oxidation, and energy expenditure and attenuates hepatic de novo lipogenesis. Together, these traits protect *mINDY*$^{-/-}$ mice from the adiposity and insulin resistance that evolve with high-fat feeding and aging. Our studies demonstrate a profound effect of *mIndy* on mammalian energy metabolism and suggest that mINDY might be a therapeutic target for the treatment of obesity and type 2 diabetes.

INTRODUCTION

Energy balance and insulin action are both closely related to life span. Whereas caloric excess leads to obesity, insulin resistance, and increased mortality, caloric restriction reduces adiposity and increases lipid oxidation, insulin sensitivity, and mitochondrial biogenesis. In addition, caloric restriction reverses obesity and type 2 diabetes, delays aging, and prolongs life in many species, including primates (Hursting et al., 2003; Lopez-Lluch et al., 2006; Hunt et al., 2006; Fontana and Klein, 2007; Colman et al., 2009). Mediators of the beneficial effects of caloric restriction include decreased plasma concentrations of anabolic hormones and growth factors, i.e., insulin and insulin-like growth factors (Fontana and Klein, 2007). Reduced expression of the *Indy* (for *I'm Not Dead, Yet*) gene in *D. melanogaster* and its homolog in *C. elegans* has been shown to promote longevity in a manner akin to caloric restriction; however, the cellular mechanism by which reduced expression of *Indy* leads to increased survival is unknown (Rogina et al., 2000; Fei et al., 2003, 2004; Wang et al., 2009).

In *D. melanogaster*, *Indy* encodes a nonelectrogenic dicarboxylate and citrate transporter (Knauf et al., 2002, 2006), and it is mainly expressed in the fat body, midgut, and oenocyte (Rogina et al., 2000), thus, in the major organs of intermediary metabolism in flies. In mammals, the gene product of *SLC13A5*, the sodium-coupled citrate transporter NaCT (mINDY), shares the highest sequence and functional similarity with *D. melanogaster INDY* (Inoue et al., 2002a), and it is predominantly expressed in liver cells (Inoue et al., 2002b; Gopal et al., 2007).

In order to examine the effect that INDY might have on energy metabolism and insulin sensitivity in mammals, we created a knockout mouse model (*mINDY*$^{-/-}$ mice) of the mammalian *Indy* homolog *SLC13A5* (*mIndy*) and assessed the effect of *mIndy* deletion on energy homeostasis and energy storage in vivo. Furthermore, we assessed the impact of *mIndy* deletion on insulin action in young, chow-fed mice, as well as after exposing them to two perturbations known to induce insulin resistance, specifically, high-fat feeding and aging.

LETTER

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Control of T_H17 cells occurs in the small intestine

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Interleukin (IL)-17-producing T helper cells (T_H17) are a recently identified $CD4^+$ T cell subset distinct from T helper type 1 (T_H1) and T helper type 2 (T_H2) cells[1]. T_H17 cells can drive antigen-specific autoimmune diseases and are considered the main population of pathogenic T cells driving experimental autoimmune encephalomyelitis (EAE)[2], the mouse model for multiple sclerosis. The factors that are needed for the generation of T_H17 cells have been well characterized[3–6]. However, where and how the immune system controls T_H17 cells *in vivo* remains unclear. Here, by using a model of tolerance induced by CD3-specific antibody, a model of sepsis and influenza A viral infection (H1N1), we show that pro-inflammatory T_H17 cells can be redirected to and controlled in the small intestine. T_H17-specific IL-17A secretion induced expression of the chemokine CCL20 in the small intestine, facilitating the migration of these cells specifically to the small intestine via the CCR6/CCL20 axis. Moreover, we found that T_H17 cells are controlled by two different mechanisms in the small intestine: first, they are eliminated via the intestinal lumen; second, pro-inflammatory T_H17 cells simultaneously acquire a regulatory phenotype with *in vitro* and *in vivo* immune-suppressive properties (rT_H17). These results identify mechanisms limiting T_H17 cell pathogenicity and implicate the gastrointestinal tract as a site for control of T_H17 cells.

