Regulation of Muscle Glucose Uptake

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Regulation and Assessment of Muscle Glucose Uptake

- Processes and Reactions Involved
- Methods of Measurement
- Physiological Regulation by Exercise and Insulin
- Metabolic Control Analysis
To Understand Glucose Metabolism In Vivo, One must Understand Regulation by Muscle

- Muscle is ~90% of insulin sensitive tissue
- Muscle metabolism can increase ~10x in response to exercise
- Muscle is a major site of insulin resistance in diabetes
Muscle Glucose Uptake in Three Easy Steps

- Extracellular
  - blood flow
  - capillary recruitment
  - spatial barriers

- Membrane
  - transporter #
  - transporter activity

- Intracellular
  - hexokinase #
  - hexokinase compartmentation
  - spatial barriers
Glucose is not “officially” taken up by muscle until it is phosphorylated. Glucose phosphorylation traps carbons in the muscle and primes it for metabolism.
Feedback Inhibition of HK II by Glc 6-P distributes Control of Muscle Glucose Uptake to Glycogen Synthesis/Breakdown and Glycolytic Pathways

\[
\frac{d[Glc\ 6-P]}{dt} = \text{Flux}_{\text{HK}} + \text{Flux}_{\text{Phos}} - \text{Flux}_{\text{GS}} - \text{Flux}_{\text{Gly}}
\]
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Tools for Studying Muscle Glucose Uptake *in vivo*

- Why *in vivo*?
- Why conscious?
Methods to Assess Muscle Glucose Uptake in vivo

• Arteriovenous differences
• Isotope dilution ([3-\(^3\)H]glucose)
• \([\(^3\)H]2\)-deoxyglucose
• Positron emission tomography ([\(^{18}\)F]deoxyglucose)
Arteriovenous Differences

- Multiple measurements over time
- Invasive, but applicable to human subjects
- Requires accurate blood flow measure
- Not applicable to small animals
- Sensitive analytical methods are needed to measure small arteriovenous difference
Isotope dilution ([3-\(^3\)H]glucose)

- Minimal invasiveness
- Applicable to human subjects
- Applicable with 6,6\(^2\)H]glucose also
- Whole body measure; not muscle specific
$[{}^3H]2$-deoxyglucose

- Sensitive
- Tissue specific
- 2-deoxyglucose metabolism may differ from that for glucose
- Usually single endpoint measure
- Generally not applied to people.
Positron emission tomography ([¹⁸F]deoxyglucose)

- Minimal invasiveness
- Applicable to human subjects
- 2-deoxyglucose metabolism may differ from that for glucose
- Single point derived from curve fitting/compartamental analysis
- Requires expensive equipment
- ROI must be stationary (i.e. cannot study during exercise)
Regulation and Assessment of Muscle Glucose Uptake

• Processes and Reactions Involved
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You can’t Study Muscle Glucose Metabolism 

*in vivo* without a Sensitizing Test

**Reason:** In the fasted, non-exercising state muscle glucose metabolism is too low.

**Sensitizing Tests:** Insulin clamp, Exercise
Fasted

GLUT4

HK II

Insulin or Exercise

HK II
Relationship of Muscle Cell Surface GLUT4 to Uptake of a Glucose Analog

From Lund et al. PNAS 92: 5817, 1995
Insulin signals the translocation of GLUT4 to the cell membrane by a series of protein phosphorylation reactions.
GLUT4 Translocation

Glucose

Insulin-stimulated Muscle

PI3K
PIP3

PI3K
PIP2

PDK1

IRS-1

P85/P110

PI3K

AKT

mTOR

GSK3

stimulate protein syn

stimulate gly syn
Exercise and Insulin act through Different Mechanisms
Glucose → GLUT4 Translocation → Working Muscle

AMPKK → AMPK

AMPK → [AMP] → CaMKKK → CaMKII → FAT Acetyl-CoA Carboxylase (ACC) → ↑ Fat Metabolism

CaMKII - Acetyl-CoA Carboxylase (ACC)

NOS, p38, ERK? → aPKC

[Ca++]
Contraction- and Insulin-stimulated Muscle Glucose Uptake use Separate Cell Signaling Pathways

• Contraction does not phosphorylate proteins involved in early insulin signaling steps.

