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

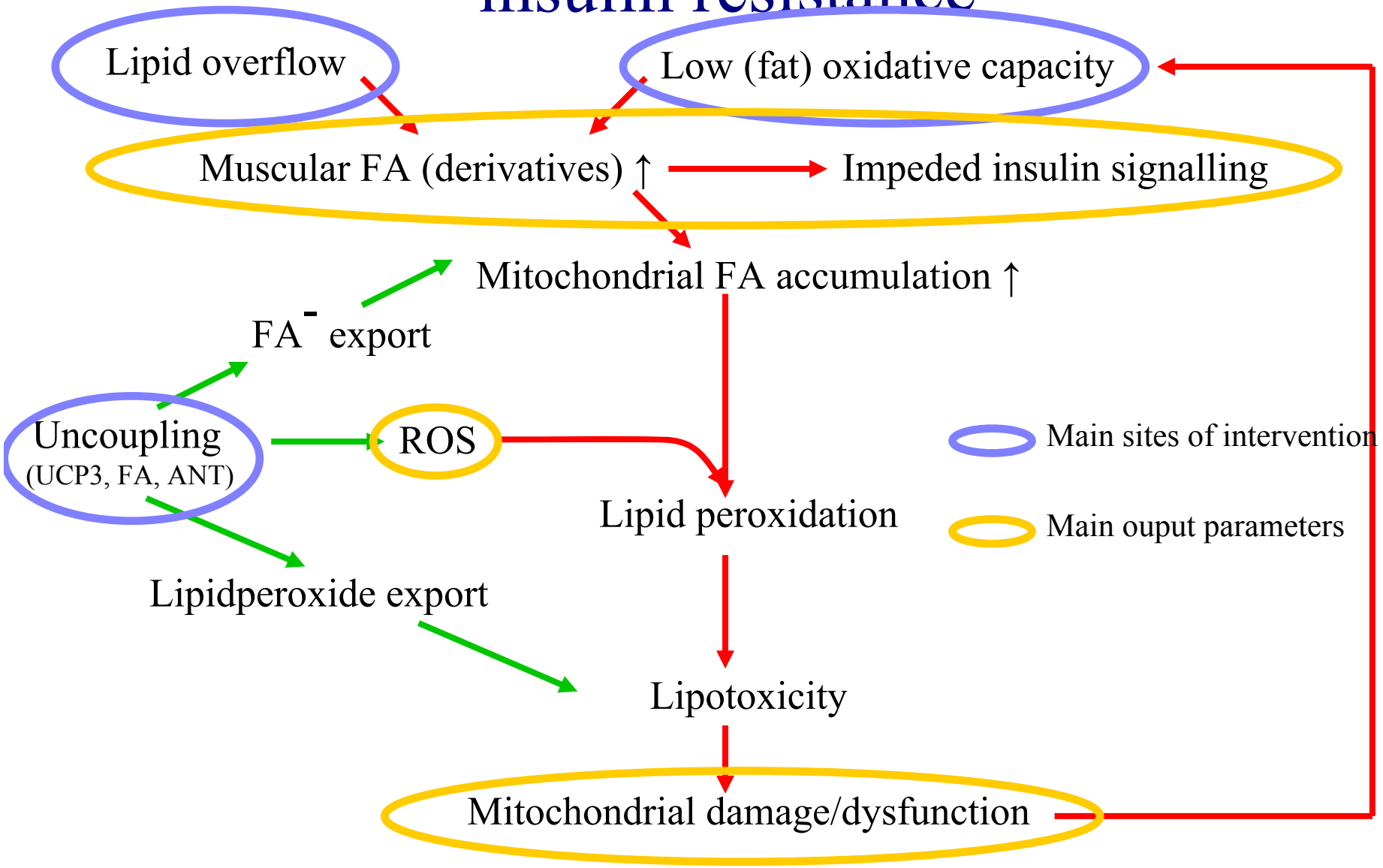
Q: Why mitochondria?

Mitochondria & insulin sensitivity

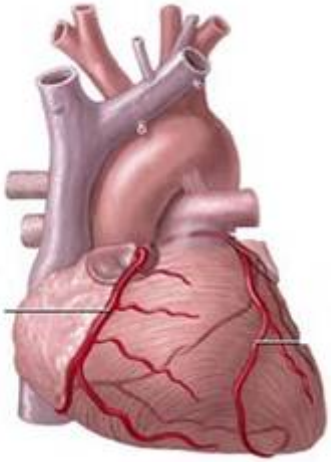
- Oxidative degradation of nutrients occurs in mitochondria
- Mitochondria are the major site of production of reactive oxygen species
- Mitochondria are vital organelles for (myo)cellular function and are regulators of apoptosis

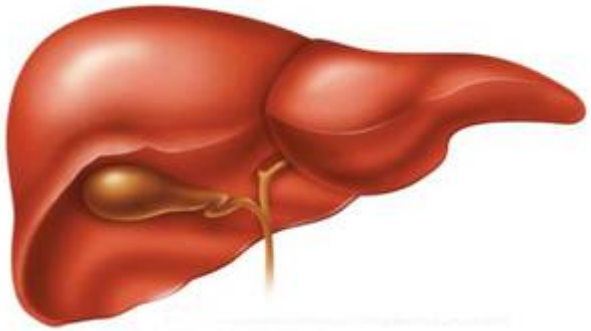
- Mitochondrial dysfunction may result in muscular fat storage and insulin resistance

Lipotoxicity, mitochondrial function and insulin resistance

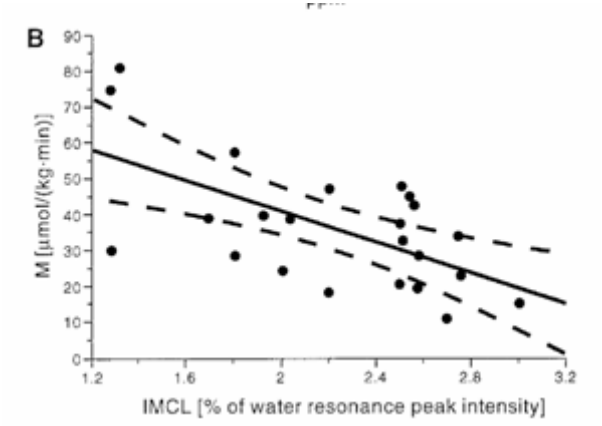


Tissues examined & Models used

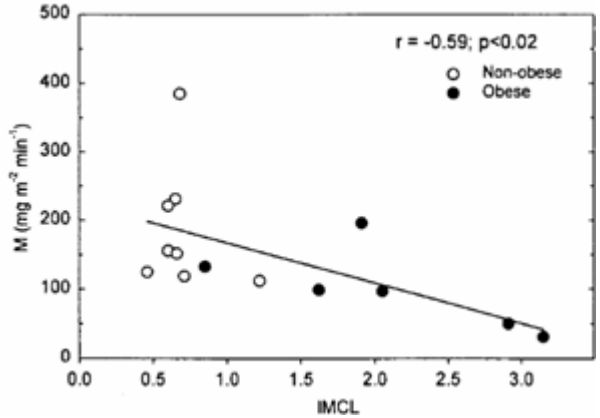




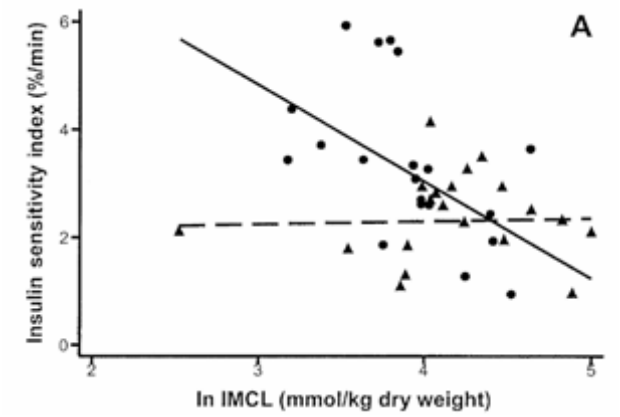
IMCL is negatively associated with insulin sensitivity



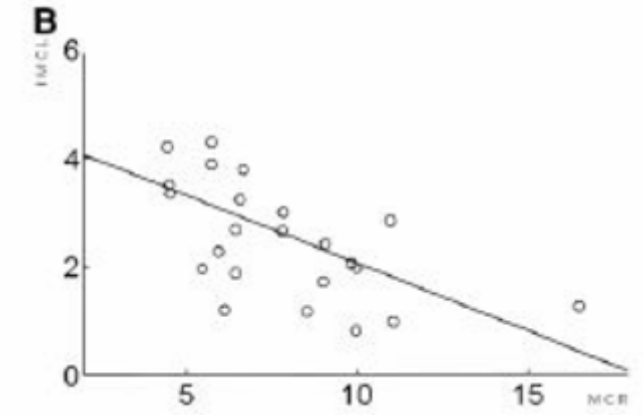
Krssak et al., Diabetologia 2002



Sinha et al., Diabetes 2002

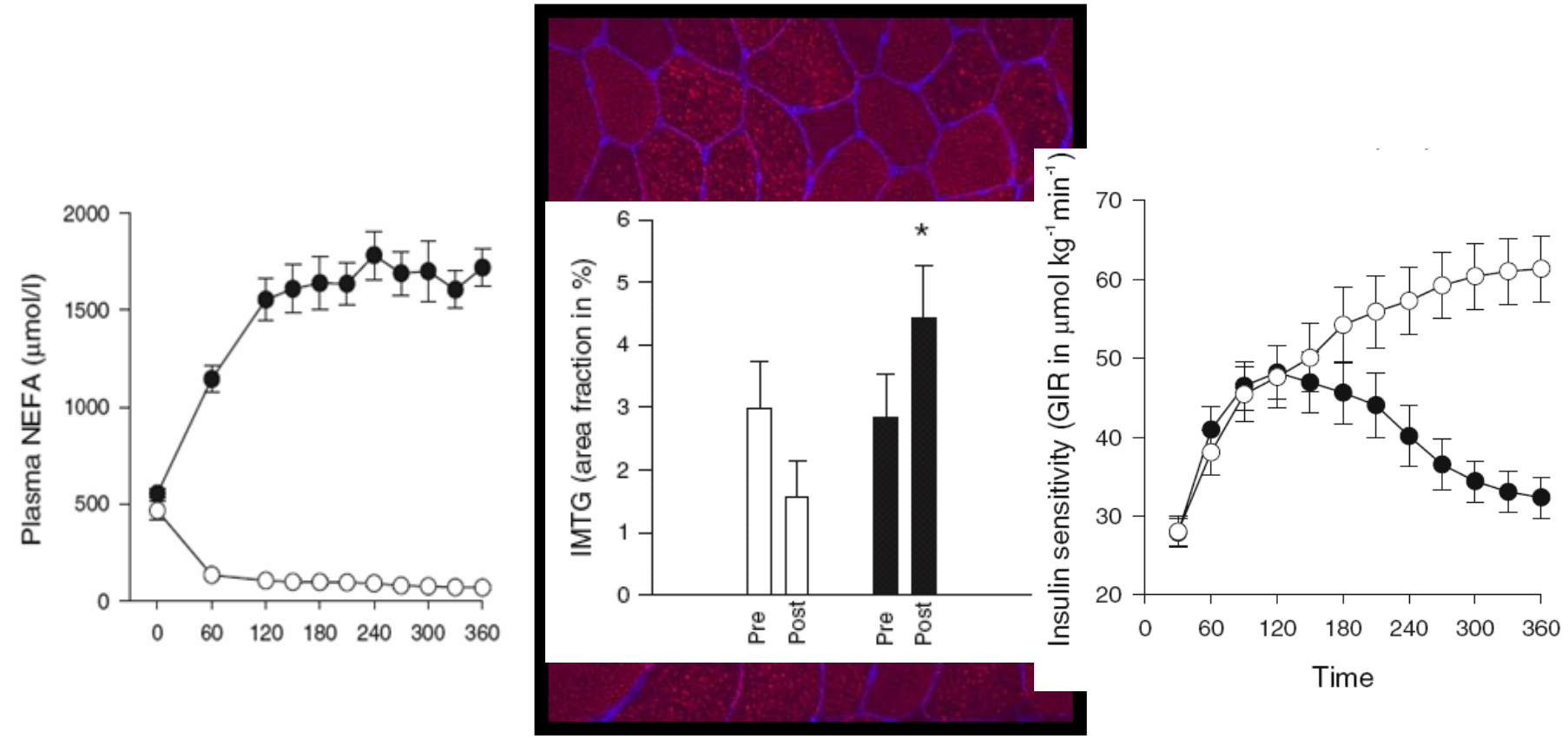


Fourouhi et al., Diabetologia 1999



Jacob et al., Diabetes 1999

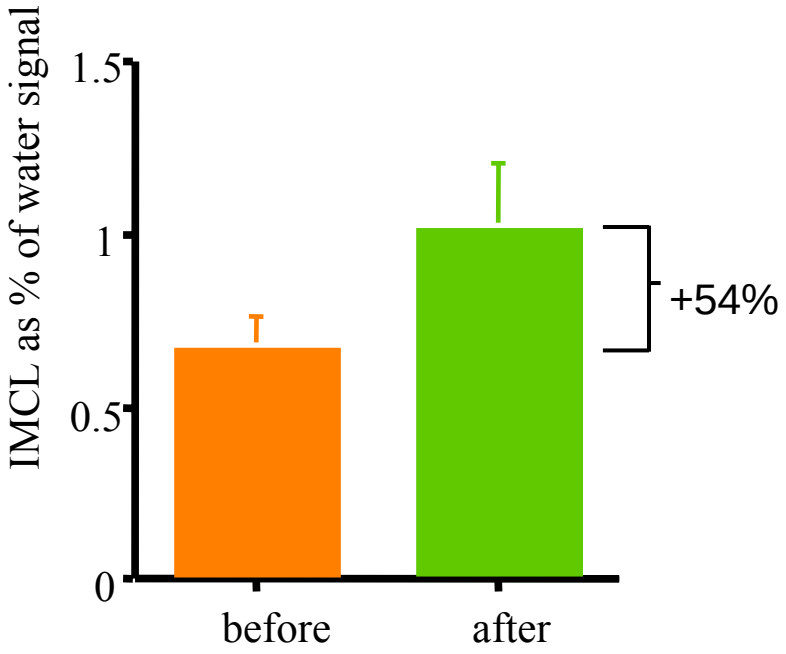
High FFA levels acutely induce muscle TG accumulation and insulin resistance



