Exercise as a Regulator of Endocrine Dysfunction in Type 2 Diabetes

John P. Kirwan, Ph.D., FACSM

Director, Metabolic Translational Research Center
Endocrinology and Metabolism Institute, Cleveland Clinic

Staff, Department of Pathobiology
Lerner Research Institute, Cleveland Clinic

Professor, Molecular Medicine
Lerner College of Medicine, Cleveland Clinic

Professor, Physiology
Case Western Reserve University
Katarina Borer, Ph.D.

Seminal contributions to exercise, energy regulation, and bioenergetics
OBJECTIVES TODAY

Provide a broad overview of exercise as it relates to the pathophysiology of type 2 diabetes

• Identify the effect of high intensity exercise on endocrine function in type 2 diabetes
• Describe a skeletal muscle contraction model and its use to interrogate insulin resistance
• Examine skeletal muscle mitochondrial dynamics and its role in insulin resistance
**Diabetes Mellitus** Prevalence

Type 2 Diabetes (T2D) >90%

1980 - 108 million (4.7%) – Global

2014 – 422 million (8.5%) – Global

USA 2017 - 30.3 million (9.4%)

*(WHO 2014, CDC 2017)*

(CDC’s Division of Diabetes Translation, National Diabetes Surveillance System)
“You shall earn your bread by the sweat of your brow.”

Kirwan et al., 2017
Pathophysiology of Type 2 Diabetes

**Healthy**  →  **IGT**  →  **Type 2 diabetes**

- **Increased insulin resistance**
- **Hyperinsulinemia, then β-cell failure**
- **Hyperglycemia**
- **Abnormal glucose tolerance**

- **Insulin Sensitivity**
- **Insulin secretion**
- **Fasting glucose**
- **Post-prandial glucose**

*IGT = impaired glucose tolerance
*OGTT = Oral Glucose Tolerance Test (75 gram)

Adapted from *Type 2 Diabetes BASICS*. International Diabetes Center (IDC), Minneapolis, 2000.
Exercise Acutely Improves Insulin Sensitivity

Figure 1. Study design.

Figure 2. Changes in (A) insulin sensitivity and (B) fatty acid uptake after exercise at 50% (EX50) and 65% (EX65) of VO$_{2\text{MAX}}$. 
Functional High Intensity Training Improves Pancreatic β-cell Function in Adults with Type 2 Diabetes

Stephan Nieuwoudt, Ciarán E Fealy, Julie A Foucher, Amanda R. Scelsi, Steven K. Malin, Mangesh R. Pagadala, Michael Rocco, Bartolome Burguera, John P. Kirwan

American Journal of Physiology - Endocrinology and Metabolism Published 16 May 2017 Vol. no.
DOI: 10.1152/ajpendo.00407.2016
Delineating Factor: Residual β-Cell Capacity

Aerobic Exercise (5 days/week)
  - “lack of time” – Korkiakangas et al., 2011

High Intensity Training (HIT)
CrossFit® training

- Functional High Intensity Training (F-HIT)
- Constantly varied workouts (8-20 minutes)
- Structured, Accountability, Personal Trainer
- Introductory program: 3 days/week for 6 weeks
- Great Lakes CrossFit Gym (Bedford, Ohio)
How to measure β-cell function?

Insulin Secretion on the Background of Insulin Sensitivity

β-Cell Function = Insulin Secretion x Insulin Sensitivity
- Disposition Index

Oral Glucose Tolerance Test (OGTT)

Early Phase

Secretion Index = ΔInsulin/ΔGlucose
Glucose Stimulated Insulin Secretion

Insulin Sensitivity Index
Modified Stumvoll Equation
Study Participants Recruited:
- Adults Diagnosed with Type 2 Diabetes
  - (non-insulin dependent)
- Sedentary, Weight Stable

PRE POST
6 weeks, 3 days/week

Body Composition (DXA)
Oral Glucose Tolerance Test
Performance and VO\textsubscript{2}\text{max}

Blood Analysis
Glucose, Pancreatic Hormones
Liver Enzymes, Gut Hormones
# CrossFit Training Results