• Wortmannin eliminates insulin- but not contraction-stimulated increases in glucose transport.

• Insulin and contraction recruit GLUT4 from different pools.

• Effects of insulin and exercise on glucose transport are additive.

• Insulin resistant states are not always ‘contraction resistant’.
Insulin Stimulation

Glucose 6-Phosphate -> Glycogen Synthesis

Glycolysis

Exercise

Glucose 6-Phosphate -> Glycogen Synthesis

Glycolysis
Somatostatin (SRIF) infusion creates an insulin-free environment

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Basal</th>
<th>Exercise</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SRIF plus Insulin</td>
<td></td>
</tr>
<tr>
<td>10 ± 1</td>
<td>10 ± 1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>8 ± 1</td>
<td>7 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>7 ± 2</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>&lt;2</td>
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</tr>
<tr>
<td></td>
<td>SRIF minus Insulin</td>
<td></td>
</tr>
<tr>
<td>13 ± 1</td>
<td>10 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>13 ± 1</td>
<td>10 ± 2</td>
<td>9 ± 1</td>
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Exercise stimulates glucose utilization independent of insulin action \textit{in vivo}
What is the stimulus/stimuli that signal the working muscle to take up more glucose?

- Shift in muscle Ca\(^{++}\) stores
- Increase in muscle adenine nucleotide levels
- Increase in muscle blood flow
Insulin-stimulated glucose utilization is increased during exercise.

Glucose Disappearance (mg·kg⁻¹·min⁻¹)

Insulin (µU/ml)
Proposed mechanisms by which acute exercise enhances insulin sensitivity

- Increased muscle blood flow
- Increased capillary surface area
- Direct effect on working muscles
- Indirect effect mediated by insulin-induced suppression of FFA levels
Regulation and Assessment of Muscle Glucose Uptake

• Processes and Reactions Involved
• Methods of Measurement
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Muscle Glucose Uptake (MGU) Requires **Three** Steps

1. **Delivery**
   - 
   - Blood

2. **Transport**
   - **Glut4**

3. **Phosphorylation**
   - **HK II**
Ohm’s Law

\[ I \cdot \text{Resistor}_1 = V_1 - V_2 \]
\[ I \cdot \text{Resistor}_2 = V_2 - V_3 \]
\[ I \cdot \text{Resistor}_3 = V_3 - V_4 \]
Mouse Model

- Catheterization of jugular vein and carotid artery
- Experiment performed ~5-7 days postoperative
- Sampling is performed 5 h after food is removed
- [2\textsuperscript{-3}H]deoxyglucose infused to measure an index of muscle glucose influx
Excise Tissues

Acclimation

Hyperinsulinemic Euglycemic Clamp

Mice are 5 h fasted at the time of the clamp

Insulin (0 or 4 mU·kg\(^{-1}\)·min\(^{-1}\))

Euglycemic Clamp

[2-3H]DG Bolus

Excise Tissues

0 5 30 min
Ohm’s Law

Glucose Influx

Transgenics

GLUT4^{Tg}

HK^{Tg}

GLUT4^{Tg} HK^{Tg}
Saline-infused and Insulin-clamped Mice

Muscle Glucose Uptake
($\mu$mol·100g$^{-1}$·min$^{-1}$)

Soleus

Gastrocnemius

WT
GLUT$^{Tg}$
HK$^{Tg}$
HK$^{Tg}$ + GLUT$^{Tg}$

Saline
Insulin-clamped

*
†
Metabolic Control Analysis of MGU

• Control Coefficient $E$
  $= \partial \ln R_g / \partial \ln [E]$

• Sum of Control Coefficients in a Defined Pathway is 1
  i.e. $C_d + C_t + C_p = 1$
Control Coefficients for MGU by Mouse Muscle Comprised of Type II Fibers

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<td>Insulin  (~80 µU/ml)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.4</td>
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Functional Barriers to MGU during Exercise
Sedentary and Exercising Mice