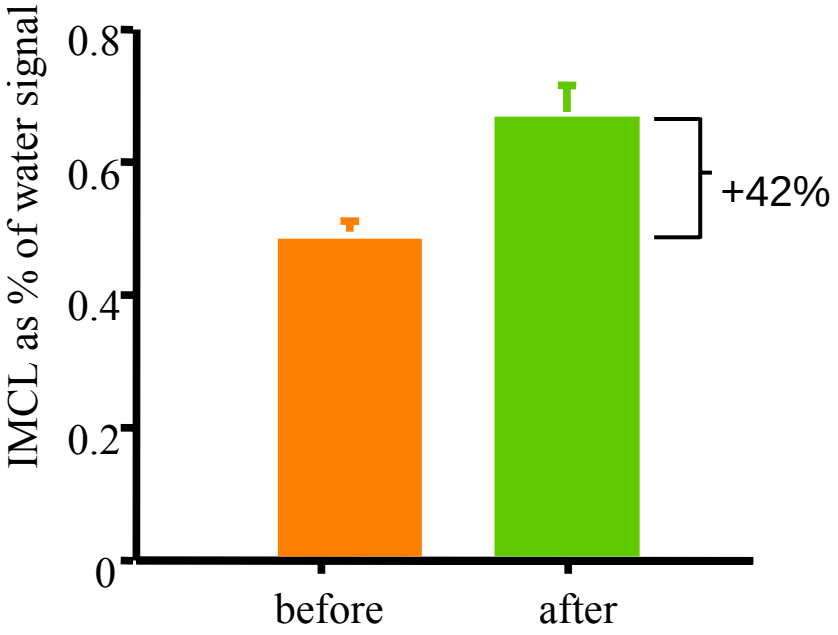
Hoeks et al, Diabetologia 2006

Paradox: endurance training also increases IMCL content

7 days high-fat diet



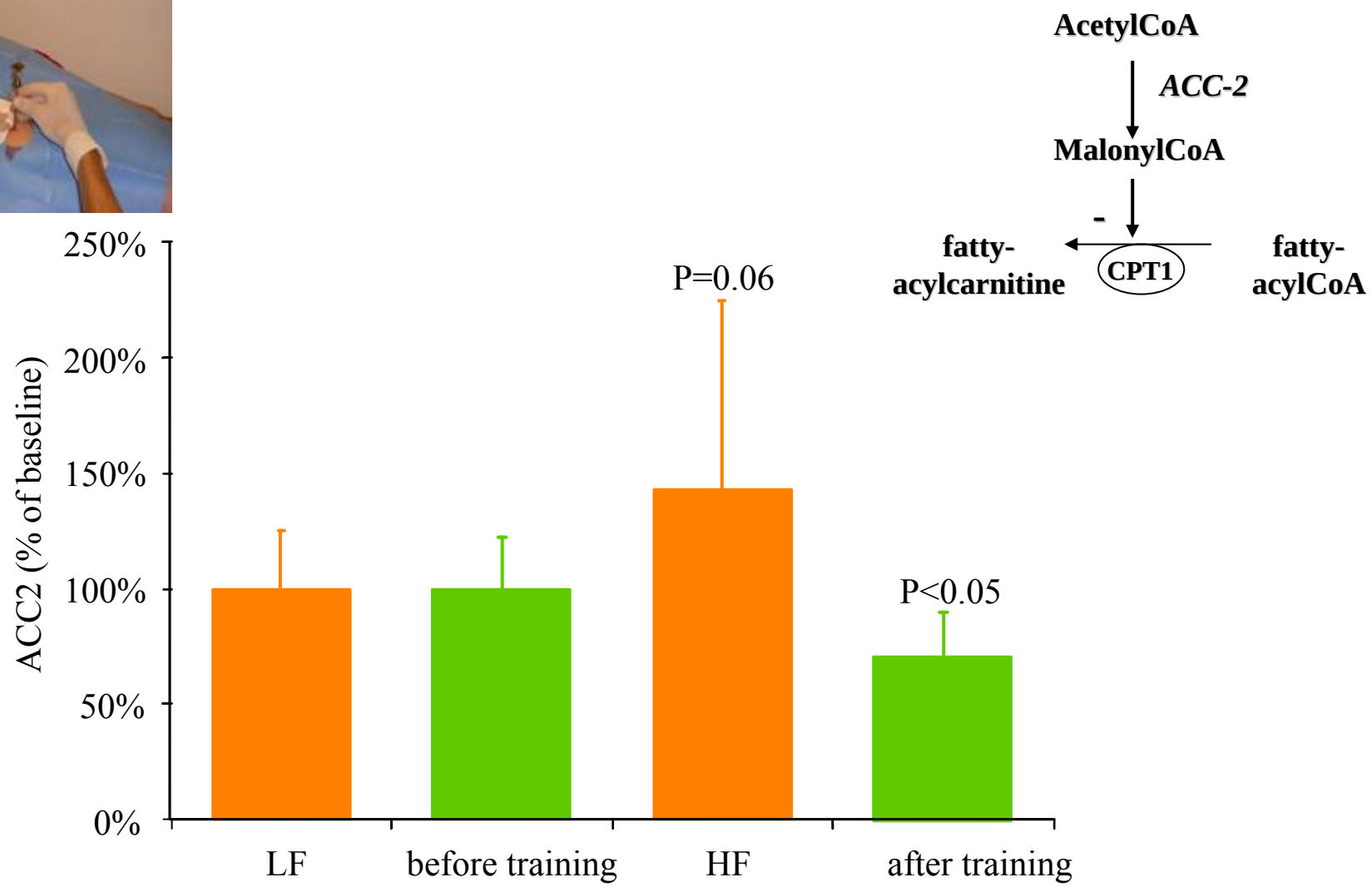
14 days endurance training



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?

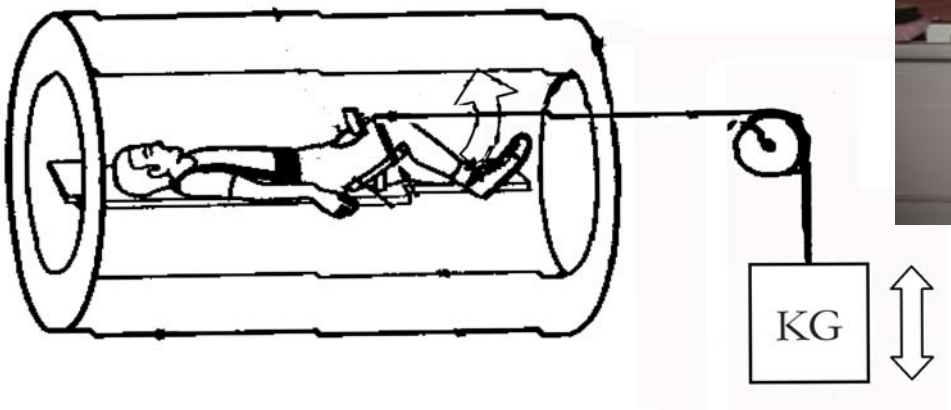
Muscular mitochondrial function in T2DM and controls, matched for BMI

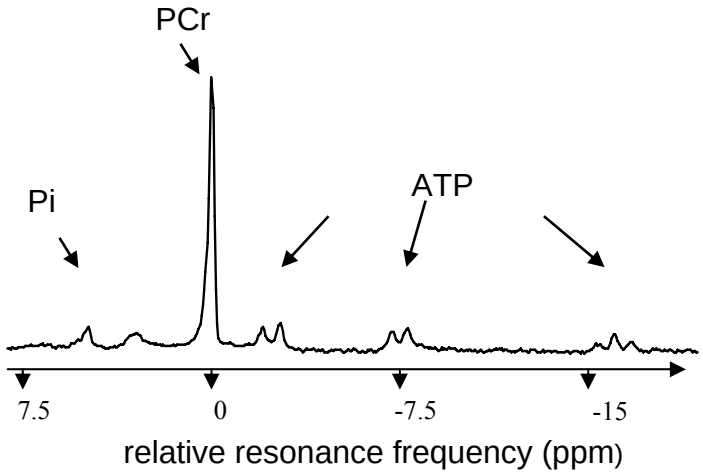
	Diabetic patients (n=12, all males)	Controls (n=9, all males)	p-value
BMI (kg/m2)	29.4 ± 3.3	29.3 ± 2.7	n.s.
Age (years)	61.8 ± 4.4	56.1 ± 6.8	P < 0.05
VO_{2max} ($ml*min^{-1}*kg^{-1}$)	30.6 ± 5.8	34.6 ± 4.8	n.s.
plasma glucose (mM)	9.56 ± 2.12	5.73 ± 0.37	P < 0.01
insulin sensitivity (GIR, $\mu mol*min^{-1}*kg^{-1}$)	18.9 ± 8.1	26.0± 6.7	P < 0.05

(mean ± stdev)

Schrauwen-Hinderling et al., Diabetologia 2007

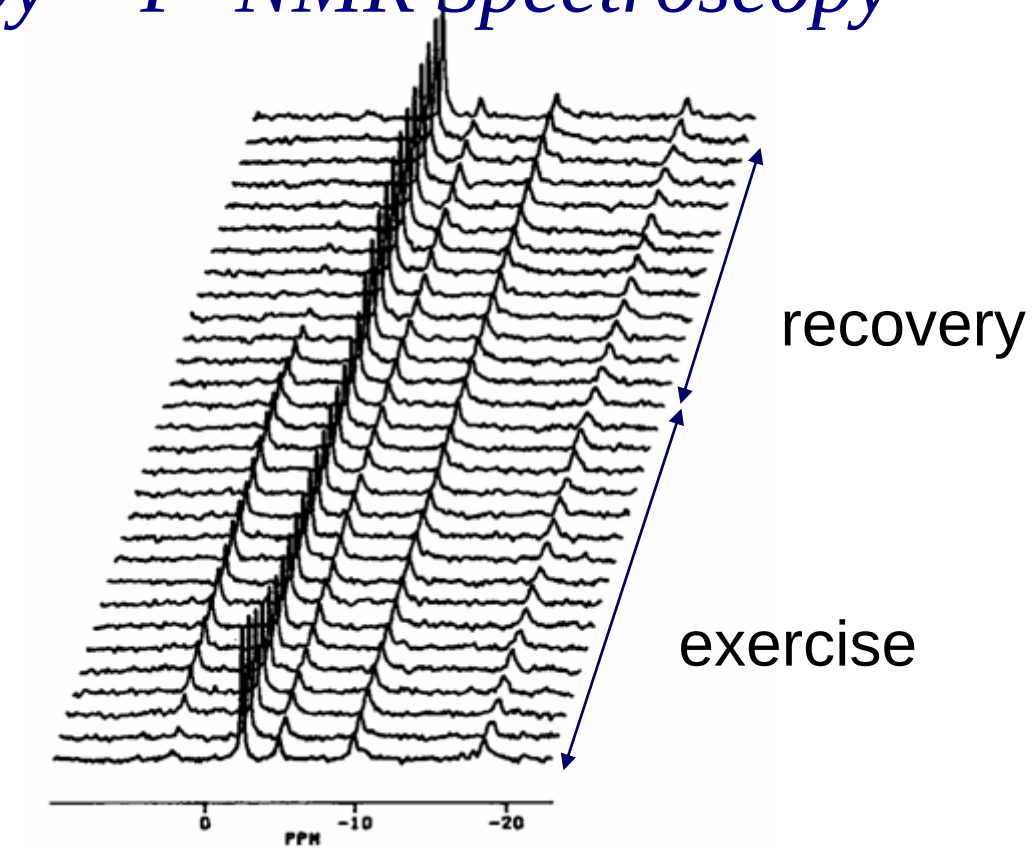
Muscle mitochondrial function *in vivo*
by ^{31}P NMR Spectroscopy





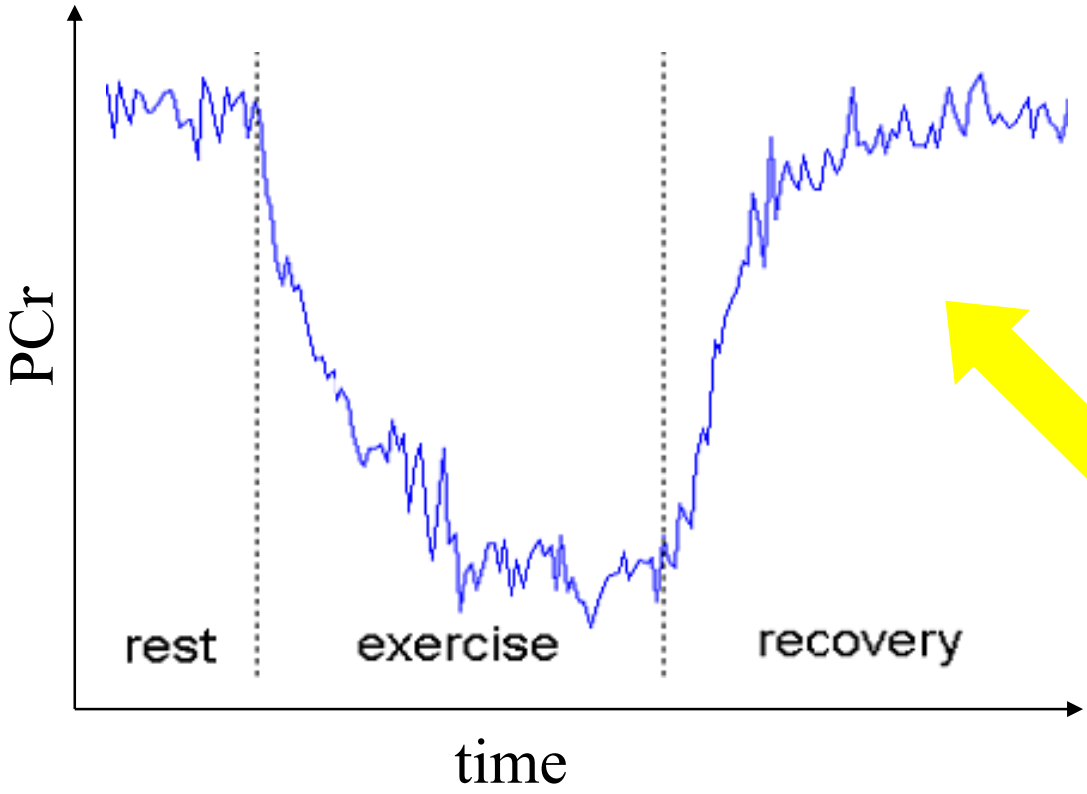
Muscle mitochondrial function *in vivo*

by ^{31}P NMR Spectroscopy



Meyer et al., 1988

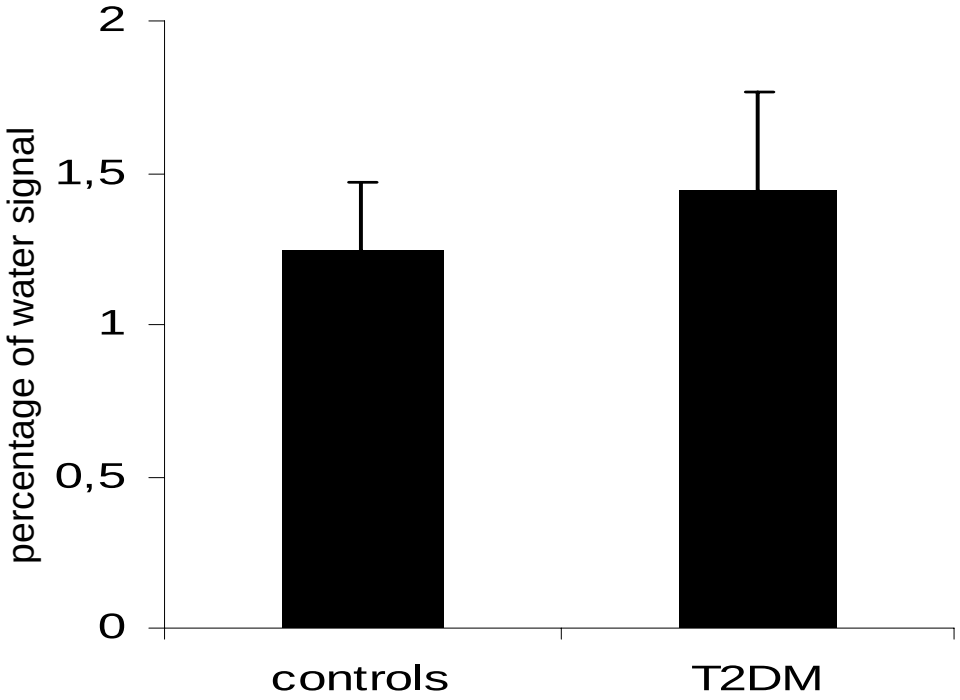
Muscle mitochondrial function *in vivo*
by ^{31}P NMR Spectroscopy