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>POST</th>
<th>Δ</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n (M/F)</strong></td>
<td>12 (5/7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>54 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>98.0 ± 3.7</td>
<td>96.1 ± 2.7</td>
<td>-1.8 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Total fat, %</strong></td>
<td>43.6 ± 1.8</td>
<td>42.5 ± 1.8</td>
<td>-1.1 ± 0.3</td>
<td>0.002*</td>
</tr>
<tr>
<td><strong>Abdominal fat, %</strong></td>
<td>56.2 ± 1.8</td>
<td>55.3 ± 1.7</td>
<td>-0.9 ± 0.7</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Physical performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VO_{2\text{max}}, L/min</strong></td>
<td>2.43 ± 0.12</td>
<td>2.81 ± 0.15</td>
<td>0.38 ± 0.08</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Session 2 (PRE) vs. 18 (POST), reps</strong></td>
<td>223 ± 12</td>
<td>282 ± 11</td>
<td>59 ± 8</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>
CrossFit Training Results

**Disposition Index**

- PRE: Disposition Index (Early phase, a.u.) 7 ± 3
- POST: Disposition Index (Early phase, a.u.) 15 ± 2

* Significant difference between PRE and POST

**Delta Disposition Index**

- Disposition Index Change (Early phase, a.u.)
  - PRE: 0
  - POST: 8 ± 3

+6 to +12
-6 to -12
CrossFit Training Results

Change in β-cell Function (POST – PRE)

ΔDisposition Index (Early phase, a.u.)

Change in Insulin Secretion (POST – PRE)

ΔSecretion Index (Early phase, ng/mL/min)

Change in Insulin Sensitivity (POST – PRE)

ΔInsulin Sensitivity Index (a.u.)

<table>
<thead>
<tr>
<th>Change in β-cell Function (POST – PRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔDisposition Index (Early phase, a.u.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in Insulin Secretion (POST – PRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSecretion Index (Early phase, ng/mL/min)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in Insulin Sensitivity (POST – PRE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔInsulin Sensitivity Index (a.u.)</td>
</tr>
</tbody>
</table>

x10³
<table>
<thead>
<tr>
<th>Glucose Tolerance</th>
<th>Responders</th>
<th>Non-responders</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose tAUC (0-180min), g/dL*min</td>
<td>33.6 ± 2.2</td>
<td>44.2 ± 4.1</td>
<td>0.05*</td>
</tr>
<tr>
<td>β-Cell Secretory Capacity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-peptide tAUC (0-180min), ng/mL*min</td>
<td>792 ± 54</td>
<td>551 ± 74</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

**PRE-Intervention**

![Graph showing VO2_max and Physical Performance](image-url)
Correlations with β-Cell Function
Abdominal Fat %
Alkaline Phosphatase (ALP)
F-HIT Increases $\beta$-cell function in adults with T2D*

Improvements are driven by increased insulin secretion, not sensitivity

Improvements in function correlate with reduced Abdominal Fat and ALP

*Responders vs. Non-responders
  ◦ Severity of Diabetes

Limitations
  ◦ Molecular Mechanisms
  ◦ Isolate the function of exercise alone
Exercise in a Petri Dish Model

Muscle cells
- C2C12 myocytes
- Differentiation

Electrical Pulse Stimulation (EPS)
- Electrodes (Platinum) → Electrical Field
- Electrophoresis
- Polarity switching
Electrical Pulse Stimulation of C2C12 Myotubes
In vitro Contraction Protects Against Palmitate-Induced Insulin Resistance in C2C12 Myotubes

Stephan Nieuwoudt, Anny Mulya, Ciaran E. Fealy, Elizabeth Martelli, Srinivasan Dasarathy, Sathyamangla V. Naga Prasad, John P. Kirwan

American Journal of Physiology - Cell Physiology Published 23 August 2017 Vol. no., DOI: 10.1152/ajpcell.00123.2017
Figure 1. Quantification of reductions in in vitro C2C12 myotube contractility with ammonium acetate. (A) Directional 2D optical flow map of pixel movement from a single contraction over 10 frames with no contraction control (B). Direction of and intensity of pixel movement is represented by the color wheel insert (C). Spot noise map depicting total pixel movement for each point over 10 frames with a single contraction and no contraction control (D). (E) Average spot noise histogram density for each myotube (n=4) relative to no contraction control. (F) Average spot noise histogram density (n=10) of control myotubes (circle) or 10mM ammonium acetate incubated myotubes (square). (*) P <0.01.
Experimental Design

No Treatment (Control) -> Control - Insulin
EPS -> EPS - Insulin
No Treatment -> Control + Insulin
EPS -> EPS + Insulin
Palmitate (PA) -> PA - Insulin
EPS -> PA + Insulin
PA -> EPS PA - Insulin
EPS PA + Insulin

