

Lipotoxicity and muscular mitochondrial function in insulin resistance

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Q: Why muscle?

Muscle & insulin sensitivity

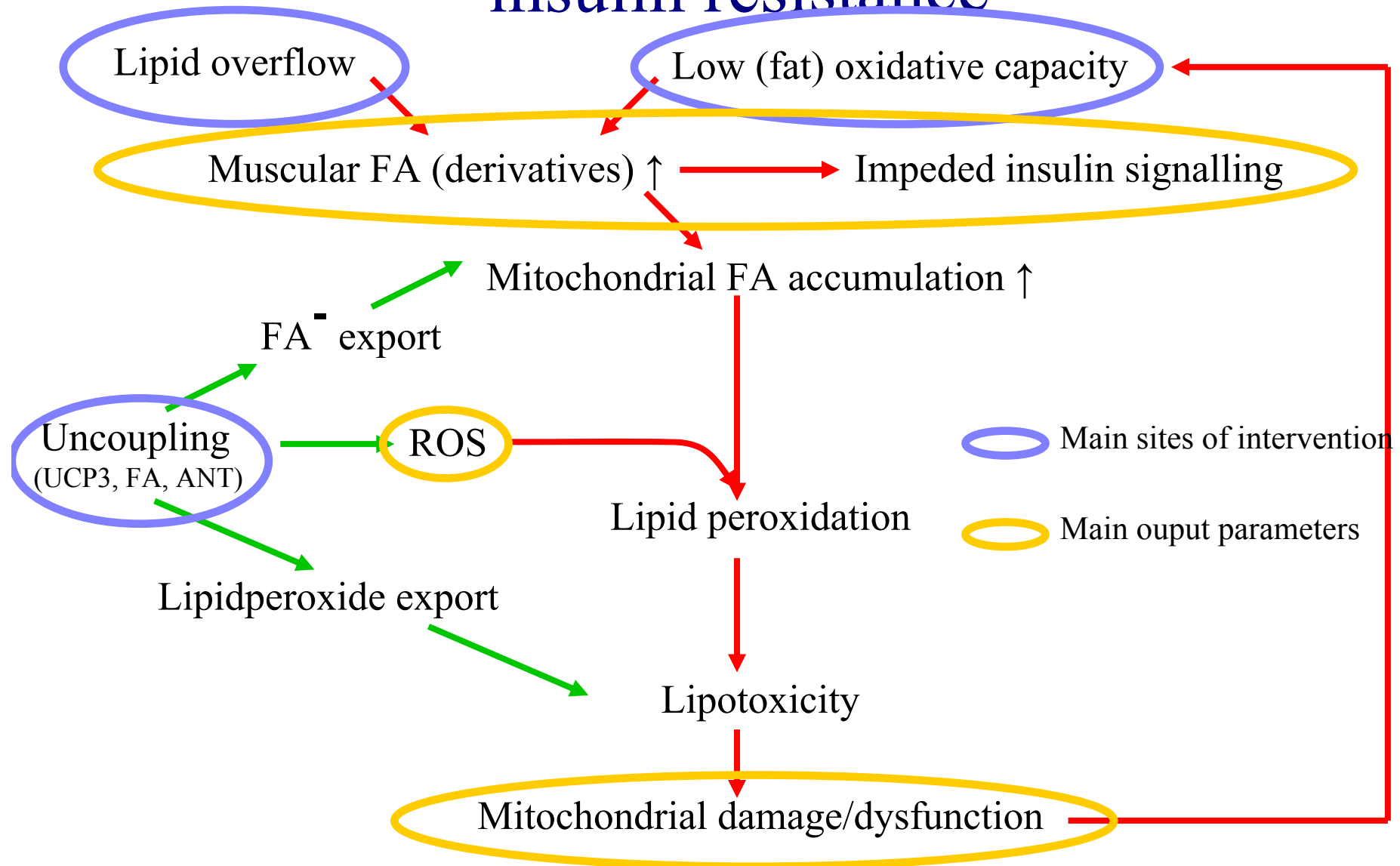
- ~ 80% of post-prandial glucose uptake occurs in muscle
- Lipid overflow to non-adipose tissue results in muscular fat storage, which associates with insulin resistance

Q: Why mitochondria?

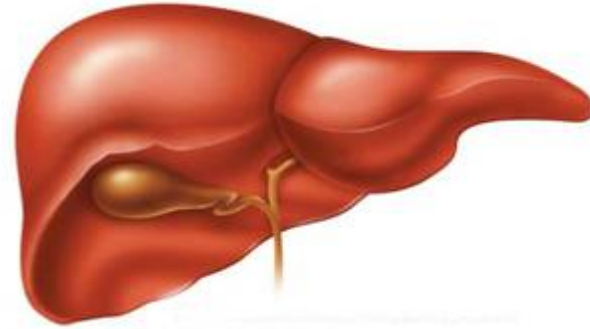
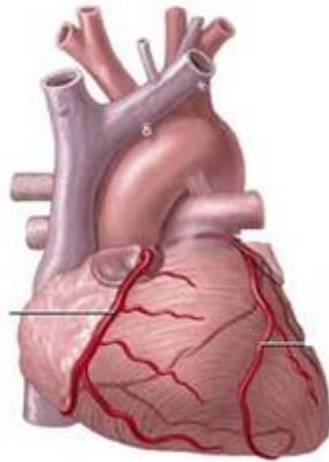
Mitochondria & insulin sensitivity