PCr resynthesis is
almost purely aerobic

PCr recovery half-time
reflects oxidative capacity

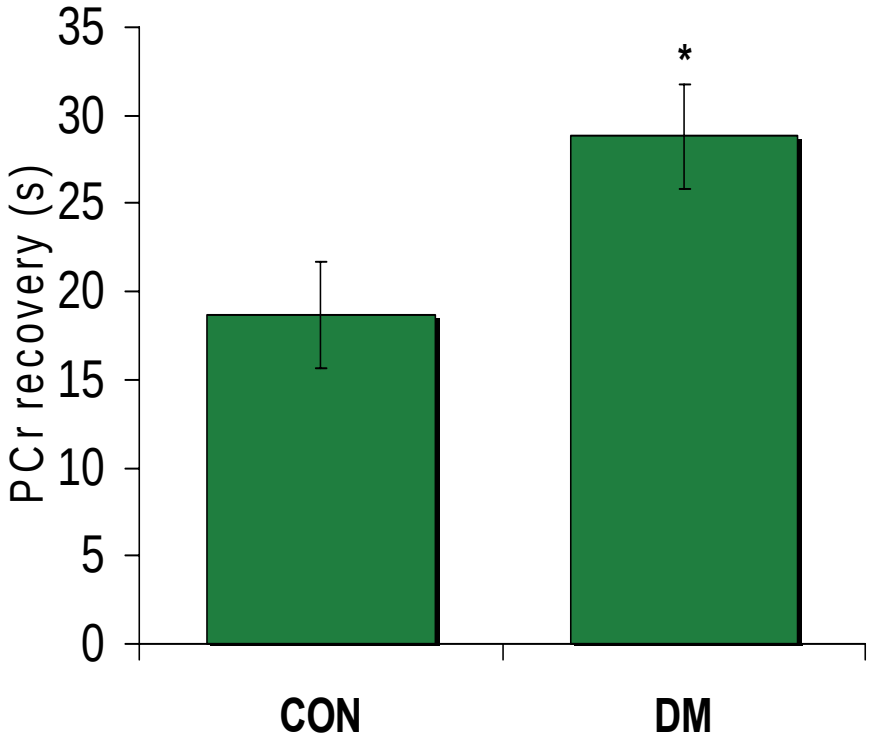
Muscular fat content in T2DM and BMI matched normoglycemic controls is similar

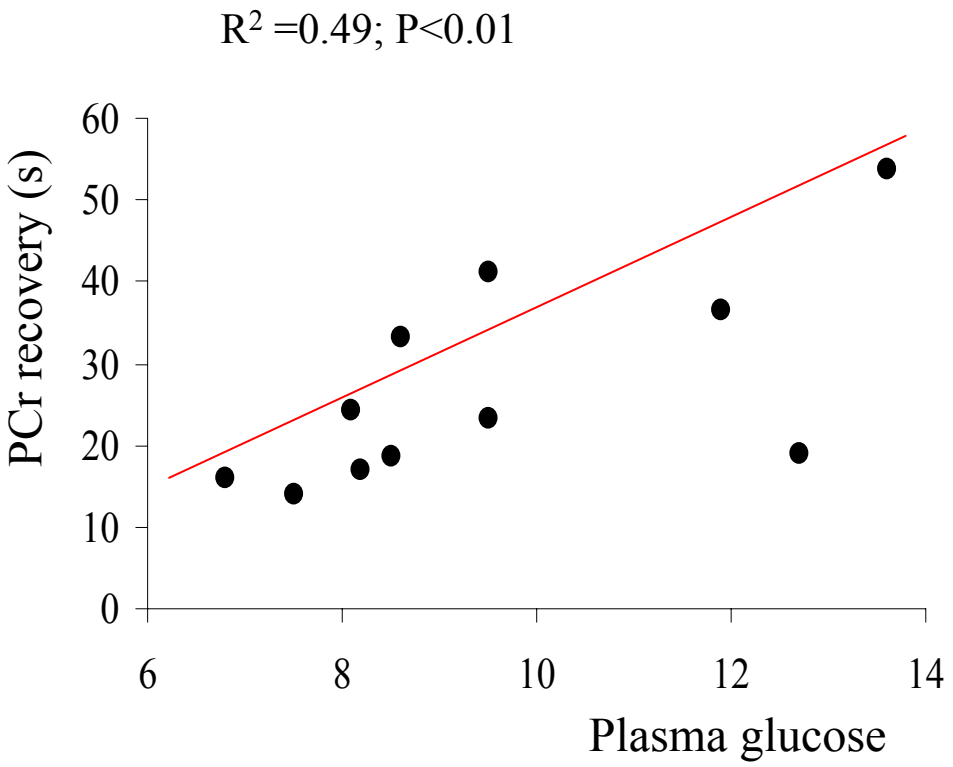


Schrauwen-Hinderling et al., Diabetologia 2007

Q: How about mitochondrial function?

In vivo mitochondrial function reduced in T2DM, correlates with glucose and HbA1C





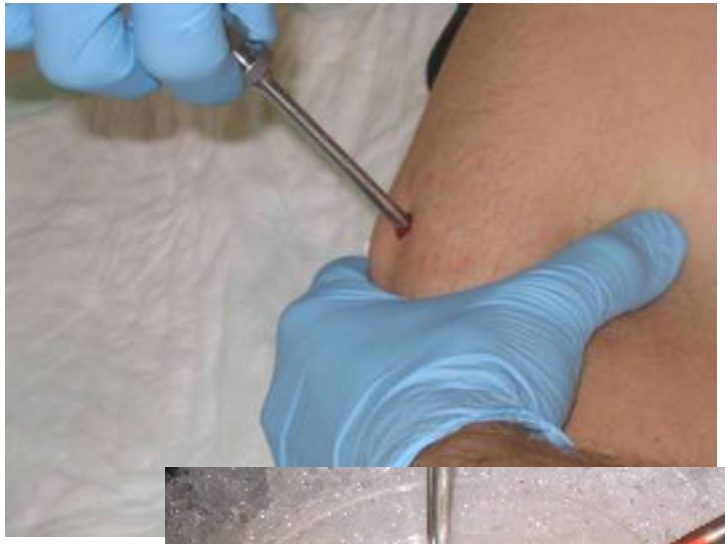
Schrauwen-Hinderling et al., Diabetologia 2007

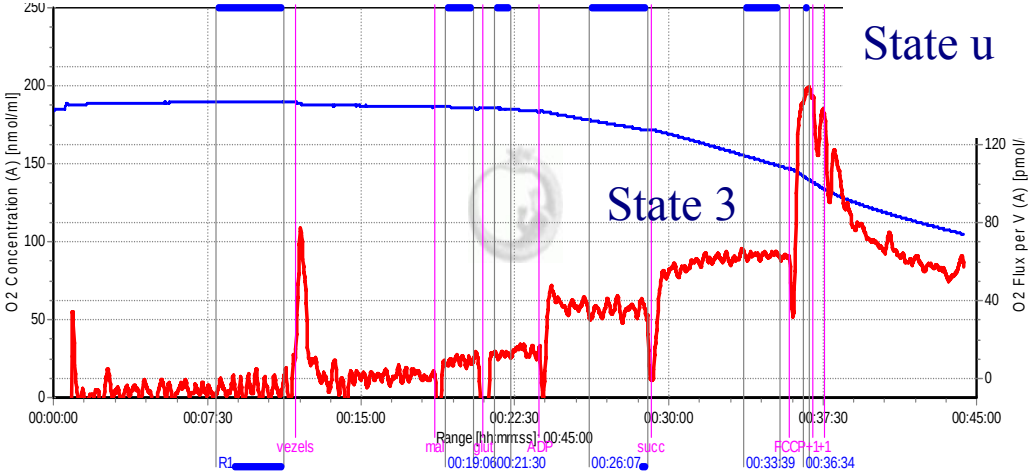
Q1:is mitochondrial dysfunction already present in the pre-diabetic state?

Q2:does intrinsic mitochondrial dysfunction underlie reduced *in vivo* mitochondrial function?

Intrinsic mitochondrial function *ex vivo*

using high-resolution respirometry





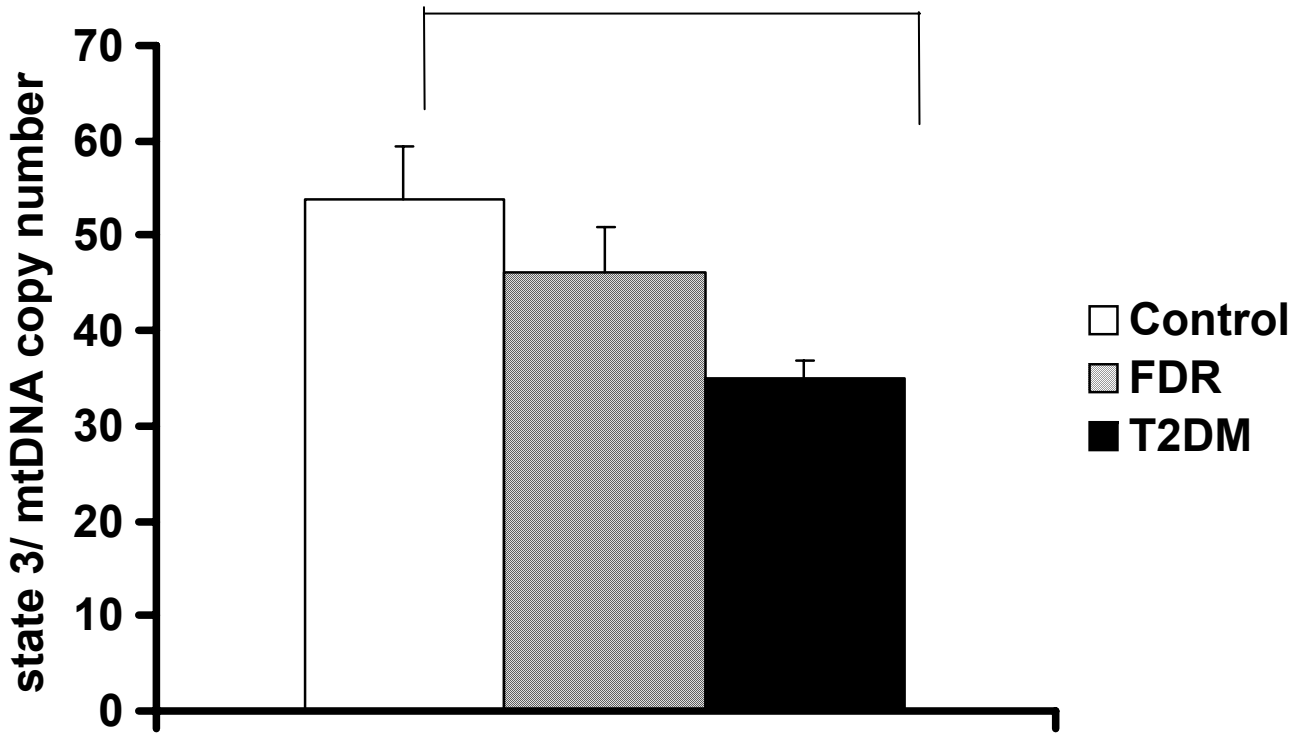
Pre-diabetic subject Characteristics

	Controls N=16	**FDR** N=12	**T2DM** N=10	
Age (y)	59.2 ± 0.7	60.1 ± 0.9	61.4 ± 1.6	n.s.
BMI (kg/m^2)	29.1 ± 0.7	30.1 ± 1.2	28.9 ± 0.7	n.s.
VO_2max (ml/kg/min)	31.2. ± 1.6	32.6 ± 9.6	28.2 ± 2.3	n.s.
Ins-stim R_d (umol/kg/min)	28.9 ± 3.7	22.1 ± 3.4	11.2 ± 2.8	* $p<0.05$