Glucose uptake
Phospho-Akt
PI3K activity
How Does Insulin Regulate Glucose Uptake
Contraction Model Validation
Glucose Uptake
Contraction Model Validation - Akt Phosphorylation

Thr308

Insulin Receptor

Insulin

Free Fatty Acids

Glucose

Muscle Contraction

[Long Chain Acyl-CoA]

(3-Oxoyglycerol)

PKC

IRS-1

IRAP

Glucose Transporter Vesicles

IRAP

PKC

PDK1

Akt

Thr308

[Insulin]

[PDH]

[Oxaloacetate]

[Glutamate]
Contraction Model Validation
Whole-cell PI3K activity

pan-PI3Kα-IP Activity

- Insulin

+ Insulin

Control  EPS  PA  EPS PA
Relative Activity (A.U.)

- Control  EPS  PA  EPS PA
Relative Activity (A.U.)

Δ% Glucose Uptake

- Control  EPS  PA  EPS PA

*  *  *

Insulin Receptor

Free Fatty Acids

Muscle Contraction
Contraction Model Validation

Conclusions

Model is validated by a known phenomenon

Contraction alone can provide protection against lipid-induced insulin resistance

Protective mechanism is evident within the canonical insulin signaling pathway

Non-canonical activation of PI3K may also mediate protective effect
What is Mitochondrial Dynamics?

Figure 1. Treatment with PA induces mitochondrial fragmentation in C2C12 cells. Jheng et al. (2011)
Mitochondrial Dynamics: A Primer

Mitochondrial Fission:
- DRP1 (Cystosol)
- MFF (OMM)
- Mid49 (OMM)
- Mid51 (OMM)

Mitochondrial Fusion:
- MFN1 (OMM)
- MFN2 (OMM)
- OPA1 (IMM)

Mitophagy:
- PINK1 (Cytosol/OMM/IMM)
- Parkin (OMM)

Mitochondrial Dynamics and Metabolic Disease

Starvation; Exercise
Fusion > Fission
Demand > Supply

Normal
Fusion > Fission
Homeostasis

Cardiometabolic; Neurological; Pulmonary; Alzheimer's
Supply > Demand
Fusion > Fission

Nutrient Status of the Cell
Mitochondrial Fission and Insulin Resistance

Nutrient oversupply leads to:
- Opening of the permeability transition pore (mPTP)
  - Inhibits insulin-stimulated glucose uptake
- Loss of mitochondrial membrane potential ($\Delta \psi_m$)
- Fragmentation of the mitochondrial network
- Loss of mitochondrial function
  - Impaired $O_2$ consumption rates
  - Uncoupled respiration
  - Slowed ATP synthesis

Hyperinsulinemia results in order to accommodate inadequate energy production

With continual overload on the mitochondria, insulin action worsens
Exercise as Molecular Medicine

Aerobic exercise leads to number adaptations in the mitochondria

- Number (mtDNA copies, biogenesis)
- Size (network & individual mitochondrion)
- Density (product of size and number)
- Function (ATP synthesis, Respiratory chain, ROS scavenging)

The effect of exercise training on mitochondrial dynamics is currently unknown
Does exercise training restore mitochondrial dynamics in insulin resistant individuals?

Hypothesis 1:
Exercise training will alter the mitochondrial phenotype such that there will be enhanced fusion and reduced fission

Hypothesis 2:
Metabolic improvements from exercise training related to changes in mitochondrial dynamics.
Study Design

12 Weeks Treadmill Walking/Jogging @ 85% HR_{\text{MAX}}
5X/weekly

Clamp Study
Muscle Biopsies
Indirect Calorimetry
{\text{VO}}_{2\text{MAX}}\text{ Testing}
DEXA Scan

