Lipotoxicity and muscular mitochondrial function in insulin resistance

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Q: Why muscle?
Muscle & insulin sensitivity

- ~ 80% of post-prandial glucose uptake occurs in muscle
- Lipid overflow to non-adipose tissue results in muscular fat storage, which associates with insulin resistance
Q: Why mitochondria?
Mitochondria & insulin sensitivity

- Oxidative degradation of nutrients occurs in mitochondria
- Mitochondria are the major site of production of reactive oxygen species
- Mitochondria are vital organelles for (myo)cellular function and are regulators of apoptosis
- Mitochondrial dysfunction may result in muscular fat storage and insulin resistance
Lipotoxicity, mitochondrial function and insulin resistance

- Lipid overflow
- Low (fat) oxidative capacity
- Muscular FA (derivatives) $\uparrow$
- Impeded insulin signalling
- Mitochondrial FA accumulation $\uparrow$
- Uncoupling (UCP3, FA, ANT)
- FA$^-$ export
- FA$^-$ export
- ROS
- Lipid peroxidation
- Lipid peroxide export
- Lipotoxicity
- Mitochondrial damage/dysfunction

Main sites of intervention
Main output parameters
Tissues examined & Models used
IMCL is negatively associated with insulin sensitivity

Krssak et al., Diabetologia 2002

Sinha et al., Diabetes 2002

Fourouhi et al., Diabetologia 1999

Jacob et al., Diabetes 1999
High FFA levels acutely induce muscle TG accumulation and insulin resistance

Hoeks et al, Diabetologia 2006
Paradox: endurance training also increases IMCL content

7 days high-fat diet

14 days endurance training

Schrauwen-Hinderling et al., Obesity 2005

Schrauwen-Hinderling et al., JCEM 2003
Training, not high-fat diet downregulates ACC2

Schrauwen et al., Diabetes 2002
Q: is IMCL only ‘harmful’ in combination with a low oxidative capacity?
Muscular mitochondrial function in T2DM and controls, matched for BMI

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (n=12, all males)</th>
<th>Controls (n=9, all males)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2)</td>
<td>29.4 ± 3.3</td>
<td>29.3 ± 2.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.8 ± 4.4</td>
<td>56.1 ± 6.8</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(\text{VO}_{2\text{max}})</td>
<td>30.6 ± 5.8</td>
<td>34.6 ± 4.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>(ml*min(^{-1})*kg(^{-1}))</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>9.56 ± 2.12</td>
<td>5.73 ± 0.37</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Insulin sensitivity (GIR, (\mu\text{mol*min}^{-1})*kg(^{-1}))</td>
<td>18.9 ± 8.1</td>
<td>26.0 ± 6.7</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

(mean ± stdev)

Schrauwen-Hinderling et al., Diabetologia 2007
Muscle mitochondrial function *in vivo*

*by* $^{31}P$ NMR Spectroscopy
Muscle mitochondrial function *in vivo*

by $^{31}$P NMR Spectroscopy

Meyer et al., 1988
Muscle mitochondrial function \textit{in vivo} by $^{31}$P NMR Spectroscopy

PCr resynthesis is almost purely aerobic.

PCr recovery half-time reflects oxidative capacity.
Muscular fat content in T2DM and BMI matched normoglycemic controls is similar.
Q: How about mitochondrial function?
In vivo mitochondrial function reduced in T2DM, correlates with glucose and HbA1C

Schrauwen-Hinderling et al., Diabetologia 2007
Q1: is mitochondrial dysfunction already present in the pre-diabetic state?

Q2: does intrinsic mitochondrial dysfunction underlie reduced *in vivo* mitochondrial function?
Intrinsic mitochondrial function *ex vivo* using high-resolution respirometry
# Pre-diabetic subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=16)</th>
<th>FDR (N=12)</th>
<th>T2DM (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>59.2 ± 0.7</td>
<td>60.1 ± 0.9</td>
<td>61.4 ± 1.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.1 ± 0.7</td>
<td>30.1 ± 1.2</td>
<td>28.9 ± 0.7</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>31.2 ± 1.6</td>
<td>32.6 ± 9.6</td>
<td>28.2 ± 2.3</td>
</tr>
<tr>
<td>Ins-stim Rₜ (umol/kg/min)</td>
<td>28.9 ± 3.7</td>
<td>22.1 ± 3.4</td>
<td>11.2 ± 2.8</td>
</tr>
</tbody>
</table>

Phielix et al., Diabetes 2008
Lower *ex vivo* mitochondrial function in T2DM irrespective of mitochondrial density (mtDNA)

* Phielix et al., Diabetes 2008
Lower *in vivo* mitochondrial function in T2DM and first-degree relatives

* P=0.08

Phielix et al., Diabetes 2008
Q: Is compromised mitochondrial function in the pre-diabetic state required to develop insulin resistance?
Transition of pre-diabetes to T2D during maturation of ZDF rats

Lenaers et al., submitted
No mitochondrial dysfunction during the development of T2D in ZDF rats

Lenaers et al., submitted

Oxidation of a lipid substrate

* p < 0.05 : compared to 6 Weeks
# p < 0.05 : compared to 12 Weeks

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No mitochondrial dysfunction during the development of T2D in ZDF rats

O2 consumption (nmol/min/mg mito)

6 weeks | 12 weeks | 19 weeks

ZDF | control

* * #

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Lenaers et al., submitted
Increased UCP3 protein blunted in ZDF rats

Lenaers et al., submitted
Uncoupling protein 3 (UCP3)