- 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

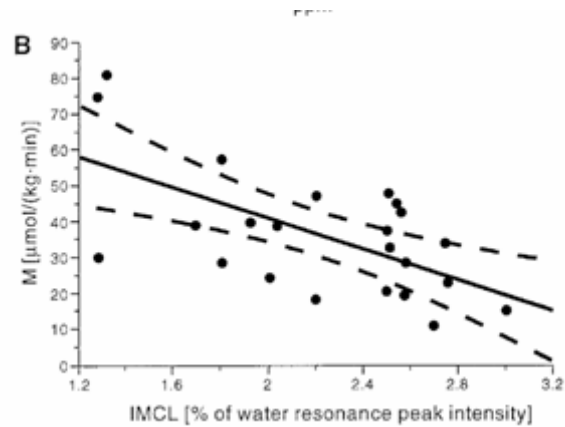
Lipotoxicity, mitochondrial function and insulin resistance



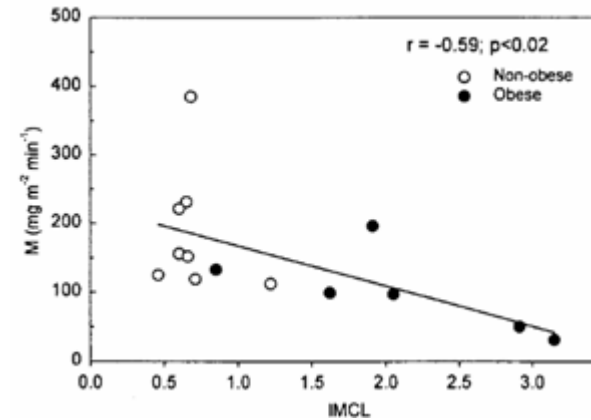
Tissues examined & Models used



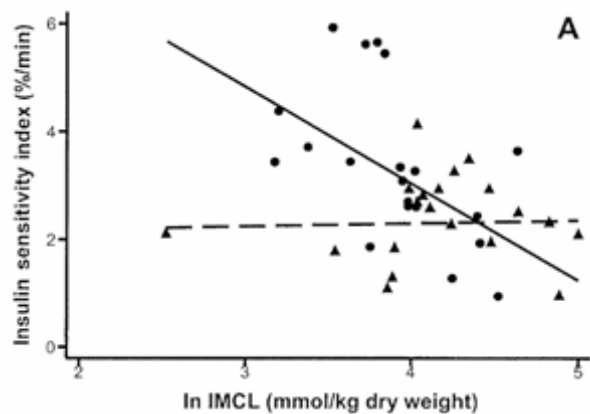
IMCL is negatively associated with insulin sensitivity



