Skeletal muscle and insulin resistance

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The prevalence of diabetes in 2010

World-wide prevalence of diabetes is > 300 million.
- 90% of cases are accounted for by Type 2 diabetes
- Genetic factors
- Obesity and physical inactivity are the main non-genetic determinants of Type 2 diabetes

Skeletal Muscle is a Major Site of Insulin Resistance in Type 2 Diabetic Patients

DeFronzo, Diabetes 37:667-687, 1988
Distribution of insulin sensitivity in healthy normal glucose tolerant people

Colletta&Mandarino, Am J Physiol 2011

Blunted change in muscle glycogen in lean insulin resistant subjects following mixed meals

Petersen KF et al. PNAS 104:12587, 2007
Fate of glucose following high carbohydrate mixed-meals

Petersen KF et al. PNAS 104:12587, 2007

Reduced Insulin-Stimulated Glucose Transport in Skeletal Muscle from Type 2 Diabetic Patients

Krook, Diabetes 49:284-292, 2000
How is insulin signalling altered in human skeletal muscle in type 2 diabetes?
No differences in protein expression of key signalling molecules in skeletal muscle from NIDDM subjects and controls.

Krook, Diabetes 49:284-292, 2000

Impaired Insulin-Stimulated IRS-1 Tyrosine Phosphorylation in Type 2 Diabetic Subjects

* p<0.05 vs. control

Biörnholm, Diabetes 46:524-527, 1997
Impaired Insulin-Stimulated Tyrosine Associated PI 3-Kinase Activity in Type 2 Diabetic Subjects

![Bar chart showing comparison between control and type 2 diabetes groups for basal and insulin-stimulated activity.](chart1)

* * p<0.05 vs. control  
Björnholm, Diabetes 46:524-527, 1997

Insulin stimulated phosphorylation of Thr308 of Akt is impaired in type 2 diabetic muscle

![Western blots and bar graphs showing phosphorylation levels of Akt in control and type 2 diabetic muscle.](chart2)

Karlsson HKR et al. Diabetes 2005
Summary

- Skeletal muscle from type 2 diabetic subjects has impaired insulin-stimulated
  - IRS1 phosphorylation
  - PI3 kinase activation
  - Thr 308 phosphorylation of Akt
  - phosphorylation of Akt substrate AS160

  glucose transport is impaired
Reduced insulin-stimulated IR phosphorylation and IRS-1 associated PI3K activity in healthy subjects with high intramyocellular lipid content

Virkamäki et al., Diabetes 50:2337-43, 2001

Insulin-dependent and independent Signaling Pathways
AMP activated protein kinase (AMPK) - a master metabolic switch

**Liver**
- Increases Fatty Acid Oxidation (Ketogenesis)
- Decreases Cholesterol Synthesis
- Decreases Lipogenesis

**Skeletal Muscle**
- Increases Fatty Acid Oxidation
- Increases Glucose Uptake

**Adipocytes**
- Decreases Lipogenesis
- Decreases Lipolysis

**Pancreatic Islets**
- Modulates Insulin Secretion

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AMPK and the regulation of metabolism

Muscle Contraction Increases [5'-AMP] and Decreases [CP]

AMPK-OH → AMPKK → AMPK-OP

- Phosphorylates ACC
- Decreases Malonyl-CoA
- Increases FFA Oxidation

- Phosphorylates Unknown Target Protein(s)
- Increases Glucose Uptake
Exposure of human muscle to AICAR increases phosphorylation of Acetyl-CoA Carboxylase

Koistinen HA et al., Diabetes 52:1066, 2003

Skeletal muscle glucose transport in response to insulin and AICAR

Koistinen HA et al., Diabetes 52:1066, 2003
Glucose Transport Process in Skeletal Muscle

Assessing Cell Surface GLUT4 Content in Skeletal Muscle
1. Incubate muscle with insulin/AICAR
2. Label the cell surface GLUT4 with biotinylated cell-impermeable photolabel
3. Lyse and IP with streptavidin
4. Run electrophoresis
5. Blot with GLUT4 antibody

An example of the GLUT4 photolabelling analysis in non-diabetic muscle
Insulin-stimulated Glucose Transport and Cell Surface GLUT4 Content in Skeletal Muscle from Diabetic Subjects

Glucose transport

Cell surface GLUT4

* p<0.01 vs. control

Ryder J, Diabetes 49:647-654, 2000

Hypoxia-stimulated Glucose Transport and Cell Surface GLUT4 Content in Skeletal Muscle from Diabetic Subjects

Glucose transport

Cell surface GLUT4

*P<0.05 vs control same condition

Ryder J, Diabetes 49:647-654, 2000
The effect of AICAR on cell surface GLUT4 content in skeletal muscle

![Graph showing changes in GLUT4 content](image)

*P<0.05  
Koistinen HA et al., Diabetes 52:1066, 2003

Summary

- Skeletal muscle from type 2 diabetic subjects has impaired insulin-stimulated glucose transport and cell surface GLUT4

- Hypoxia stimulated glucose transport and cell surface GLUT4 are also impaired in type 2 diabetes

- General defect in GLUT4 translocation process?
Studies on subjects at increased risk for developing type 2 diabetes = first-degree relatives