Phielix et al., Diabetes 2008

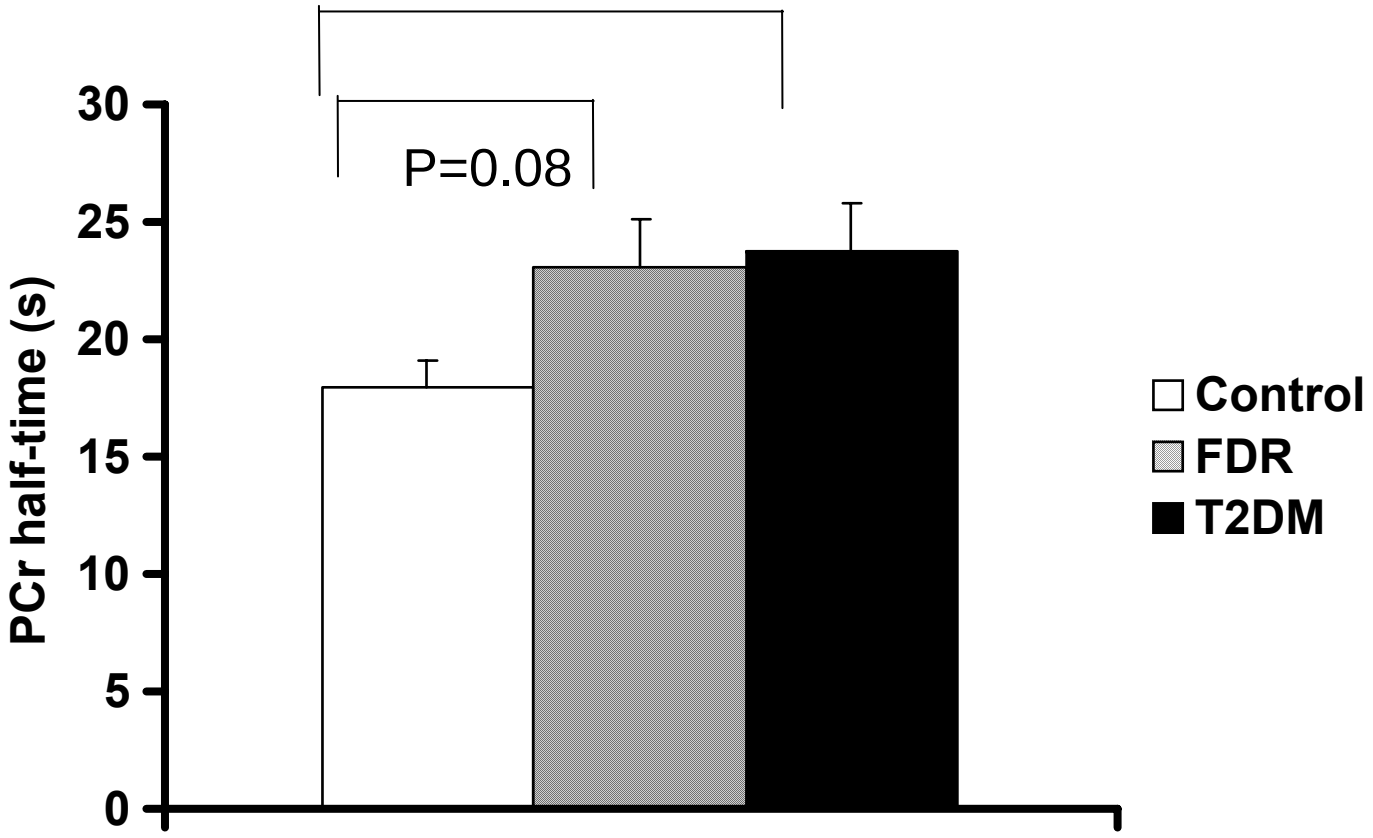
Lower *ex vivo* mitochondrial function in T2DM

irrespective of mitochondrial density (mtDNA)



Phielix et al., Diabetes 2008

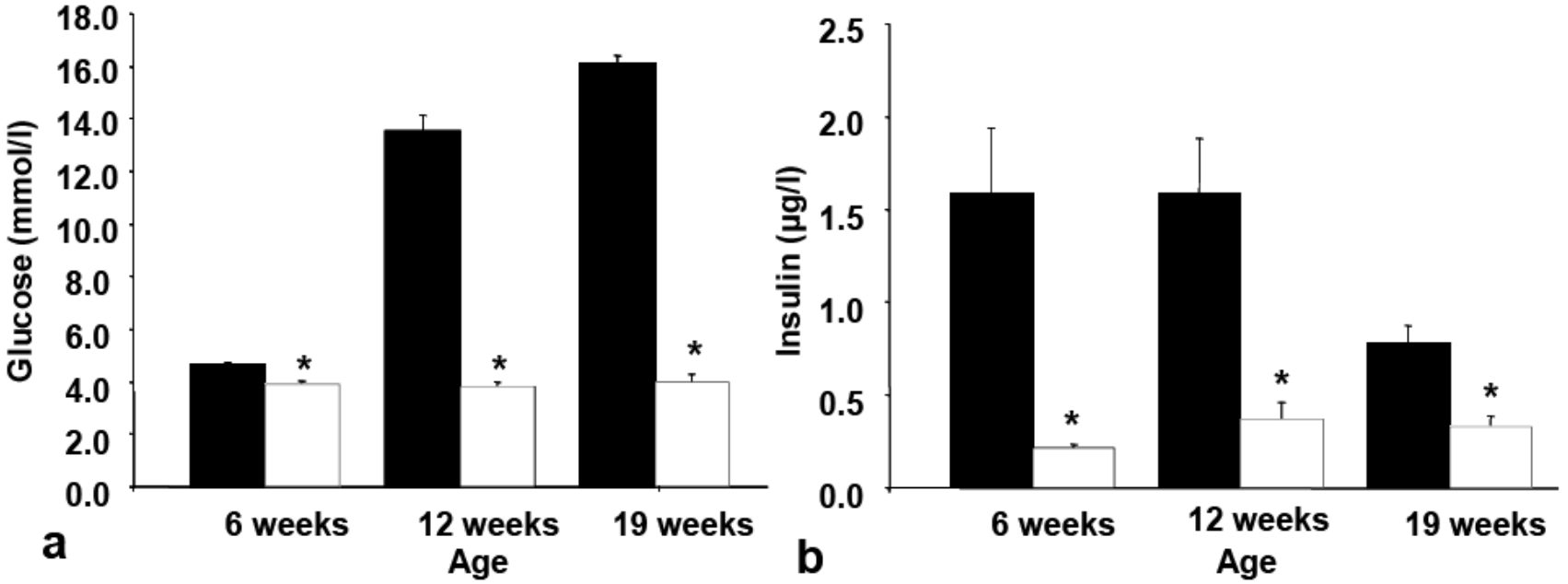
Lower *in vivo* mitochondrial function in T2DM and first-degree relatives



Phielix et al., Diabetes 2008

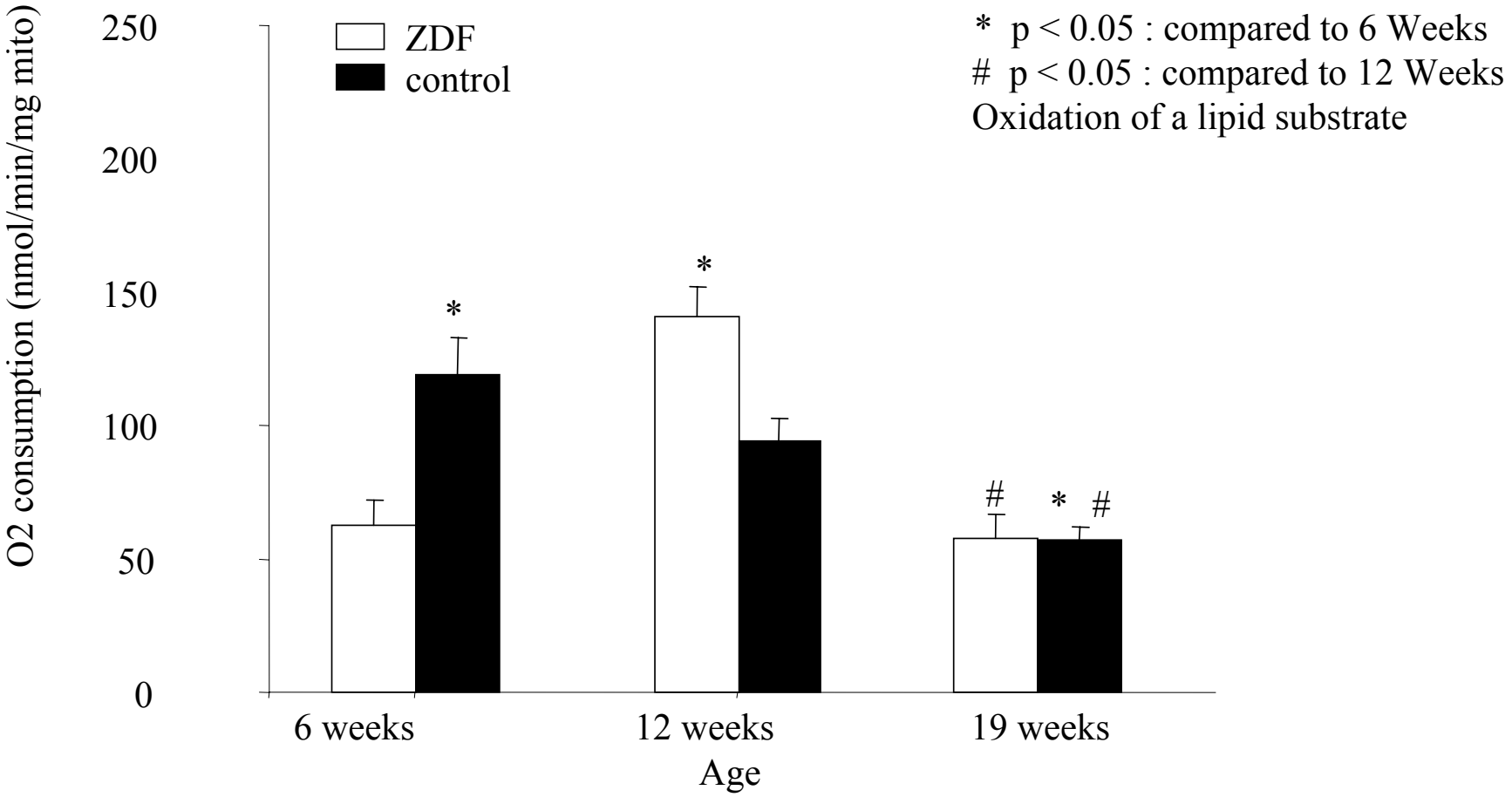
Q: Is compromised mitochondrial function in the pre-diabetic state required to develop insulin resistance?