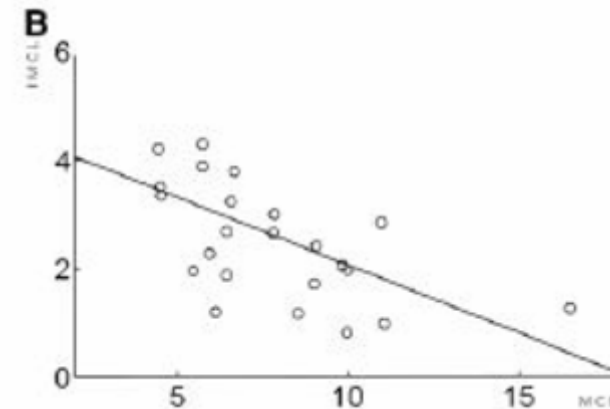
Krssak et al., Diabetologia 2002



Sinha et al., Diabetes 2002

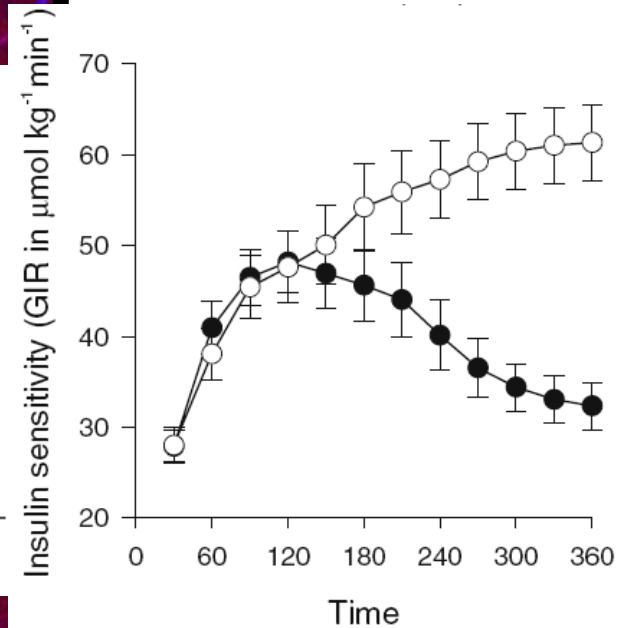
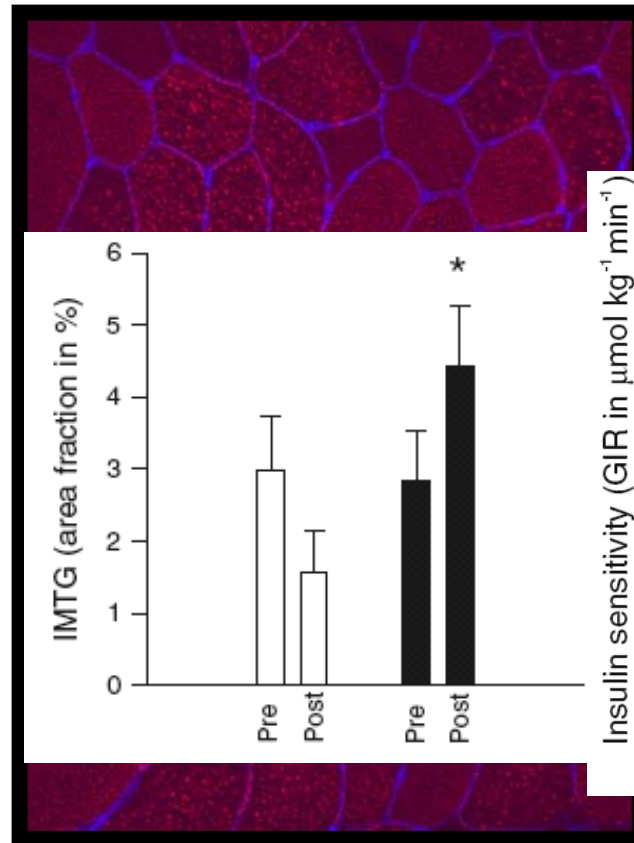
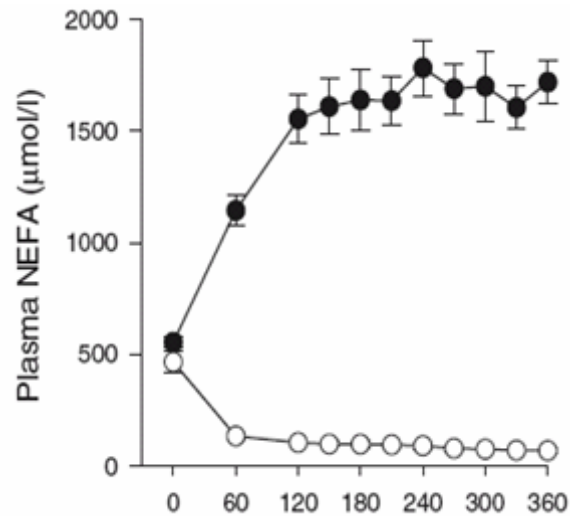