T_H17 cells have been associated with the pathogenesis of several chronic inflammatory disorders, including rheumatoid arthritis and multiple sclerosis[2,7]. To study the cellular and molecular mechanisms that control pathogenicity mediated by T_H17 cells we first used the CD3-specific antibody treatment model. It is known that CD3-specific antibody treatment induces a 'cytokine storm' and local inflammation mainly in the small intestine[8]. Despite this it has been validated as an *in vivo* model of tolerization[9] and is now under study in human clinical trials[10]. By mimicking antigen, CD3-specific antibody treatment leads to activation-induced cell death (AICD) of T cells[11,12] and consequently a systemic upregulation of IL-6 (ref. 9) and transforming growth factor-β (TGF-β1) induced by phagocyte engulfment of apoptotic T cells[13]. In line with these publications, we found that CD3-specific antibody treatment induced an immunoregulatory environment marked by simultaneous expression of TGF-β1 and IL-6 (Fig. 1a). The combination of these cytokines is important for the development of T_H17 cells *in vitro* and *in vivo* as it has been previously clearly established[3,4]. Accordingly, we found elevated levels of IL-17A in plasma of CD3-specific antibody-treated animals compared to controls (Fig. 1a).

First, we aimed to investigate the source of IL-17A. It has been reported that a few hours after injection of CD3-specific antibody, there is a rapid disappearance of the majority of T cells from the

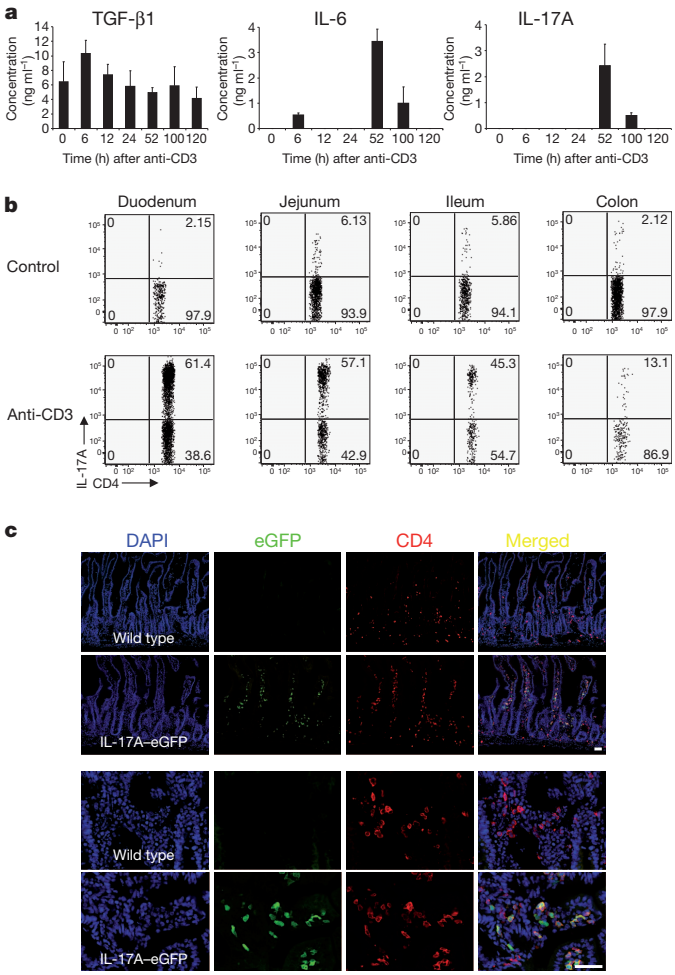

Figure 1 | Accumulation of T_H17 cells in the small intestine after CD3-specific antibody treatment. Mice were injected with CD3-specific antibody. **a**, Plasma levels of TGF-β1, IL-6 and IL-17A. Mean ± s.e.m.; $n = 4$. **b**, Flow cytometric analysis of IL-17A–eGFP expression (gated on $CD4^+TCR\beta^+$ events); numbers in quadrants indicate percent cells in each. **c**, Immunofluorescence staining of frozen sections of the small intestine after CD3-specific antibody treatment (eGFP, green; CD4, red; cell nuclei, DAPI). Scale bar, 50 μm. Data are representative of at least three independent experiments.