Transition of pre-diabetes to T2D during maturation of ZDF rats



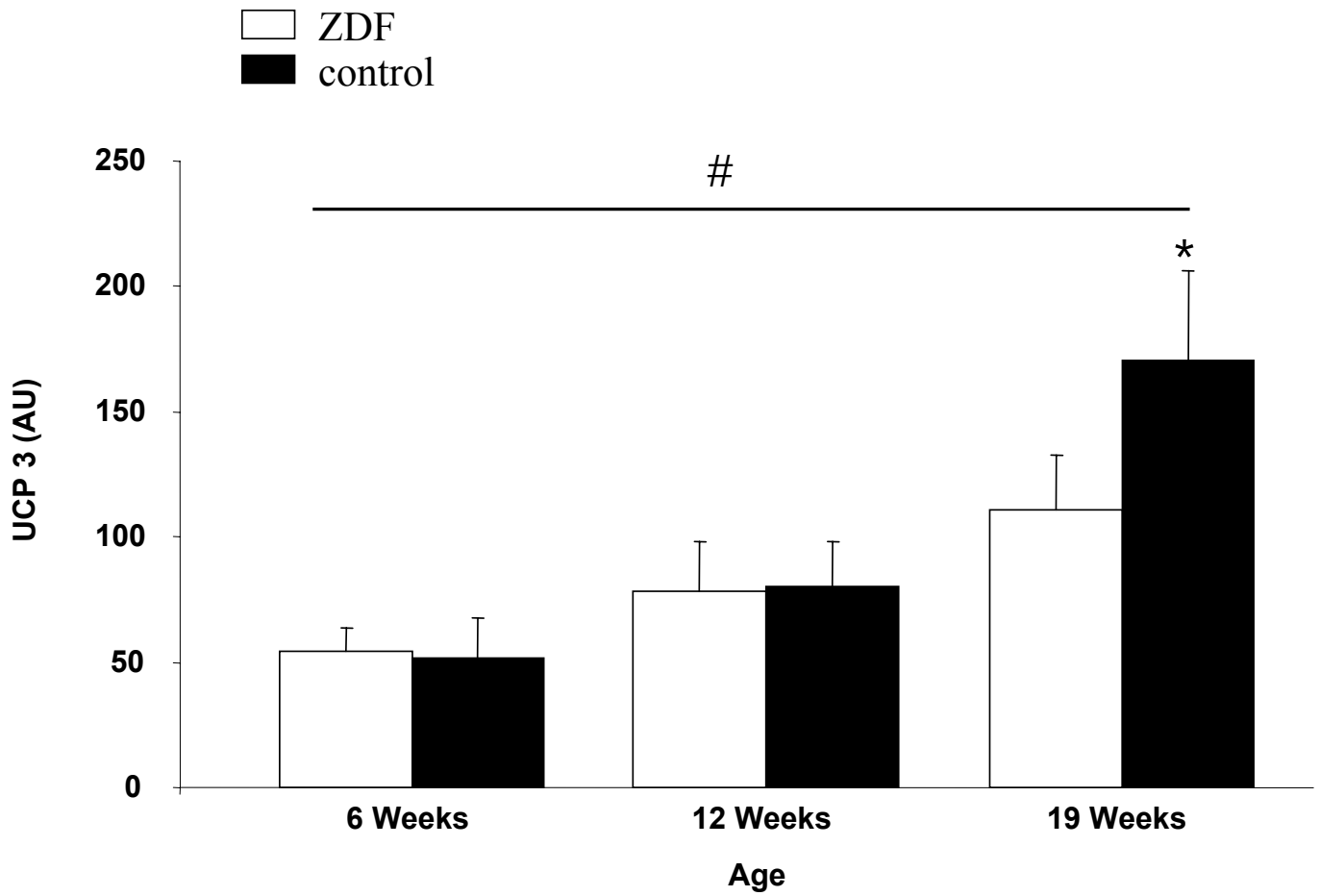
Lenaers et al., submitted

No mitochondrial dysfunction during the development of T2D in ZDF rats



Lenaers et al., submitted

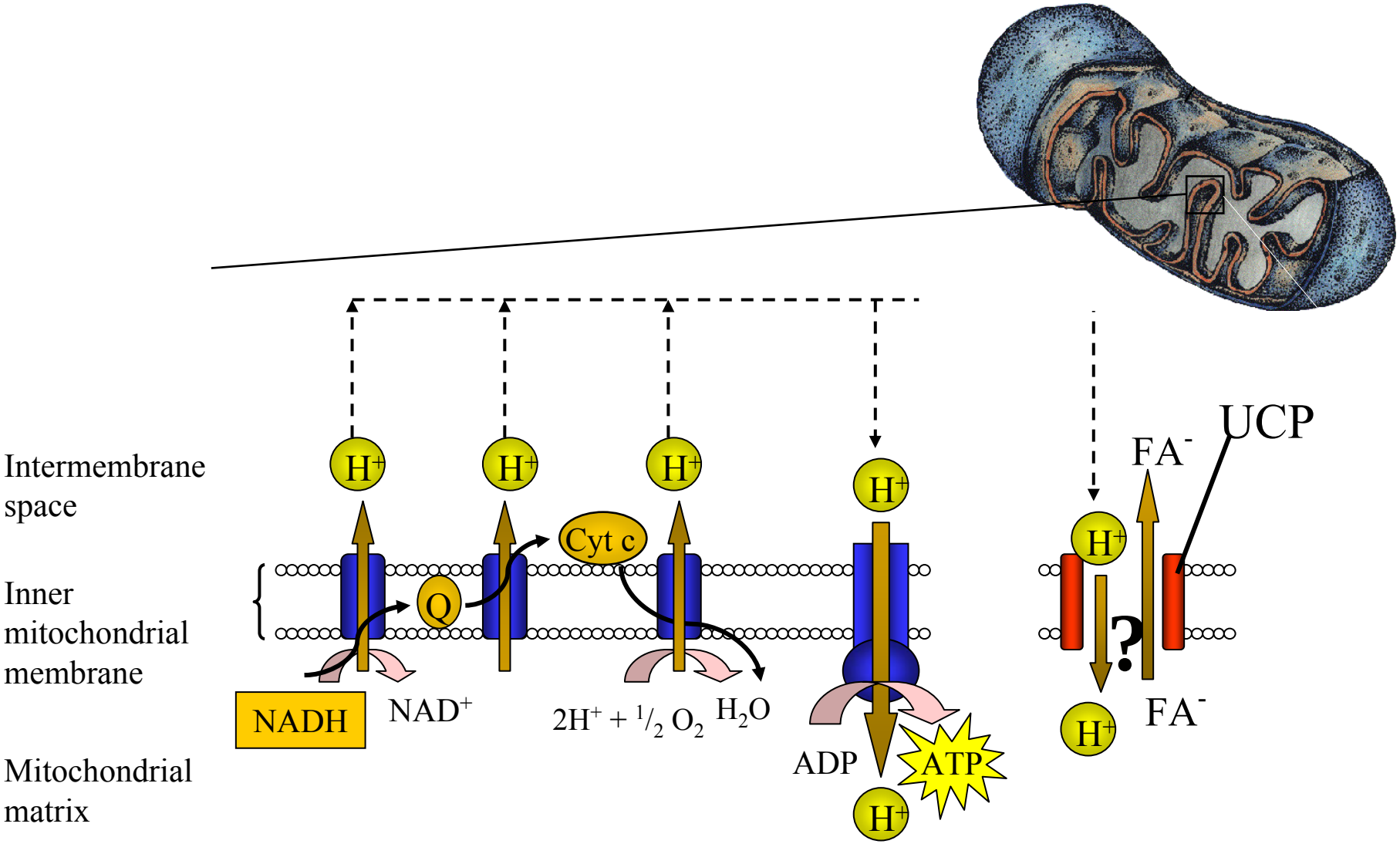
Increased UCP3 protein blunted in ZDF rats



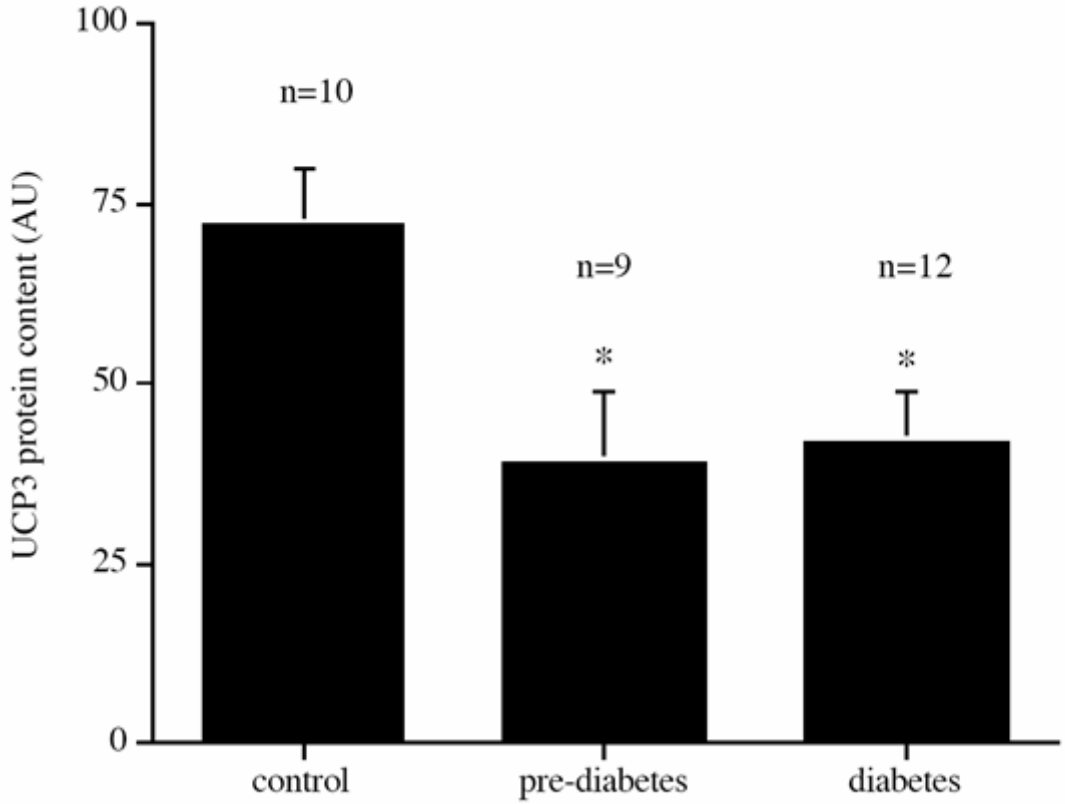
Lenaers et al., submitted

Uncoupling protein 3 (UCP3)

- ~55% homology to UCP1 which is responsible for the thermogenesis in of 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 H^+ or FA^-


Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
H+
Q
Cyt c
NADH
NAD+
2H+ + 1/2 O2
H2O
ADP
ATP
FA-
UCP
?
Electron transport chain
ATP synthase
UCP leak

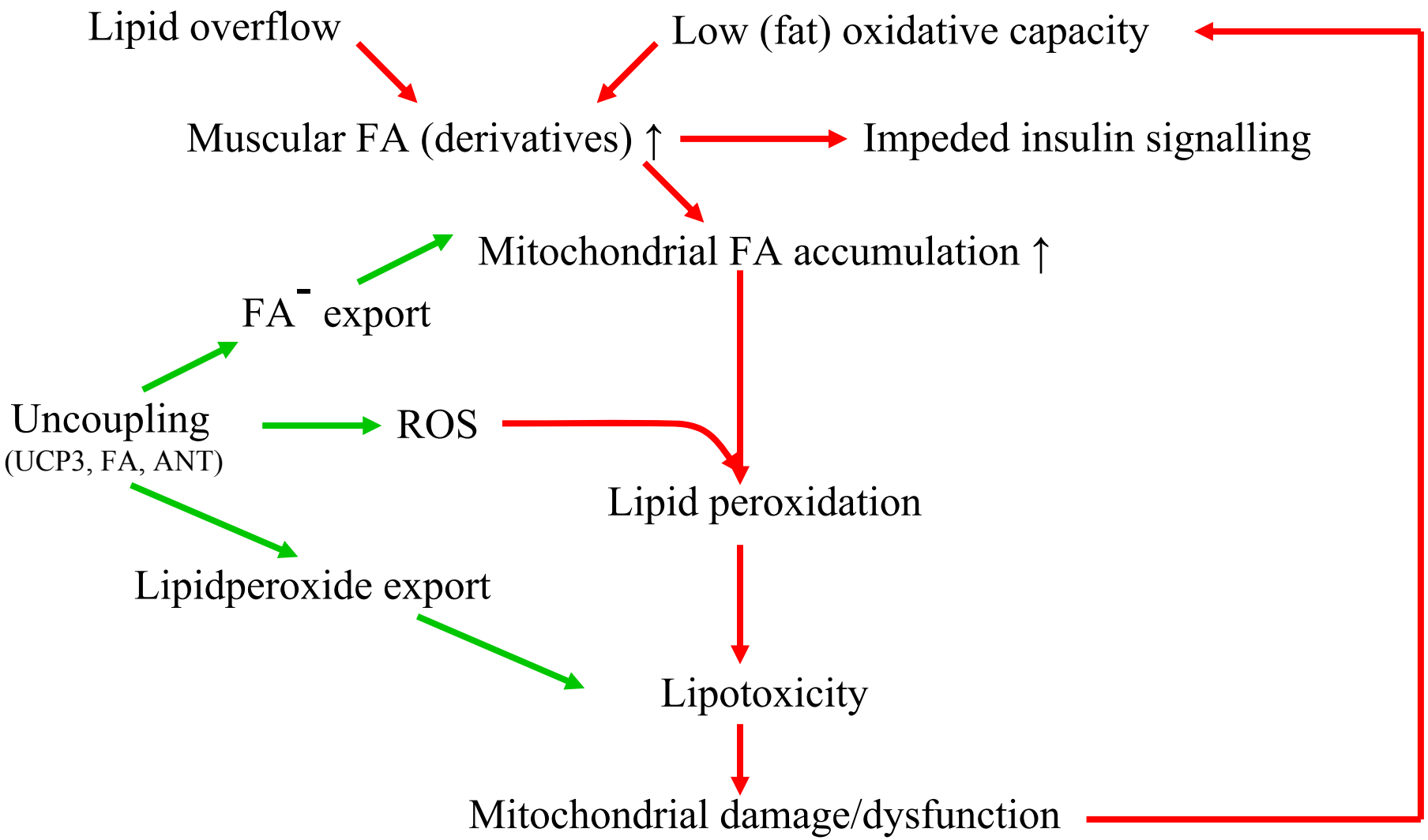
UCP3 is reduced in (pre)diabetic patients



Schrauwen et al., JCEM 2006

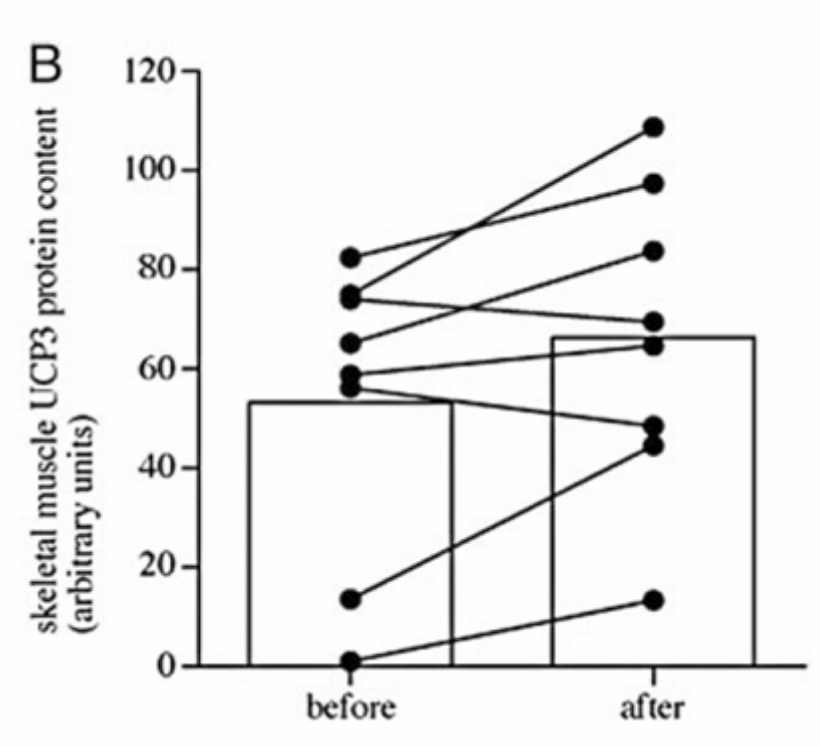
Q: Is UCP3 involved in the development of mitochondrial dysfunction in T2D?

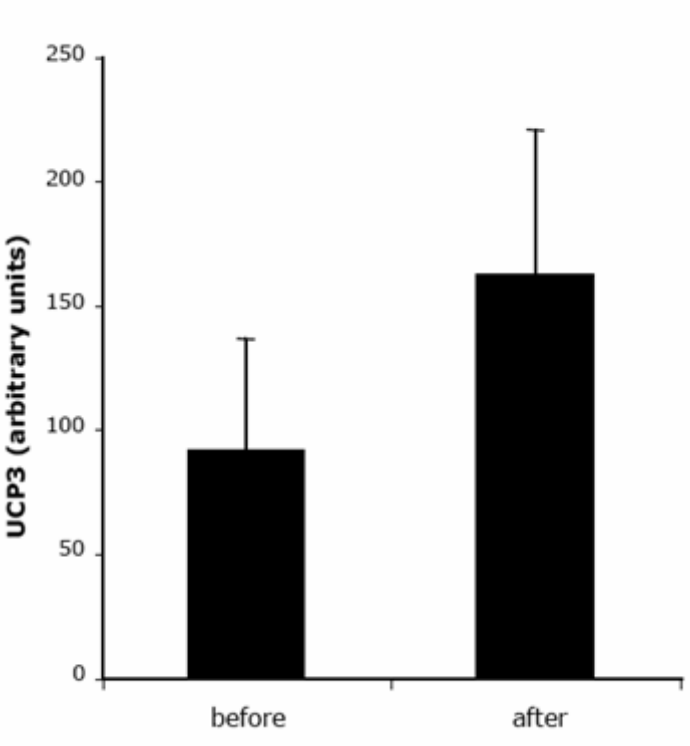
Lipotoxicity, mitochondrial function and insulin resistance



Q: Can insulin sensitizing by exercise training restore mitochondrial aberrations observed in T2D?