Fourouhi et al., Diabetologia 1999



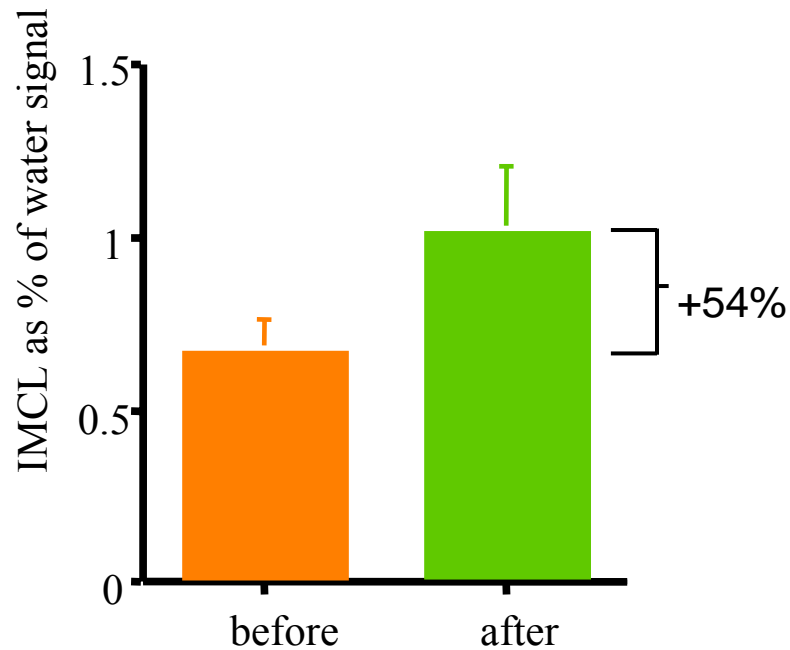
Jacob et al., Diabetes 1999

High FFA levels acutely induce muscle TG accumulation and insulin resistance

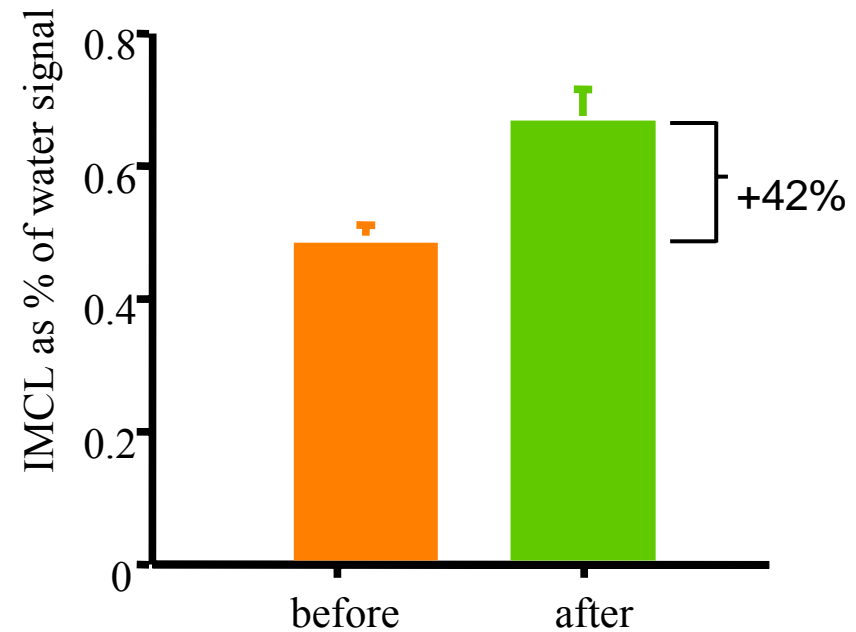


Paradox: endurance training also increases IMCL content

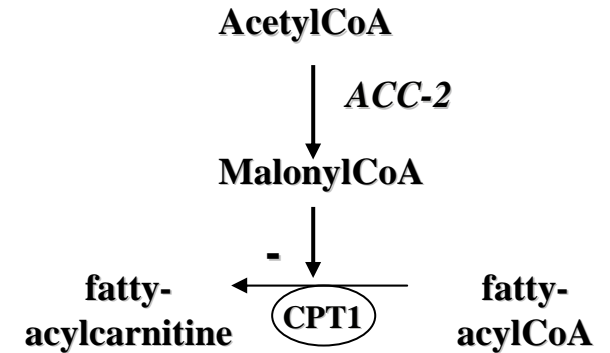
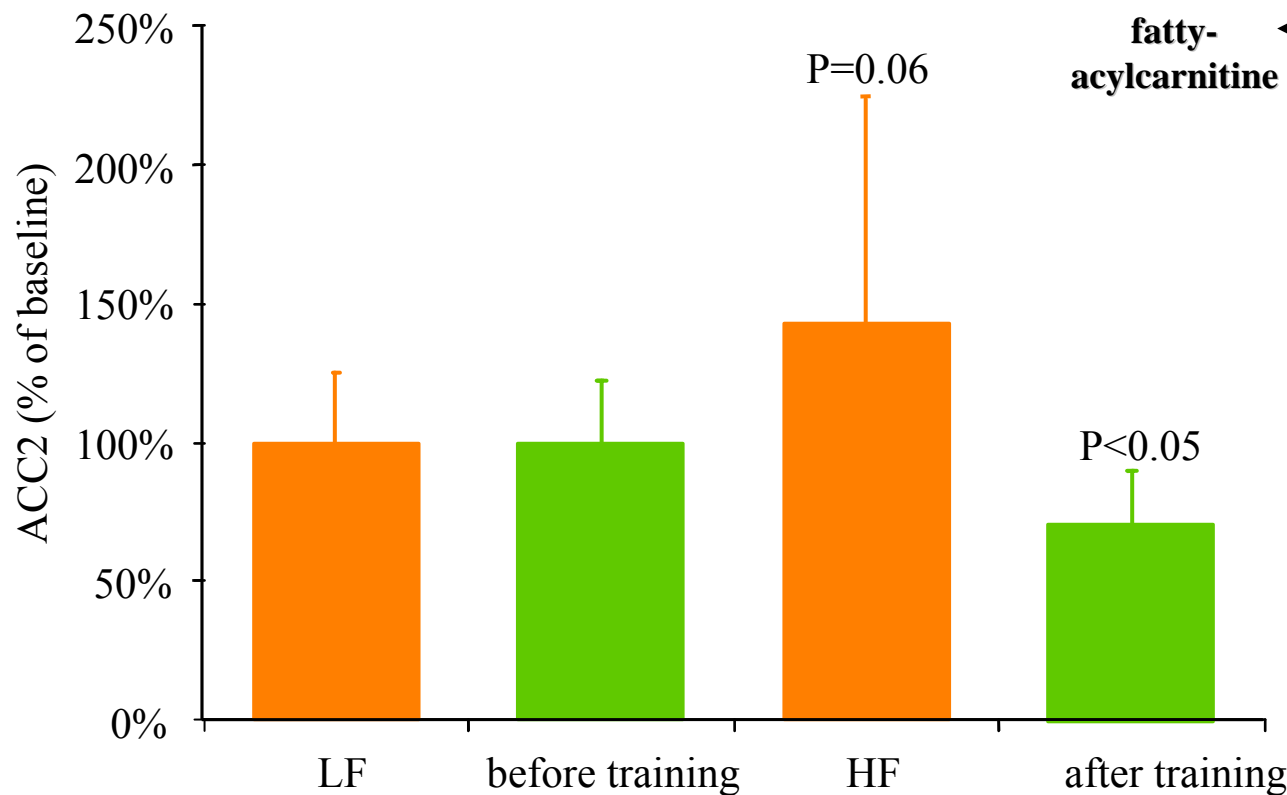
7 days high-fat diet