Accumulation of fat in skeletal muscle in insulin resistance

• Fatty acid oxidation is reduced in whole muscle, possibly due to lower content of mitochondria
• ATP synthesis and TCA flux are reduced in insulin resistant offspring of T2D patients
• Decreased PGC1α expression in insulin resistance may impair mitochondrial biogenesis


• Fatty acid transporter CD36 is increased in sarcolemma – enhanced fatty acid transport into muscle

Bonen A et al. FASEB J 18:1144, 2004
Metabolic inflexibility in skeletal muscle insulin resistance

Kelley DE & Mandarino LJ. Diabetes 49:877, 2000
Eckhardt K et al. Rev Endocr Metab Disord 12:163, 2011

Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes

Umut Özcan,¹,² Qiong Cao,¹,² Erkan Yilmaz,¹ Ann-Hwee Lee,² Neal N. Iwakoshi,² Esra Özdel,¹ Gürol Tuncman,¹ Cem Görgün,¹ Laurie H. Glimcher,²,³ Gökhan S. Hotamisligil¹†

SCIENCE VOL 306 15 OCTOBER 2004
ER stress

- The endoplasmic reticulum (ER) is a vast membranous network and the principal site of protein synthesis and maturation.
- Under disruption of ER homeostasis, the unfolded protein response (UPR) aims to restore ER homeostasis.
- Obesity and high fat diet induce ER stress in animals models.

1) PERK

- Protein kinase-like ER kinase (PERK).
- Phosphorylates the $\alpha$-subunit of the eukaryotic initiation factor 2 (eIF2).
- eIF2 is a initiation factor of protein synthesis.
- Inhibition of global protein translation.
2) IRE1

- Inositol-requiring protein 1 (IRE1).
- Processes splicing of the mRNA of XBP, (X-box-binding protein 1)
- The XBP1s protein is a transcription factor that translocates to the nucleus, where it induces expression of ER stress genes.
  - One of these is BiP, (immunoglobulin heavy-chain-binding protein), an ER chaperone.

ATF6

- Activating transcription factor 6 (ATF6).
- Activates transcription of BiP, CHOP and XBP1.
  - CCAAT/enhancer-binding protein–homologous protein (CHOP) is an apoptotic transcription factor.
Increased ER stress in the liver links JNK activation to inhibition of IRS1

Özcan, Science 2004

Improved adipose ER stress after gastric bypass

Gregor Diabetes 2009
Improved liver ER stress after gastric bypass surgery

B

Grp78

p-eIF2α

Gregor Diabetes 2009

Palmitate induces ER stress in human skeletal muscle cells

C

ATF3

D

CHOP

E

XBP1

F

XBP1s

Peter A, Diabetes 2009
**Palmitate induces inflammation in human skeletal muscle cells**

A. IL-8

B. CXCL3/MIP-1β

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Peter A, Diabetes 2009

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**ER stress may not mediate palmitate induced insulin resistance**

ER stress reducing chaperone TUDCA does not alleviate palmitate induced insulin resistance in C2C12 myotubes

TUDCA does not restore PKB signaling in palmitate treated human myotubes

Hage Hassan R et al. Diabetologia 2012
Palmitate may mediate insulin resistance via ER stress pathway

Chemical chaperone 4-PBA alleviates ER stress and improves PKB/Akt signaling in C2C12 cells


Co-incubation with palmitate and oleate
Results in larger lipid droplets and reduced active lipids

Co-exposure to palmitate and oleate alleviates ER stress and insulin resistance

C

In vivo in SD rats

D

L6 myotubes


The effect of one bout of exercise on postprandial metabolism

- 12 young lean insulin resistant subjects
- 45 min on elliptical trainer

Rabol R et al. PNAS 108:13705, 2011
ONE BOUT OF EXERCISE INCREASES MUSCLE NET GLYCOGEN SYNTHESIS FOLLOWING MEAL

A

Change in muscle glycogen content (mmol/L)

Resting  |  Exercise

P<0.001

Rabol R et al. PNAS 108:13705, 2011

One bout of exercise reduces liver triglyceride synthesis 40%

B

Change in liver triglyceride content (%)

Resting  |  Exercise

P<0.05

Rabol R et al. PNAS 108:13705, 2011
Hepatic de novo lipogenesis decreased 27%

The effect of one bout of exercise on postprandial metabolism

Rabol R et al. PNAS 108:13705, 2011
Antioxidants prevent health promoting effects of exercise in men

Insulin sensitivity

A

Previously untrained

B

Pre-trained

Ristow M et al. PNAS 106:8665, 2009

Antioxidants prevent exercise training induced PGC1α and SOD1

A

Previously untrained

B

Pre-trained

G

H

Ristow M et al. PNAS 106:8665, 2009
Physical exercise

Antioxidants

Transient increase of oxidative stress (ROS)

Target gene induction (PGC1α/β, PPARγ, SOD1/2, GPx1)

Induction of insulin sensitivity & endogenous ROS defense (Mitohormesis)

Reduced disease risk

Ristow M et al. PNAS 106:8665, 2009

Positive effects of RNOS

Antioxidant supplementation

Transduction of signalling pathways
for mitochondrial biogenesis
Upregulation of the endogenous oxidative defense system

Dose of oxidative stress

Impairment of cellular function and force production

Down-regulation of signalling pathways

Negative effects of RNOS