Insulin sensitizing by Rosiglitazone restores UCP3 protein content in T2D…

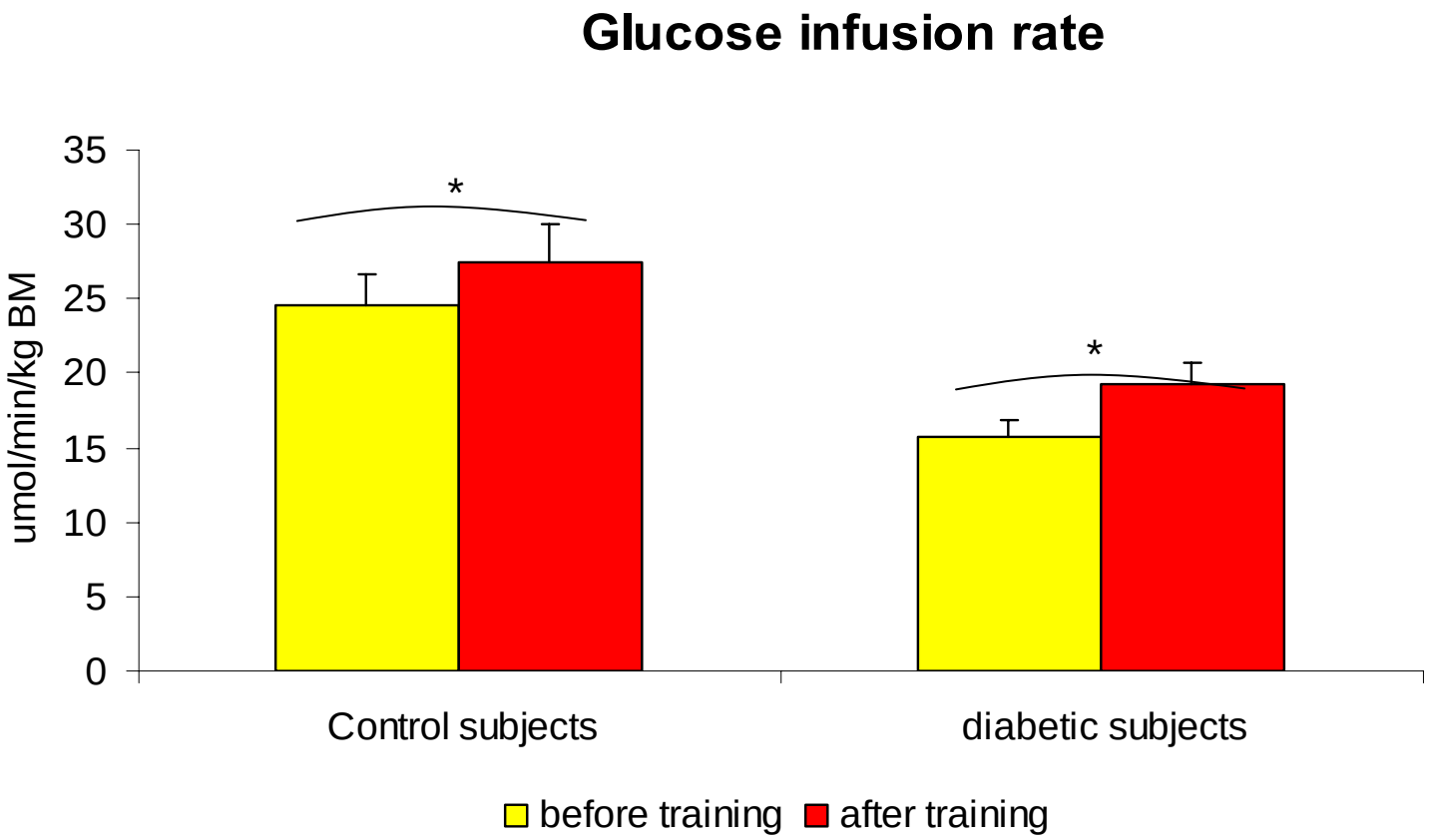




Schrauwen et al, JCEM 2006

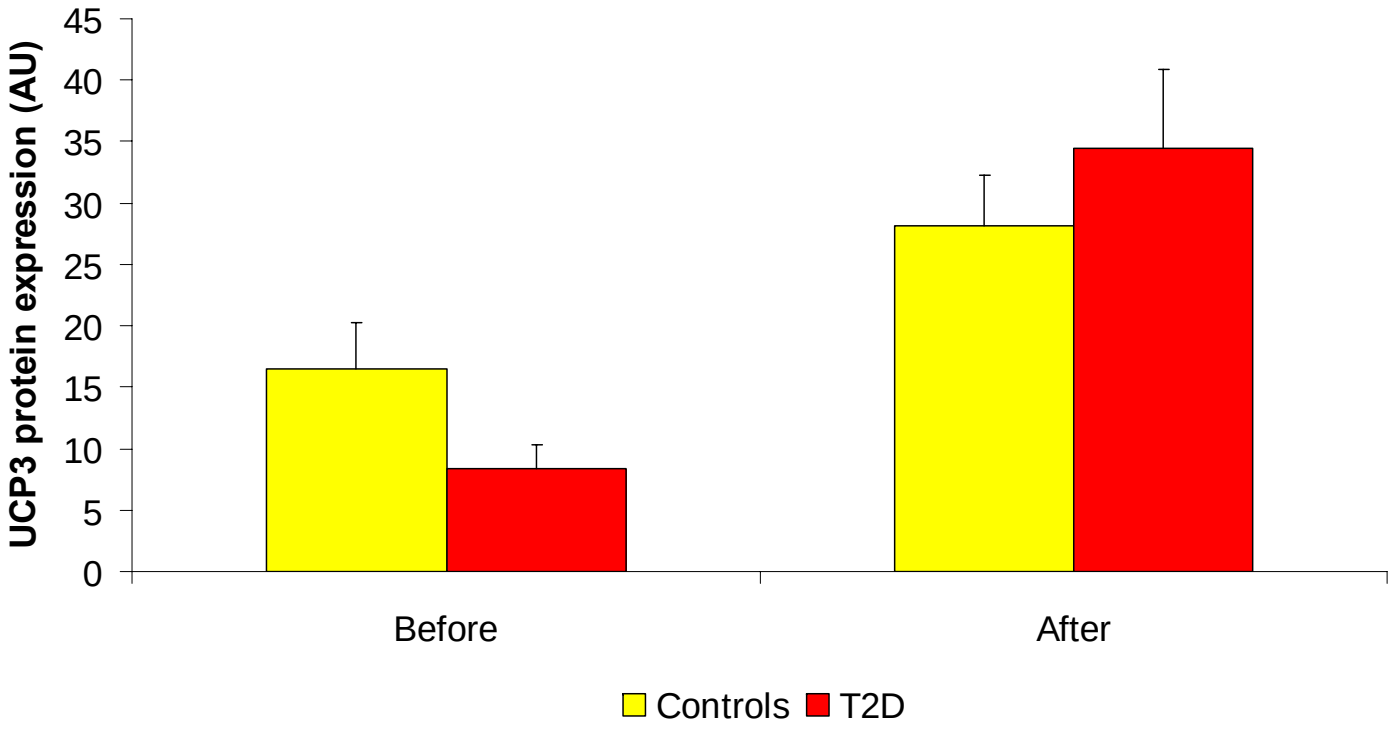
Mensink et al, Diab Obes Met 2006

Exercise training in T2D and controls improves insulin sensitivity



Meex et al., in progress

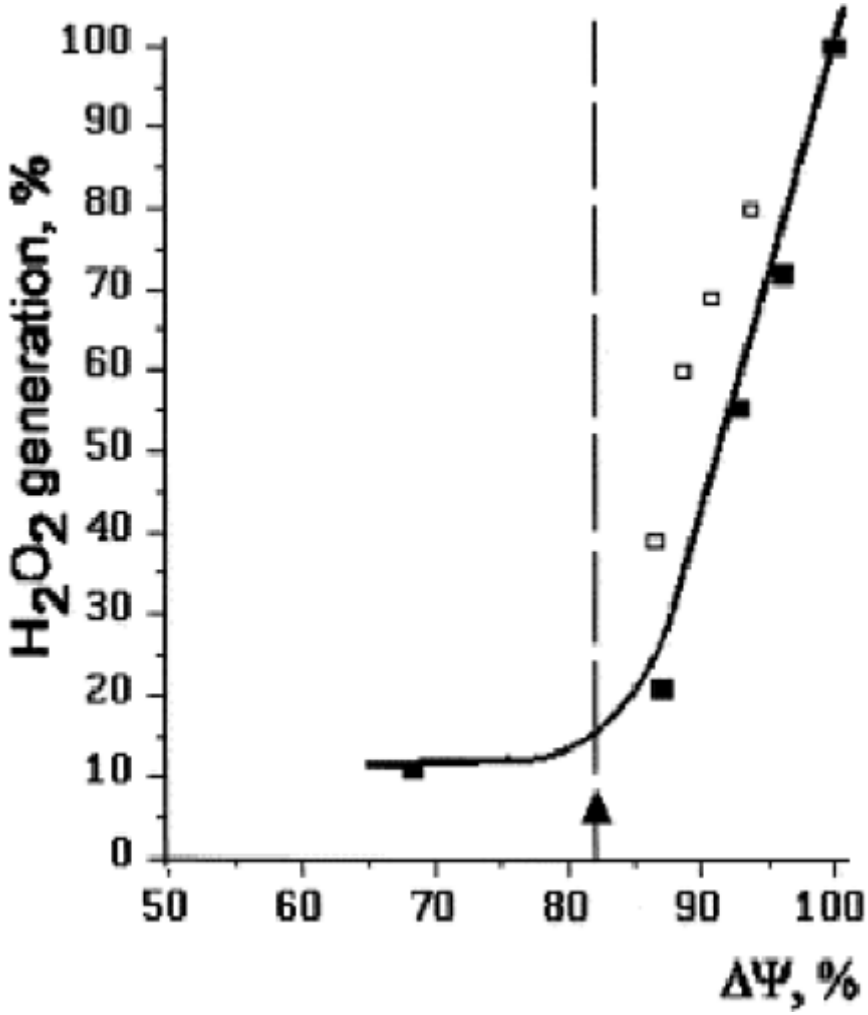
Exercise training in T2D restores UCP3 content



Meex et al., in progress

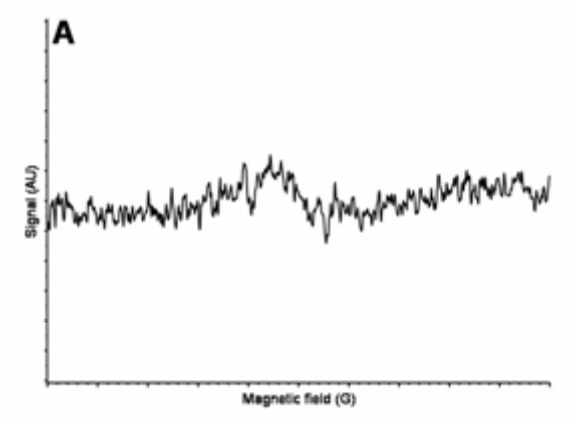
Q: Can mitochondrial uncoupling reduce superoxide production in diabetogenic conditions?

Mitochondrial proton gradient and ROS production

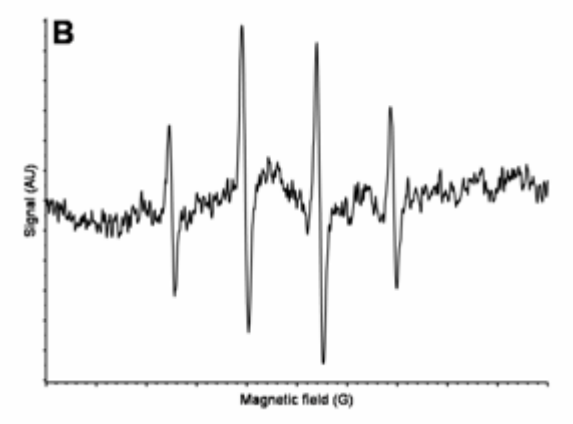


Skulachev et al., BBA 1997

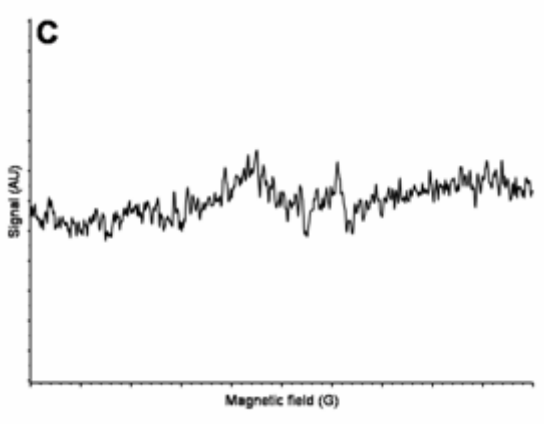
Measuring superoxide production by electron spin resonance in isolated mitos



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



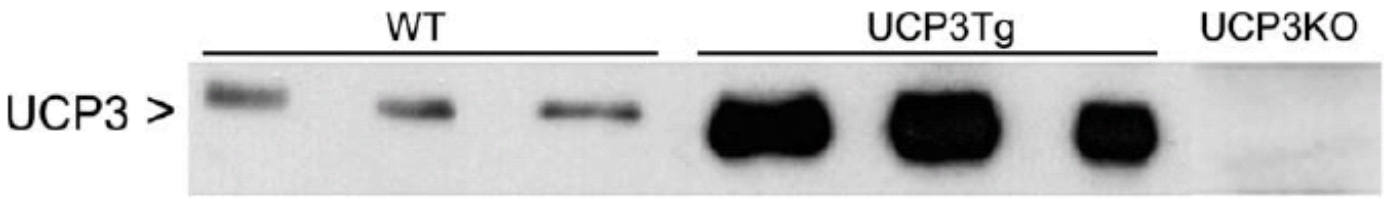
muscle mito 0.2 mg/ml
5 min 37° C
100 mM DMPO
10 mM glutamate
10 mM succinaat
3 mM malate