14 days endurance training



Training, not high-fat diet downregulates ACC2



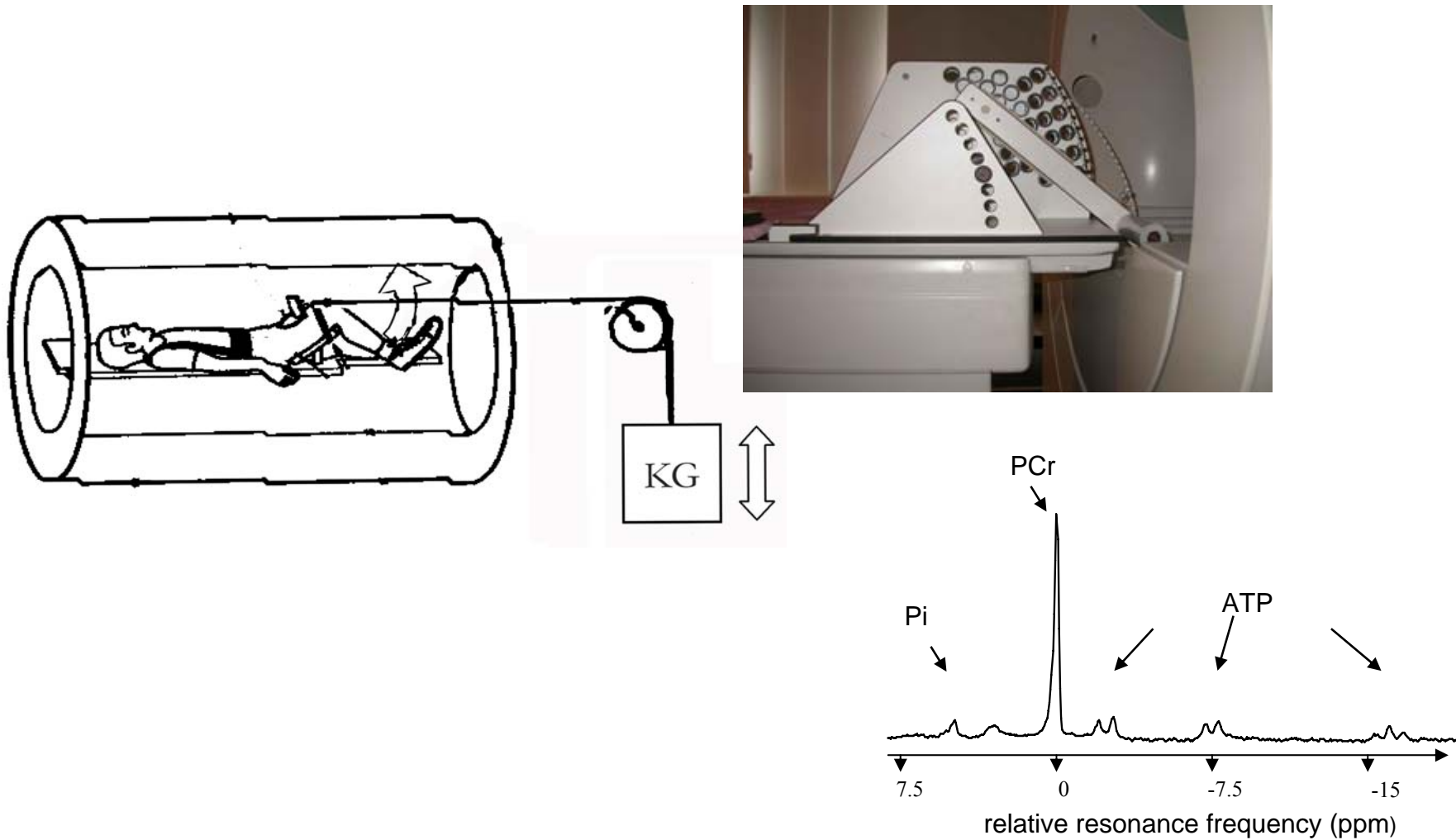
Q: is IMCL only 'harmful' in combination with a low oxidative capacity?

