Mitochondrial uncoupling and type 2 diabetes

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In muscle, fat accumulation (IMCL) is negatively associated with insulin sensitivity.

Krassak et al., Diabetologia 2002

Sinha et al., Diabetes 2002

Fourouhi et al., Diabetologia 1999

Jacob et al., Diabetes 1999
mitochondrial dysfunction may underlie muscular fat accumulation, insulin resistance and ultimately type 2 diabetes mellitus

Kelley et al., Diabetes 2002: smaller and damaged mitochondria in T2DM
Mootha, Nature Genetics 2003: downregulation of OXPHOS genes in T2DM
Patti, PNAS 2003: downregulation of OXPHOS genes in T2DM
Petersen, NEJM 2004: reduced muscular ATP synthesis rate in FDR
Q1: is mitochondrial dysfunction already present in the pre-diabetic state?

Q2: does intrinsic mitochondrial dysfunction underlie reduced \textit{in vivo} mitochondrial function?
Subject characteristics

Phielix et al., Diabetes 2008
$^{31}$P MR Spectroscopy to determine energy status in muscle
PCr resynthesis is almost purely aerobic.

PCr recovery half-time reflects oxidative capacity.
Decreased *in vivo* mitochondrial function in T2DM and first-degree relatives

* p<0.05
# p=0.08

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Phielix et al., Diabetes 2008
Intrinsic mitochondrial function using high-resolution respirometry
O₂ concentration $\rightarrow$ O₂ consumption

- **State U**
- **Substrate**
  - glutamate
  - palmCoA

- **State 3**
- **State 4**
- Add ADP
- Block ATPase
- Completely uncoupled (chemically)
Decreased *intrinsic* mitochondrial function in T2DM and FDR after normalization for mtDNA

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<th>cont</th>
<th>fdr</th>
<th>t2dm</th>
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* p<0.05
# p=0.08

Phielix et al., Diabetes 2008
Conclusions - part 1

- Both *in vivo* and *ex vivo* mitochondrial function is reduced in T2DM

- *In vivo* and *ex vivo* mitochondrial function tended to be reduced in FDR

- lower mitochondrial function in FDR and T2DM was accompanied by similar IMCL content when compared to controls

This suggests that a reduced intrinsic mitochondrial function may be implicated in the development of diabetes, but is not responsible for muscular fat accumulation
Mitochondrial dysfunction: cause or consequence of lipid accumulation?

Mitochondrial dysfunction:
- reduced oxidative capacity
  
  lipid accumulation

Mitochondrial lipotoxicity

Skeletal muscle insulin resistance
Mitochondrial morphology in T2D

Kelley et al., Diabetes, 2002
Q: does a high-fat diet lead to mitochondrial dysfunction?
Intramuscular triacylglycerols after 8 weeks HFD in rats

Hoeks et al., FEBS 2008
Carnitine + Palmitoyl-CoA

Hoeks et al., FEBS 2008
PGC-1α protein

Hoeks et al., FEBS 2008
PGC-1a and OxPhos proteins

$\text{Complex V protein levels, ND6 subunit (AU)}$

$\text{Complex III protein levels, 30 kD subunit (AU)}$

$\text{Complex II protein levels, } \alpha \text{ subunit (AU)}$

$\text{Complex I protein levels, } \alpha \text{ subunit (AU)}$

$r^2=0.66, p<0.001$

$r^2=0.70, p<0.001$

$r^2=0.68, p<0.001$

$r^2=0.66, p<0.001$
prolonged HF feeding (16 weeks) increases ROS production and reduces mitochondrial function
Conclusions - part 2

- On the short term, high-fat diets are accompanied by adaptive improvements in mitochondrial function, to adjust oxidative capacity to fat intake.

- However, prolonged high-fat feeding is associated with a reduction in mitochondrial function and increased ROS production.
Q: How are mitochondria protected against lipotoxicity?
Mitochondrial ROS production depends on proton gradient

Skulachev et al., BBA 1997
Fatty acid can lower proton gradient (fatty acid-induced uncoupling)
Fatty acid induced uncoupling and ROS production

N=3
Q: Is FA-induced uncoupling reduced in a model of type 2 diabetes mellitus?
Zucker Diabetic Fatty rats

- Mutation in the leptin receptor (shortened leptin receptor protein)
- Hyperphagic

- When put on moderate high-fat diet these animals show
  - Obesity
  - Hyperlipidemia
  - Fasting hyperglycemia
  - Full blown diabetes by the age of 12 weeks
FA-induced uncoupling in ZDF

Higher EC50 = lower FA-induced uncoupling

Unpublished observations
UCP3 and ANT levels

Unpublished observations
Uncoupling protein-3 and lipotoxicity

UCP3 is upregulated under fat-overload conditions:
- high-fat diet (Hesselink et al., JCI 2003)
- fasting (Millet et al., JCI 1997, Samec et al., Faseb J1998)
- blocking CPT1 activity (Schrauwen et al., Faseb 2002)
- acute exercise in fasted state (Schrauwen et al., AJP J 2002)

UCP3 is downregulated when fat oxidative capacity is high:
- weight reduction (Schrauwen et al., diabetologia 2000)
- type I muscle fibers (Hesselink et al., FASEB J 2001)
- endurance training (Schrauwen et al., FASEB J 2001)

reviewed in: Schrauwen et al., Prog Lipid Res 2006
lack of UCP3 increases lipid peroxidation

Hoeks et al., Febs lett 2006
Overexpressing UCP3 reduces ROS production

Nabben et al., Febs letters, 2008
UCP3 is reduced in (pre)diabetic patients

Schrauwen et al., JCEM 2006
Uncoupling: role ANT

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
FA-induced uncoupling: ANT inhibition

Unpublished observations
ANT inhibition in ZDF rats

**Without CATr**

Unpublished observations
Summary: FA-induced uncoupling

- Zucker Diabetic Fatty rats show a decreased sensitivity to FA-induced uncoupling

- ANT partly mediates FA-induced uncoupling in skeletal muscle mitochondria

- The difference between ZDF and control rats disappears upon ANT inhibition, indicating that at least part of the difference is localized in the portion of FA-induced uncoupling that is mediated by ANT

- Still, UCP3 is reduced too: role in lipotoxicity?
current working hypothesis

Plasma FFA ↑  Oxidative capacity ↓

Mitochondrial FA accumulation

Fatty acid induced uncoupling (UCP3, ANT)

ROS

Lipid peroxidation

Peroxides accumulation

Mitochondrial damage

adapted from: Schrauwen et al., Diabetes 2004
Current other projects

- Lipid droplet coating proteins: role in insulin resistance (DNA electroporation)
- Endurance training, mitochondrial function and diabetes
- Can Acipimox treatment restore mitochondrial function in diabetes
- Diabetic cardiomyopathy: role for cardiac lipid accumulation?
- Fasting-induced insulin resistance: mechanisms
- Diet and liver fat
- Polyphenols and mitochondrial function
- Brown adipose tissue
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