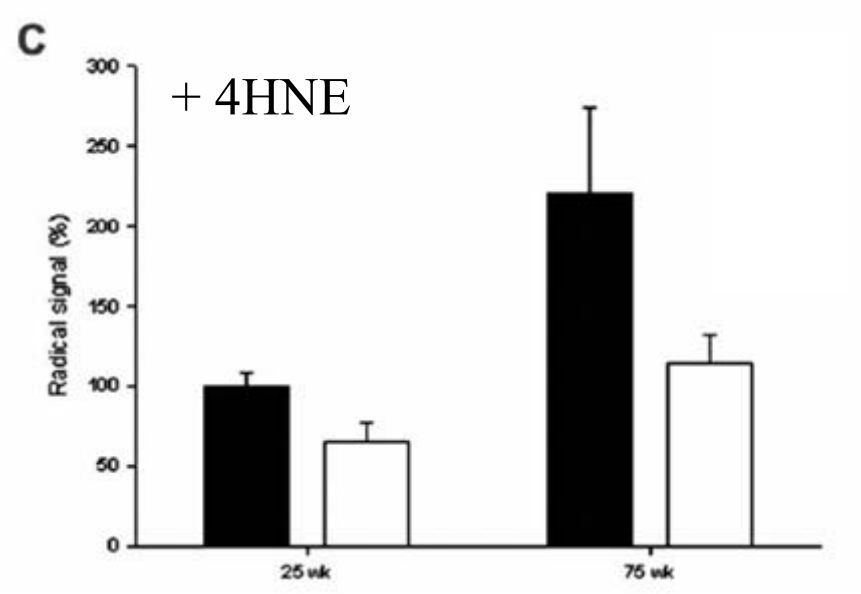


idem 2
500 U/ml SOD

Hoeks et al., FEBS 2008

Aging related increased superoxide production is blunted in UCP3 tg mice





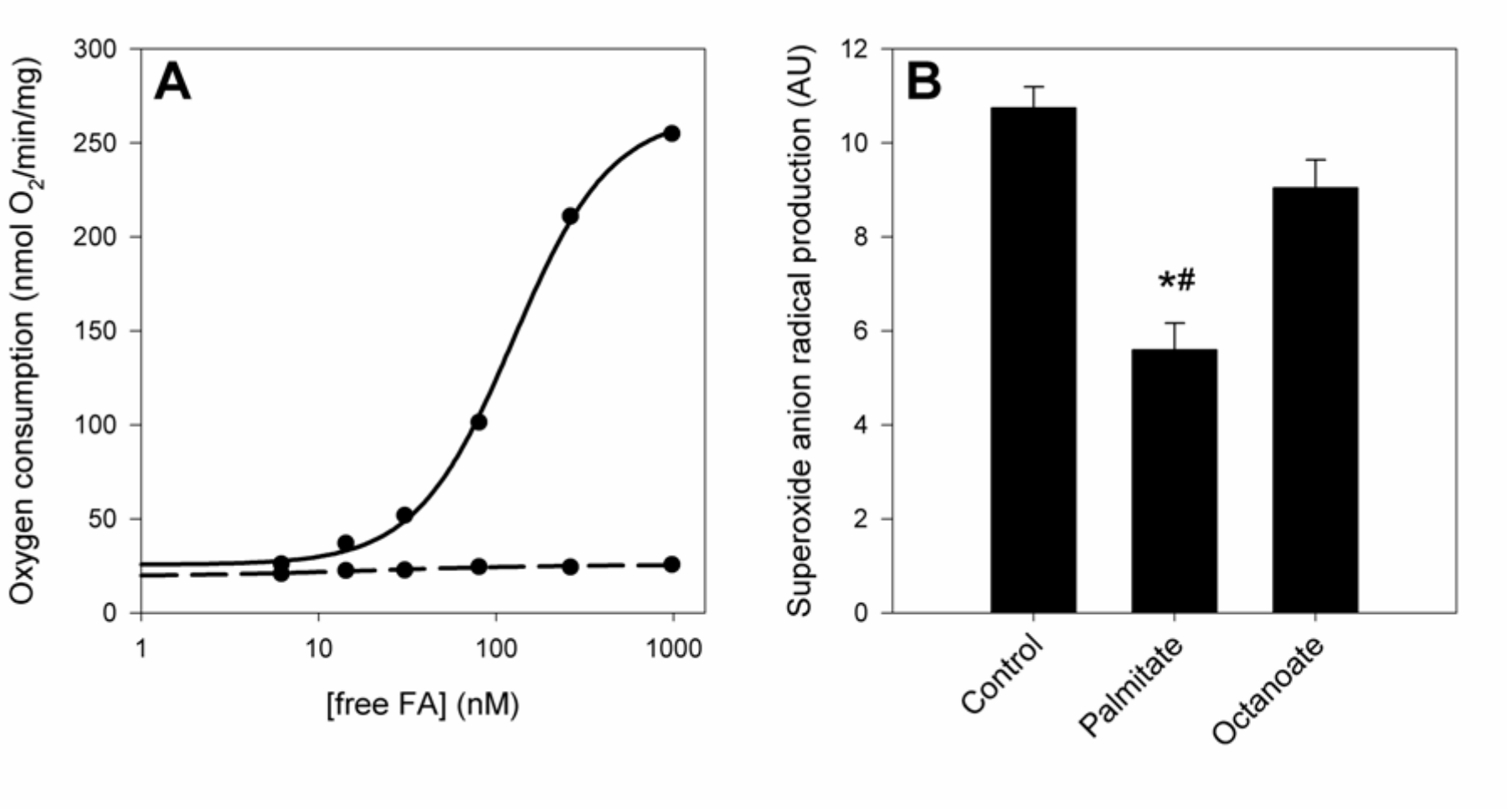
■ WT □ Overexpressor

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

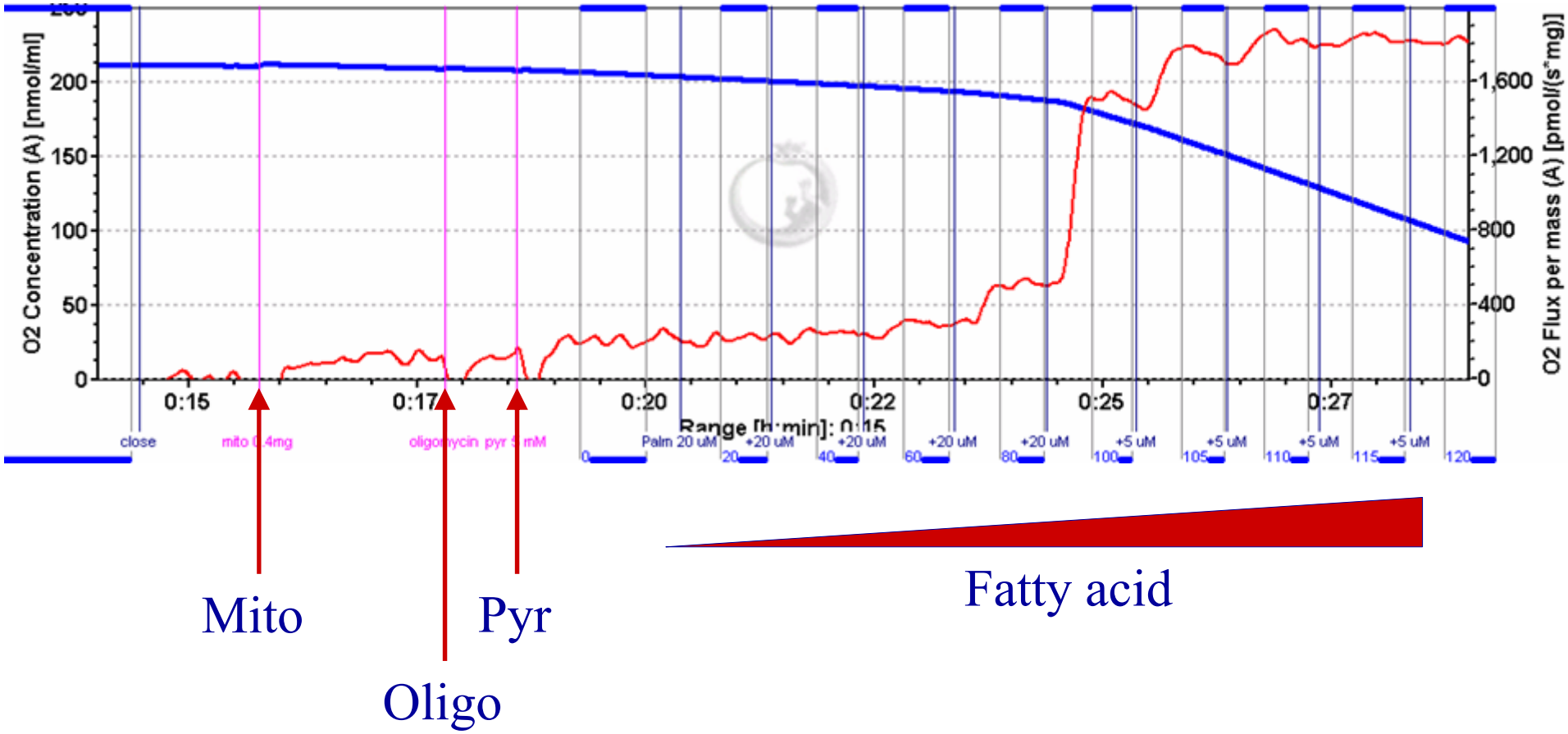
Hoeks et al., in progress

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

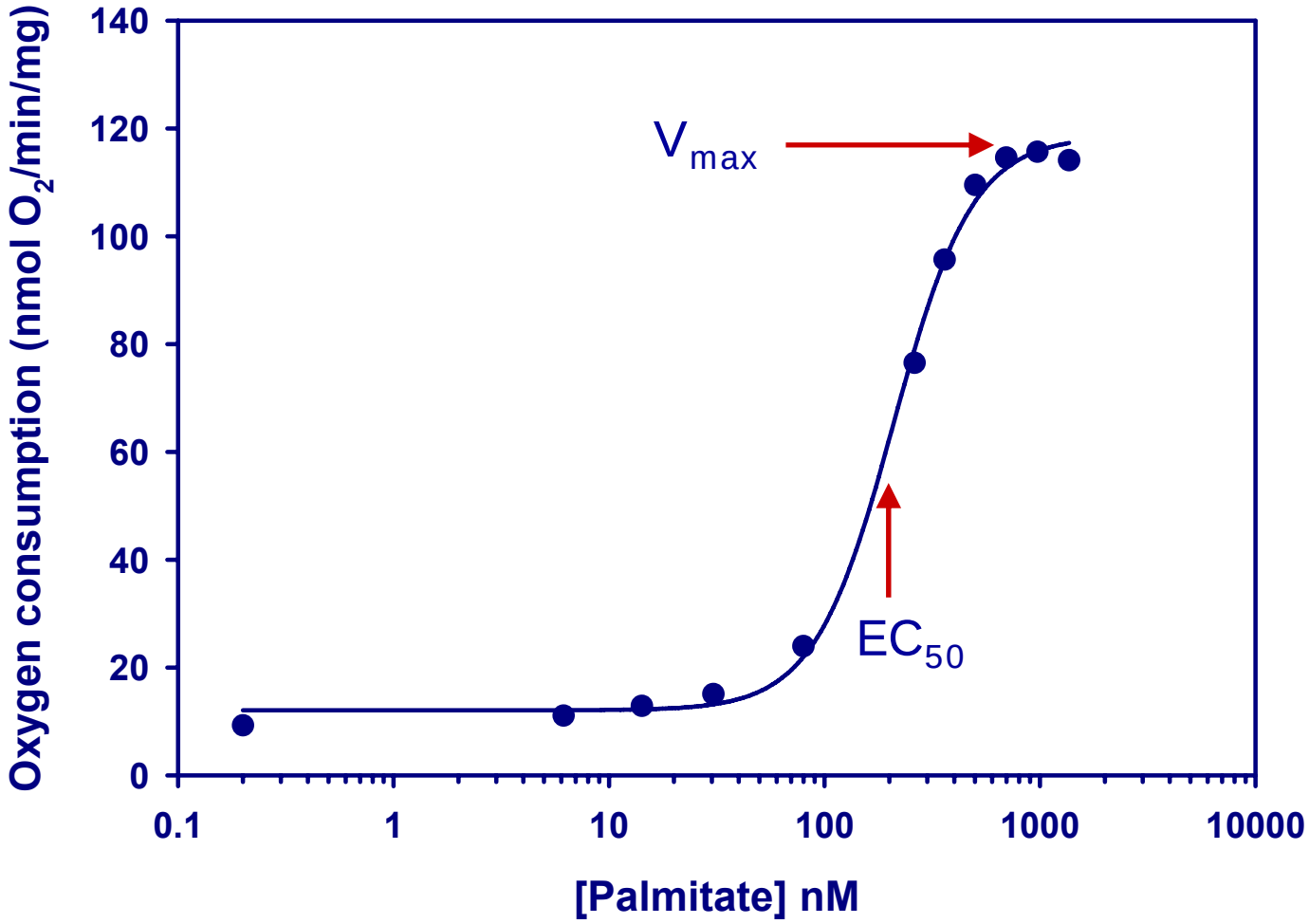
Q: Is FA-induced uncoupling lower in diabetic ZDF Rats?