Muscular mitochondrial function in T2DM and controls, matched for BMI

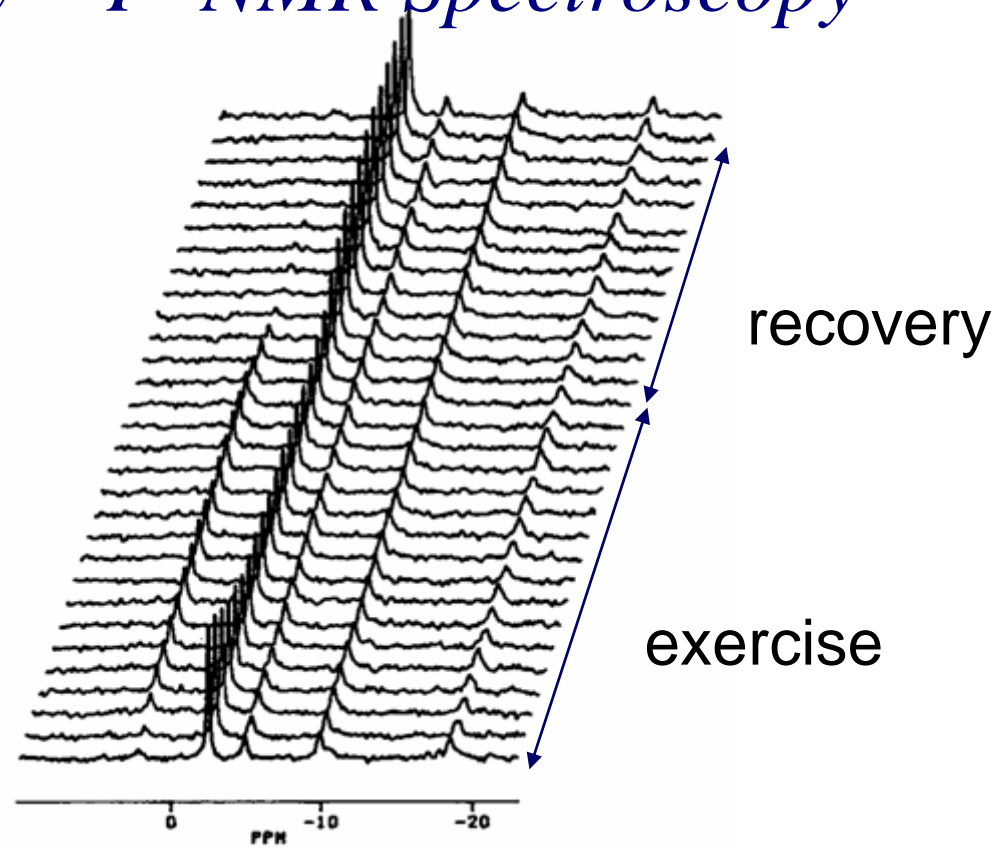
	Diabetic patients (n=12, all males)	Controls (n=9, all males)	p-value
BMI (kg/m ²)	29.4 ± 3.3	29.3 ± 2.7	n.s.
Age (years)	61.8 ± 4.4	56.1 ± 6.8	P < 0.05
VO _{2max} (ml*min ⁻¹ *kg ⁻¹)	30.6 ± 5.8	34.6 ± 4.8	n.s.
plasma glucose (mM)	9.56 ± 2.12	5.73 ± 0.37	P < 0.01
insulin sensitivity (GIR, μmol*min ⁻¹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

(mean ± stdev)

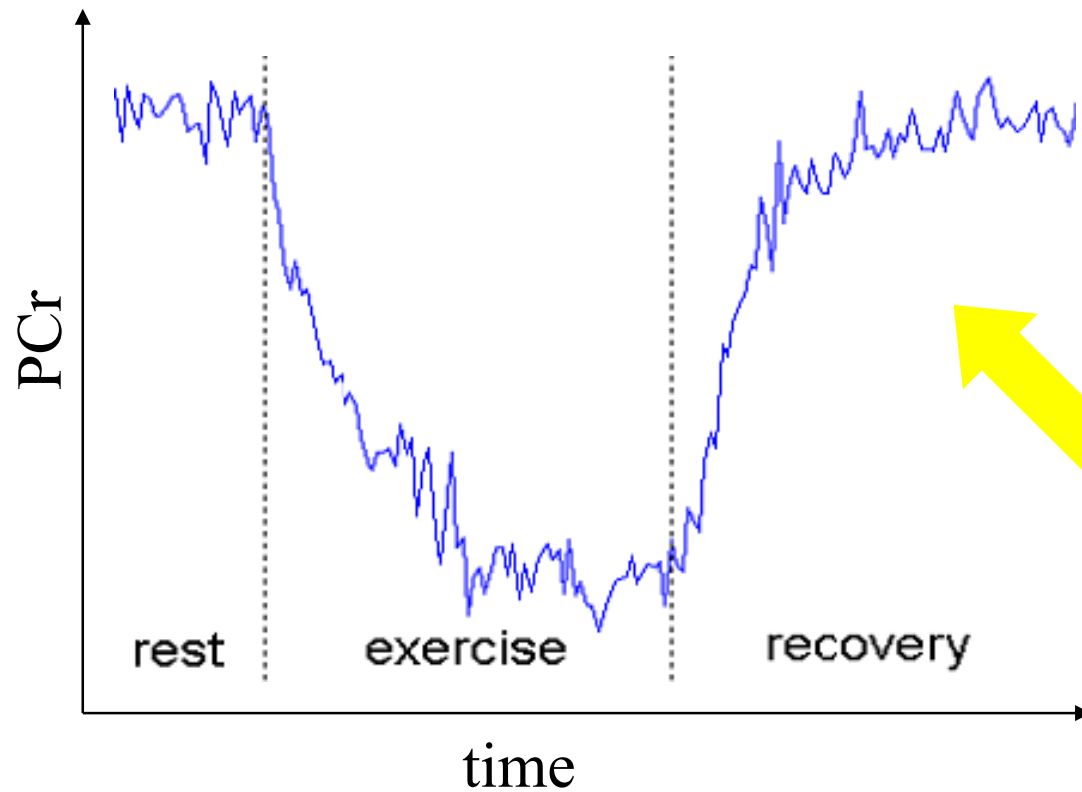
Muscle mitochondrial function *in vivo* by ^{31}P NMR Spectroscopy