- ~55% homology to UCP1 which is responsible for the thermogenesis in brown adipose tissue
- Primarily expressed in skeletal muscle and heart
- UCP3 is able to lower the mitochondrial proton gradient - upon activation by fatty acids - by transporting $\text{H}^+$ or $\text{FA}^-$
Inner mitochondrial membrane

Intermembrane space

Mitochondrial matrix

Electron transport chain

ATP synthase

UCP leak

NADH + H+ → NAD+ + H2

2H+ + 1/2 O2 → H2O

ADP + Pi → ATP

FA−
UCP3 is reduced in (pre)diabetic patients

Schrauwen et al., JCEM 2006
Q: Is UCP3 involved in the development of mitochondrial dysfunction in T2D?
Lipotoxicity, mitochondrial function and insulin resistance

- Lipid overflow
- Low (fat) oxidative capacity

Muscular FA (derivatives) \(\uparrow\) → Impeded insulin signalling

- Mitochondrial FA accumulation \(\uparrow\)
- Uncoupling (UCP3, FA, ANT)
- FA\(^{-}\) export

ROS → Lipid peroxidation → Lipotoxicity

- Lipid peroxide export

Mitochondrial damage/dysfunction
Q: Can insulin sensitizing by exercise training restore mitochondrial aberrations observed in T2D?
Insulin sensitizing by Rosiglitazone restores UCP3 protein content in T2D…

Schrauwen et al, JCEM 2006

Mensink et al, Diab Obes Met 2006
Exercise training in T2D and controls improves insulin sensitivity

Glucose infusion rate

Control subjects diabetic subjects

* Meex et al., in progress
Exercise training in T2D restores UCP3 content

Meex et al., in progress
Q: Can mitochondrial uncoupling reduce superoxide production in diabetogenic conditions?
Mitochondrial proton gradient and ROS production

Skulachev et al., BBA 1997
Measuring superoxide production by electron spin resonance in isolated mitos

muscle mito 0.2 mg/ml
5 min 37° C
100 mM DMPO

muscle mito 0.2 mg/ml
5 min 37° C
100 mM DMPO
10 mM glutamate
10 mM succinaat
3 mM malate

idem 2
500 U/ml SOD

Hoeks et al., FEBS 2008
Aging related increased superoxide production is blunted in UCP3 tg mice

Nabben et al., FEBS Lett 2008
Type 2 diabetes, fatty acid-induced uncoupling and superoxide production

- Increased supply of fatty acids to the muscle
- Decreased fat oxidative capacity in skeletal muscle
- Increased storage of lipid (intermediates) in muscle
- Increased production of reactive oxygen species (ROS)
- Increased levels of lipid peroxidation (FA + ROS)
FA-induced uncoupling and ROS

Mouse skeletal muscle mitochondria, n=3

Hoeks et al., in progress
Hypotheses

- FA-induced uncoupling controls mitochondrial ROS production when facing high intracellular fatty acid levels
- Reduced or defective FA-induced uncoupling contributes to the progression of type 2 diabetes
Q: Is FA-induced uncoupling lower in diabetic ZDF Rats?
FA-induced uncoupling

Mito

Pyr

Oligo

Fatty acid

Hoeks et al., in progress
FA-induced uncoupling

Oxygen consumption (nmol O₂/min/mg) vs [Palmitate] nM

V_max
EC_{50}

Hoeks et al., in progress
Diabetic ZDF rats less sensitive to FA-induced uncoupling

n=4

Hoeks et al., in progress
Q: What mechanisms could be responsible for mitochondrial (FFA) uncoupling?
Mitochondrial uncoupling

**Uncoupling Protein 3 (UCP3)**
- Exact physiological function not yet established
- Hypothesis: FA (peroxides) activate UCP3 protein $\rightarrow$ proton leak

**Adenine Nucleotide Translocator (ANT)**
- Primary function: mitochondrial exchange of ADP and ATP
- Hypothesis: ANT (partly) mediates FA-induced uncoupling and contributes to basal proton leak in some tissues
Lower UCP3 but comparable ANT levels in diabetic ZDF rat

n=6
ANT inhibition: Carboxy Atractyloside

Hoeks et al., in progress
After ANT inhibition similar sensitivity to FA-induced uncoupling; ANT defect?

Hoeks et al., in progress
Conclusions

- Intrinsic mitochondrial aberrations underlie mitochondrial dysfunction in T2D
- First-degree relatives (pre-diabetics) already tend to have lower mitochondrial function
- Failure to maintain adaptive improvements in mitochondrial function parallel the transition of the prediabetic state towards full blown type 2 diabetes (rats)
- Mitochondrial dysfunction in T2D does not augment muscular fat storage compared to (obese) controls
- Insulin sensitizing interventions restore UCP3 content in T2D
- Overexpression of UCP3 blunts age related superoxide production
- FA-induced uncoupling reduces superoxide production
- Diabetic ZDF rats are less sensitive to FA-induced uncoupling (need more FA for the same level of uncoupling)
- The difference in sensitivity to FA-induced uncoupling is mediated via ANT
Thanks to...

Patrick Schrauwen
Ruth Meex
Vera Schrauwen-Hinderling
Gert Schaart
Esther Kornips
Johan de Vogel
Noud van Herpen
Miranda Nabben

Joris Hoeks
Esther Phielix
Ellen Lenaers
Ronnie Minnaard
Denis van Beurden
Katarina Fredriksson
Johanna Jörgensen
Silvie Timmers