FA-induced uncoupling



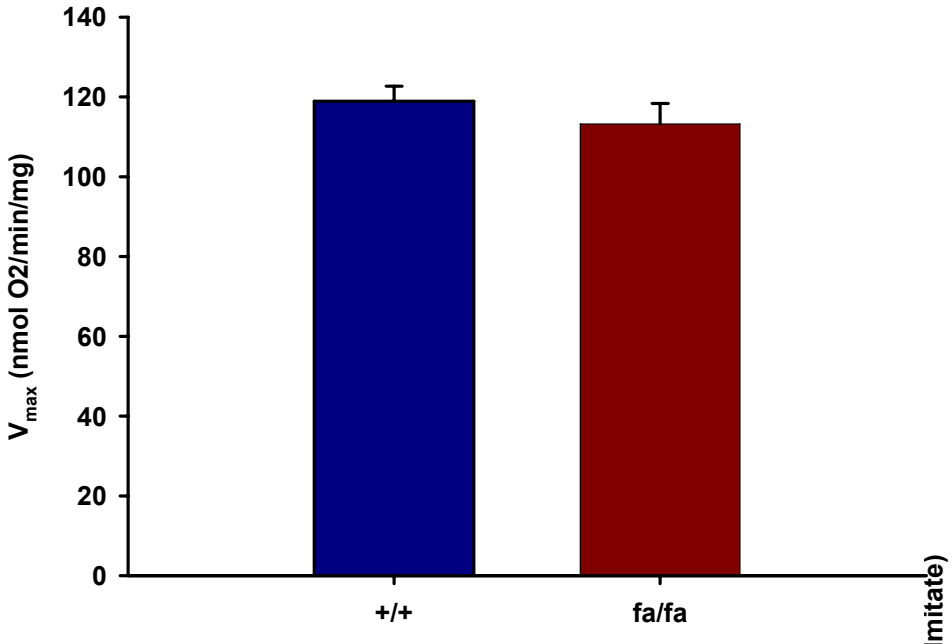
Hoeks et al., in progress

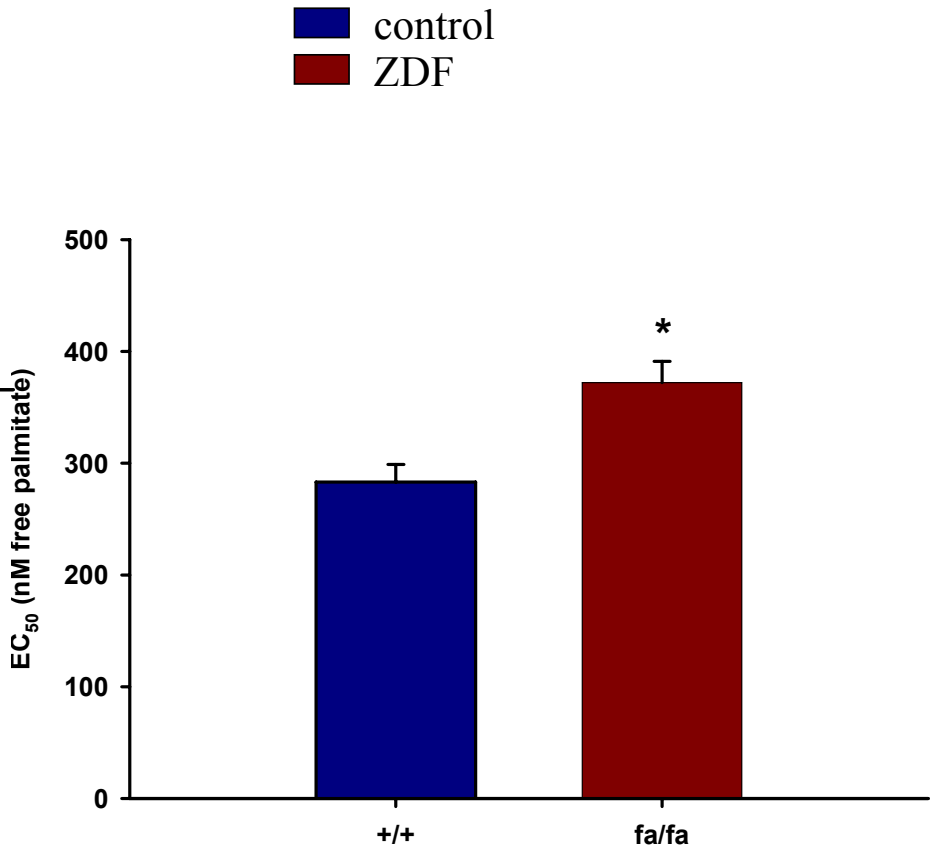
FA-induced uncoupling



Hoeks et al., in progress

Diabetic ZDF rats less sensitive to FA-induced uncoupling





Hoeks et al., in progress

n = 4

Q: What mechanisms could be responsible for mitochondrial (FFA) uncoupling?

Mitochondrial uncoupling

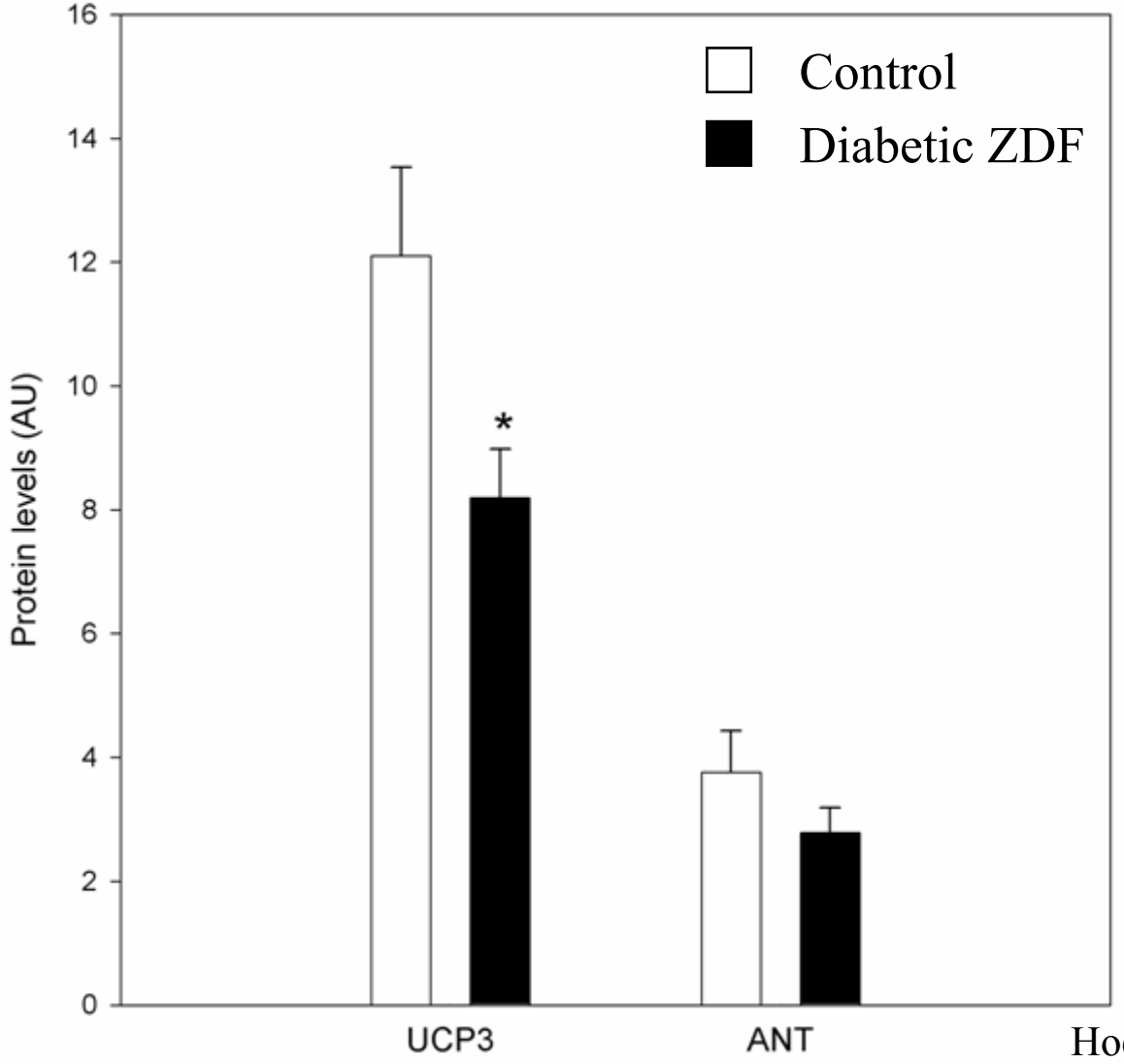
Uncoupling Protein 3 (UCP3)

- Exact physiological function not yet established
- Hypothesis: FA (peroxides) activate UCP3 protein → 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

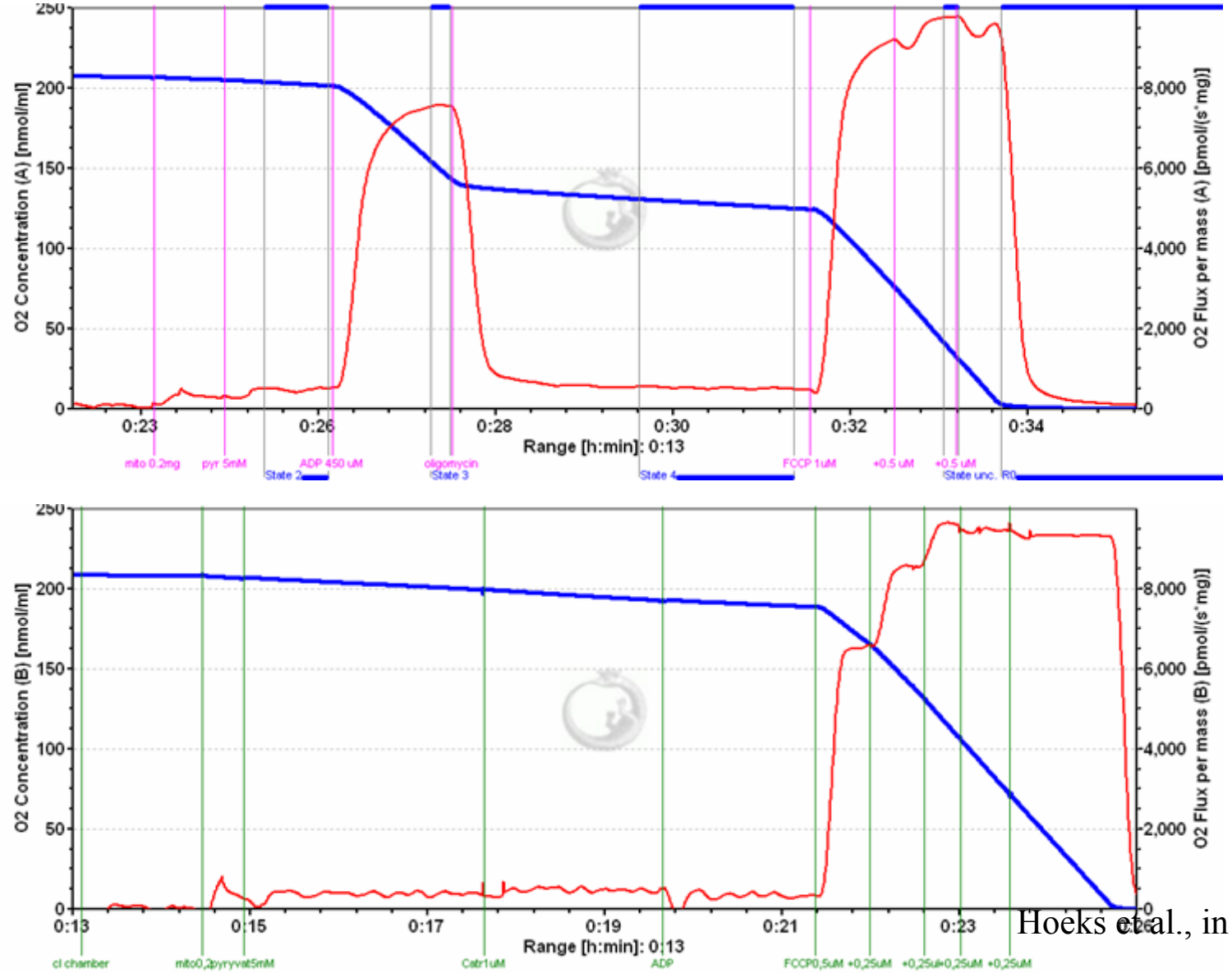
Lower UCP3 but comparable ANT levels in diabetic ZDF rat



Hoeks et al., in progress

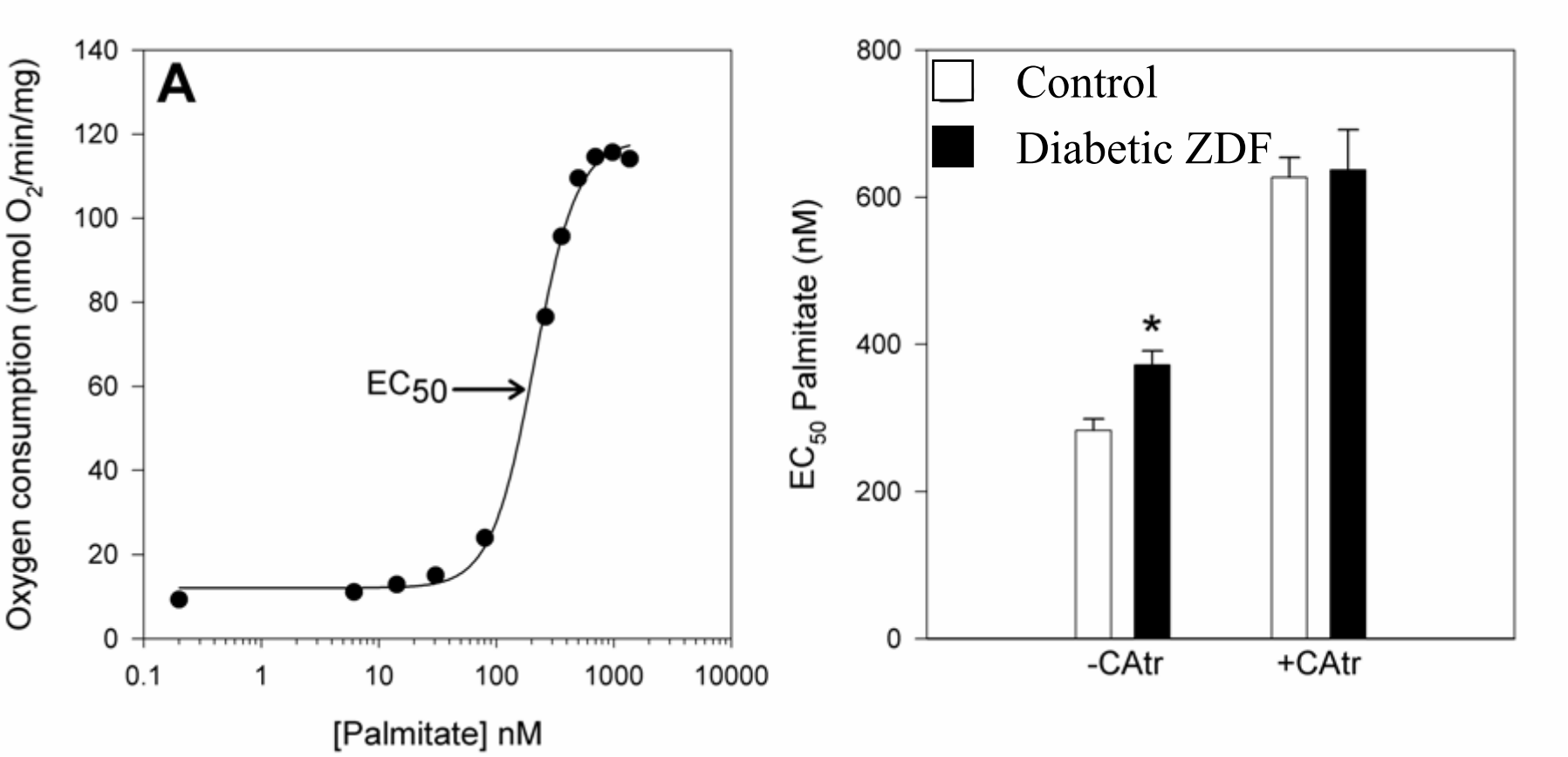
n = 6

ANT inhibition: CarboxyAtractyloside



Hoeks et al., in progress

After ANT inhibition similar sensitivity to FA-induced uncoupling; ANT defect?



Hoeks et al., in progress

Conclusions

- Intrinsic mitochondrial abberations 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

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