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



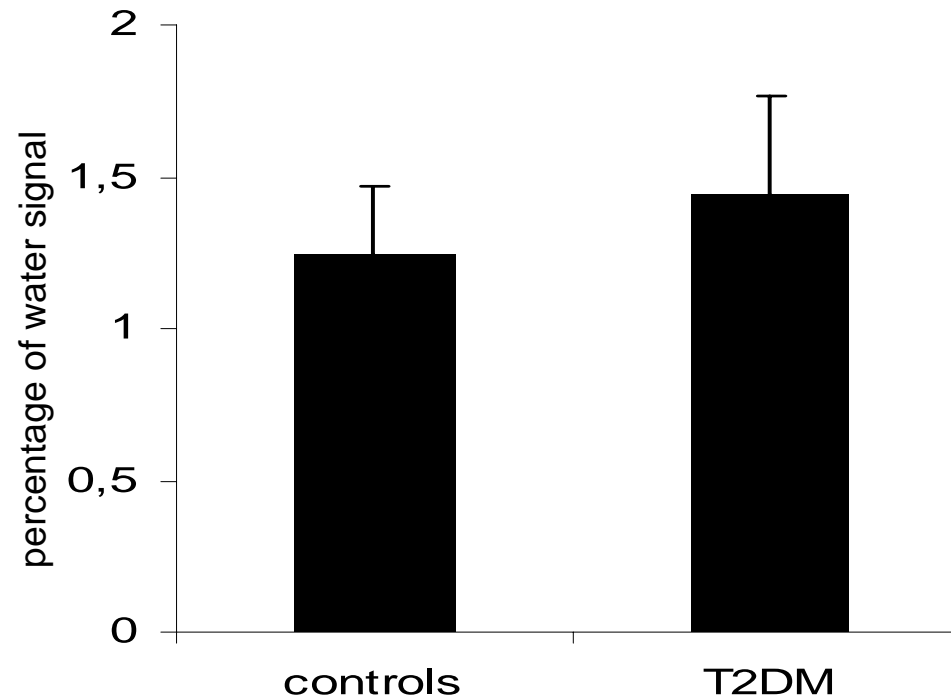
Muscle mitochondrial function *in vivo* by ^{31}P NMR Spectroscopy



PCr resynthesis is
almost purely aerobic

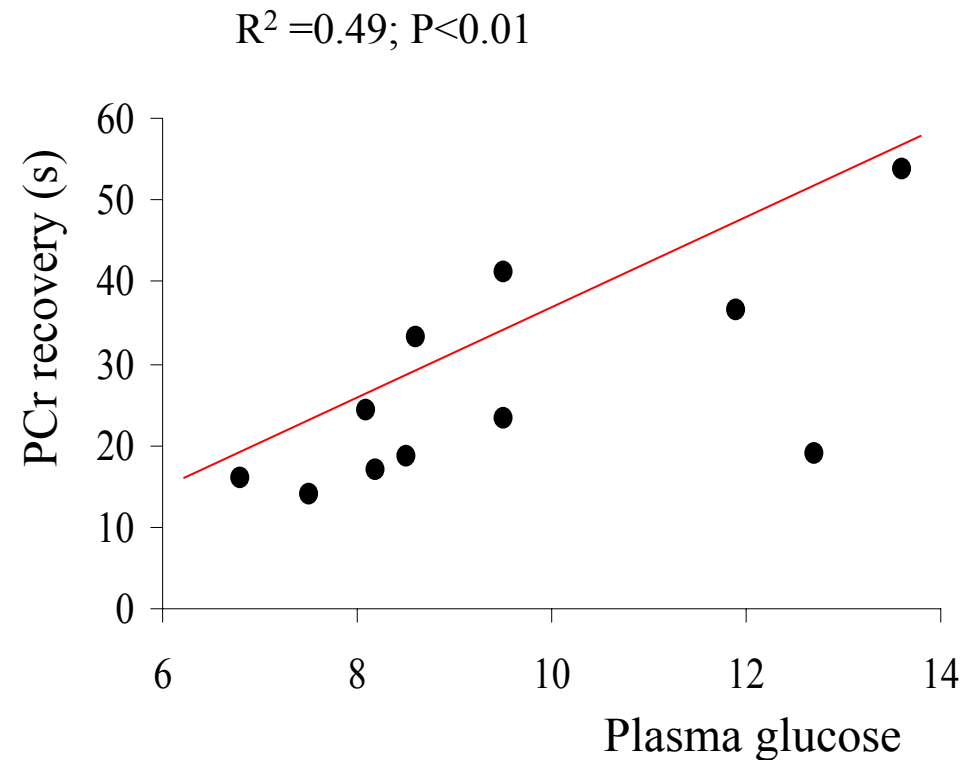
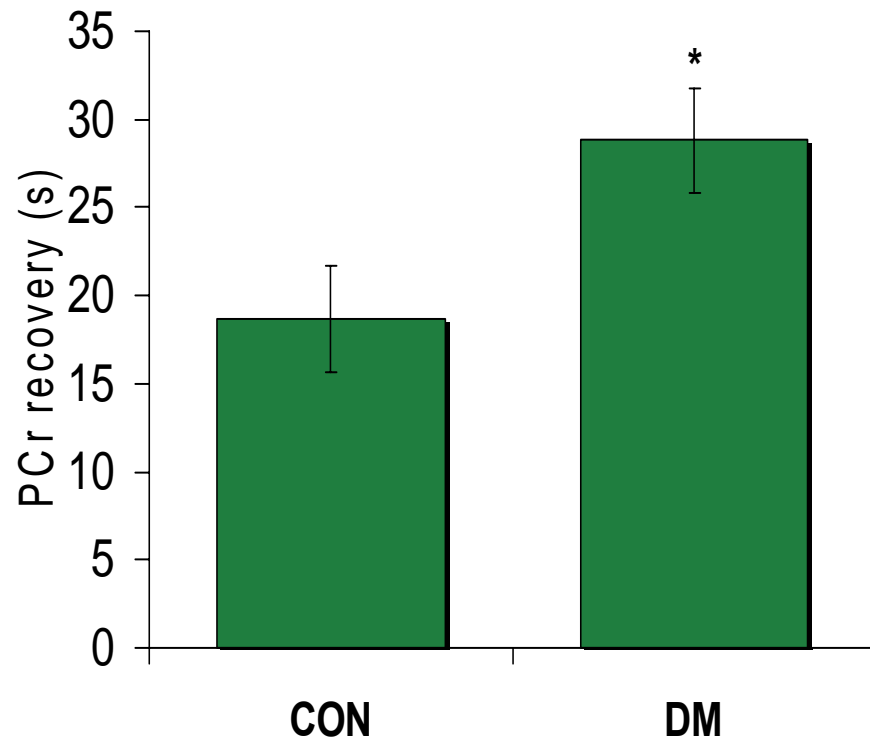
PCr recovery half-time
reflects oxidative capacity