Muscle Glucose Uptake
($\mu$mol·100g$^{-1}$·min$^{-1}$)

**Gastrocnemius**

**SVL**

**Soleus**

**WT**  **GLUT4$^Tg$**  **HK$^TG$**  **HK$^Tg$ + GLUT4$^Tg$**
Control Coefficients for MGU by Mouse Muscle Comprised of Type II Fibers

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</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exercise</strong></td>
<td>0.2</td>
<td>0.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Sedentary

Exercise

GLUT4

HK II

ptf 2002
Functional Barriers to MGU in Dietary Insulin Resistance

Extracellular → Membrane → Intracellular

Glucose → Glucose 6-phosphate
Saline-infused and Insulin-clamped Mice

Muscle Glucose Uptake
\[ \mu\text{mol} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1} \]

Chow Fed

High Fat Fed

Hypothesis

Increasing conductance of the muscle vasculature will prevent insulin resistance in high fat fed mice.
Ohm’s Law and Insulin Resistance

Glucose Influx

\[ G_a \rightarrow G_e \rightarrow G_i \rightarrow 0 \]

WT

PDE5(-)

Sildenafil was used to inhibit PDE5 activity and increase vascular conductance
PDE5 Inhibition and Vascular Conductance

Natriuretic Peptides & Guanylin

Nitric Oxide

sGC → cGMP → PKG → Blood vessel relaxation

Vascular Smooth Muscle Cell

PDE5 Inhibitor
Index of Glucose Uptake during an Insulin Clamp in High Fat Fed Mice

Muscle Glucose Uptake
µmol·100 g tissue⁻¹·min⁻¹

- Soleus
- Gastrocnemius
- SVL

Vehicle
Sildenafil/L-arginine

* indicates significant difference.
Summary

Control of Muscle Glucose Uptake is Distributed between Glucose Delivery to Muscle, Glucose Transport into Muscle, and Glucose Phosphorylation into Muscle.

_Treatment of Insulin Resistance may Involve any one or More of the Three Steps_
Gastrocnemius Akt/PKB after an Insulin Clamp in High Fat Fed Mice

**Sildenafil + L-Arginine**

**Vehicle**

- Anti-Akt/PKB
- Anti-phospho-Akt/PKB
- Anti-GAPDH

**Graphs:**
- Anti-Akt/PKB: Sildenafil/L-arginine vs Control
- Anti-phospho-Akt/PKB: Sildenafil/L-arginine vs Control
An Index of MGU (R_g) in vivo using 2-Deoxy-[^3^H]glucose

\[ R_g = \frac{[2^{-3^H}]DGP_{tissue} (t)}{AUC [2^{-3^H}] DG_{plasma}} \]

- Glucose_{plasma}

![Graph showing the decrease in [2^{-3^H}]DG over time with a bolus injection at 0 minutes.](image)
## Descriptive Characteristics

<table>
<thead>
<tr>
<th></th>
<th>WT Chow</th>
<th>WT High Fat</th>
<th>HKII\textsuperscript{TG} Chow</th>
<th>HKII\textsuperscript{TG} High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>17</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>26 ± 1</td>
<td>36 ± 2\textsuperscript{A}</td>
<td>26 ± 1</td>
<td>35 ± 1\textsuperscript{B}</td>
</tr>
<tr>
<td>Fasting Glucose (mg·dL\textsuperscript{-1})</td>
<td>166 ± 6</td>
<td>196 ± 5\textsuperscript{A}</td>
<td>167 ± 7</td>
<td>167 ± 7</td>
</tr>
<tr>
<td>Fasting Insulin (mU·mL\textsuperscript{-1})</td>
<td>20 ± 3</td>
<td>52 ± 13\textsuperscript{A}</td>
<td>21 ± 2</td>
<td>37 ± 16\textsuperscript{B}</td>
</tr>
</tbody>
</table>

\textsuperscript{A} = p < 0.001 vs. WT chow fed mice
\textsuperscript{B} = p < 0.001 vs. HKII\textsuperscript{TG} chow fed mice