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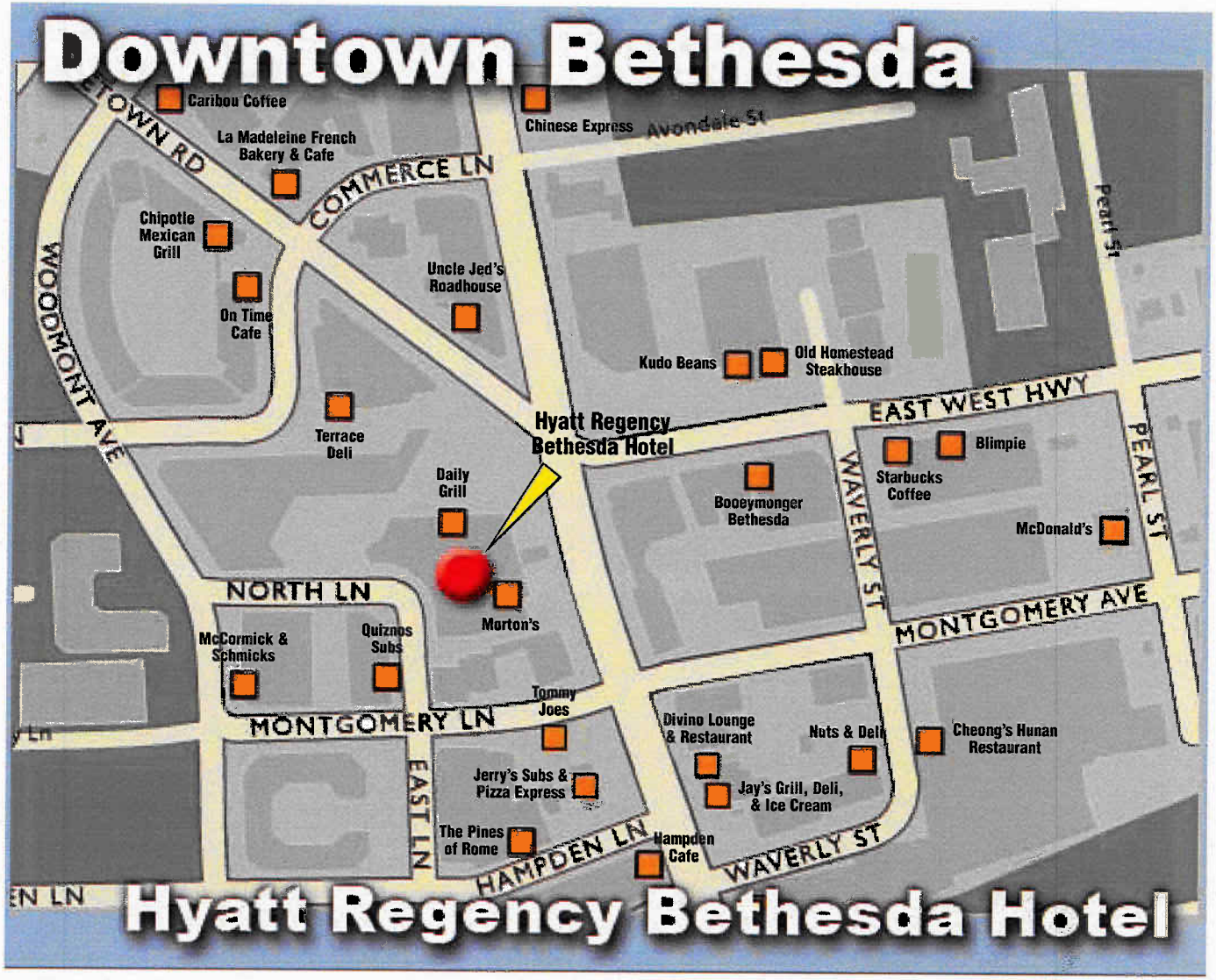
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