Week 0

Week 12

Clamp Study
Muscle Biopsy
Indirect Calorimetry
{\text{VO}}_{2\text{MAX}}\text{ Testing}
DEXA Scan
Hyperinsulinemic-Euglycemic Clamp Study
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$M$</th>
<th>$SD$</th>
<th>p-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>66.3</td>
<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>$\Delta$ Weight (kg)</td>
<td>-13.20</td>
<td>3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ BMI (kg/m²)</td>
<td>-4.23</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ Body Fat %</td>
<td>-6.80</td>
<td>3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>$\Delta$ VO₂MAX (ml/kg/min)</td>
<td>8.13</td>
<td>3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ FPG (mg/dL)</td>
<td>-4.03</td>
<td>4.3</td>
<td>0.020</td>
</tr>
<tr>
<td>$\Delta$ FPI (μU/mL)</td>
<td>-2.83</td>
<td>3.4</td>
<td>0.033</td>
</tr>
<tr>
<td>$\Delta$ Triglycerides (mg/dL)</td>
<td>-57.80</td>
<td>64.6</td>
<td>0.025</td>
</tr>
<tr>
<td>$\Delta$ Cholesterol (mg/dL)</td>
<td>-35.30</td>
<td>25.6</td>
<td>0.003</td>
</tr>
<tr>
<td>$\Delta$ HDL (mg/dL)</td>
<td>2.00</td>
<td>5.8</td>
<td>0.329</td>
</tr>
<tr>
<td>$\Delta$ VLDL (mg/dL)</td>
<td>-11.70</td>
<td>12.2</td>
<td>0.019</td>
</tr>
<tr>
<td>$\Delta$ LDL (mg/dL)</td>
<td>-25.60</td>
<td>21.6</td>
<td>0.006</td>
</tr>
<tr>
<td>$\Delta$ GDR (mg/kg/min)</td>
<td>2.32</td>
<td>1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ NOGD (mg/kg/min)</td>
<td>2.05</td>
<td>1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\Delta$ HOMA</td>
<td>-0.80</td>
<td>0.8</td>
<td>0.019</td>
</tr>
<tr>
<td>% $\Delta$ M/I</td>
<td>1.24</td>
<td>0.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Exercise training restores Mitochondrial Dynamics in Insulin Resistant Individuals

- Reductions in Drp1 phosphorylation in association with improved insulin resistance and fat oxidation supports the hypothesis that Drp1 mediated mitochondrial fission may link mitochondrial function with insulin sensitivity

Fealy et al. (2014)
Exercise Training Improves Mitochondrial Dynamics

Representative immunoblots of regulators of mitochondrial dynamics from human skeletal muscle tissue. ± indicates either pre (-) or post (+) exercise training. Quantification of protein expression expressed as fold induction relative to pre-intervention corrected to loading control (HSC70).
Mitochondrial Dynamics & Insulin Sensitivity

\[ R^2 = 0.53 \]
\[ P = 0.016 \]

\[ R^2 = 0.62 \]
\[ P = 0.011 \]

\[ R^2 = 0.54 \]
\[ P = 0.016 \]
Implications

Targeting of novel proteins and pathways regulating glucose metabolism

- Development of pharmacologic interventions
- Development of therapeutic treatments
Kirwan Lab Research Team

**Staff Scientists/Fellows/Grad Students/Residents**

Hussein Yassine, M.D.
Anny Mulya, Ph.D.
Gustavo Heresi, M.D.
Takhar Kasumov, Ph.D.
Mangesh Pagadela, M.D.
Juan Pablo del Rincon, M.D.
Sankar Navaneethan, M.D.
Ciaran Fealy, Ph.D.
David Mosinski, CCLCM Ph.D. Student
Andreea Mocanu, Univ. Bucharest, Ph.D. Student
Stephan Nieuwuodt, CWRU Ph.D. Student
Hanna Huang, CWRU Ph.D. Student
Adithya Hari, CWRU Ph.D. Student
Jessica Sacks, CCLCM Ph.D. Student
Thomas Solomon, Ph.D.
Jacob Haus, Ph.D.
Karen Kelly, Ph.D.
Emily Louis, Ph.D.
Steve Malin, Ph.D.
Melissa Erickson, Ph.D.

**Support Staff**

Chris Axelrod - Research Coordinator
Emily Huang - Lead Technologist
Debbie Paul - Research Assistant
CTSA Clinical Research Unit Team
CTSA Core Labs and Metabolic Kitchen

**Key Collaborators**

Art McCullough, Digestive Disease Institute, CCF, Cleveland
Chris Flask, Radiology, CWRU, Cleveland
Phil Schauer - Bariatric Metabolic Institute, CCF, Cleveland
Sangeeta Kashyap - Endocrinology Institute, CCF, Cleveland
Stacy Brethauer - Bariatric Metabolic Institute, CCF, Cleveland
Alastair Ross – Nestle Research Center, Lausanne
Jean-Philippe Godin – Nestle Research Center, Lausanne
Hope Barkoukis - Nutrition, CWRU, Cleveland
Richard Watanabe - Preventive Medicine, USC, Los Angeles
Patrick Catalano - Reproductive Biology, MHMC/CWRU, Cleveland

**Funding / Support**

NIH – RO1 DK089547, UL1-RR024989, R01 DK108089, U34/U01 DK107917, Metagenics Inc.
Questions?