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



Q: How about mitochondrial function?

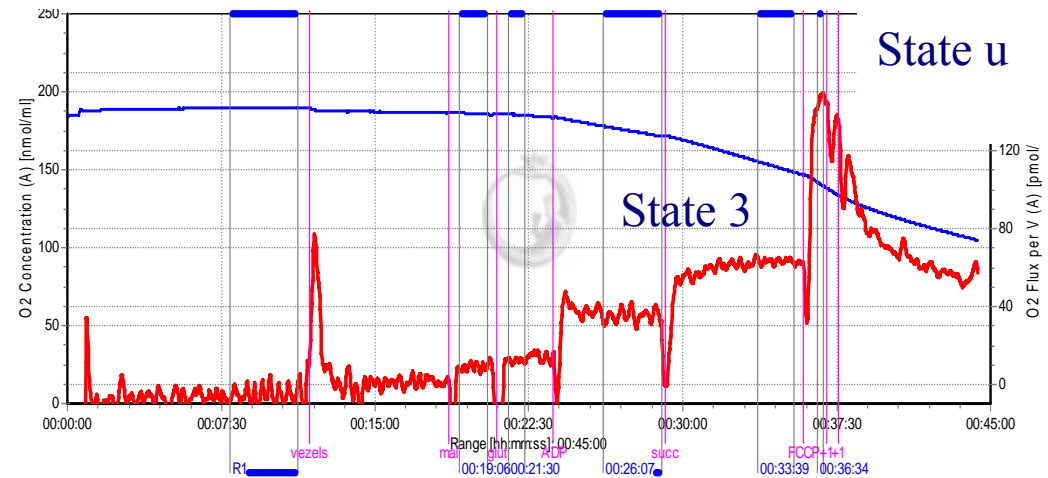
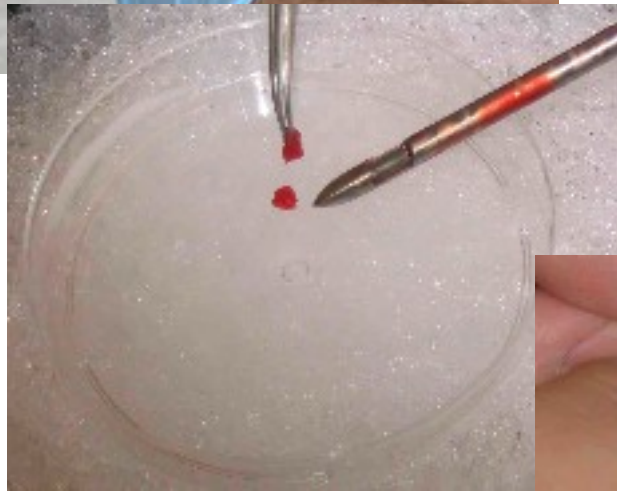
In vivo mitochondrial function reduced in T2DM, correlates with glucose and HbA1C



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

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

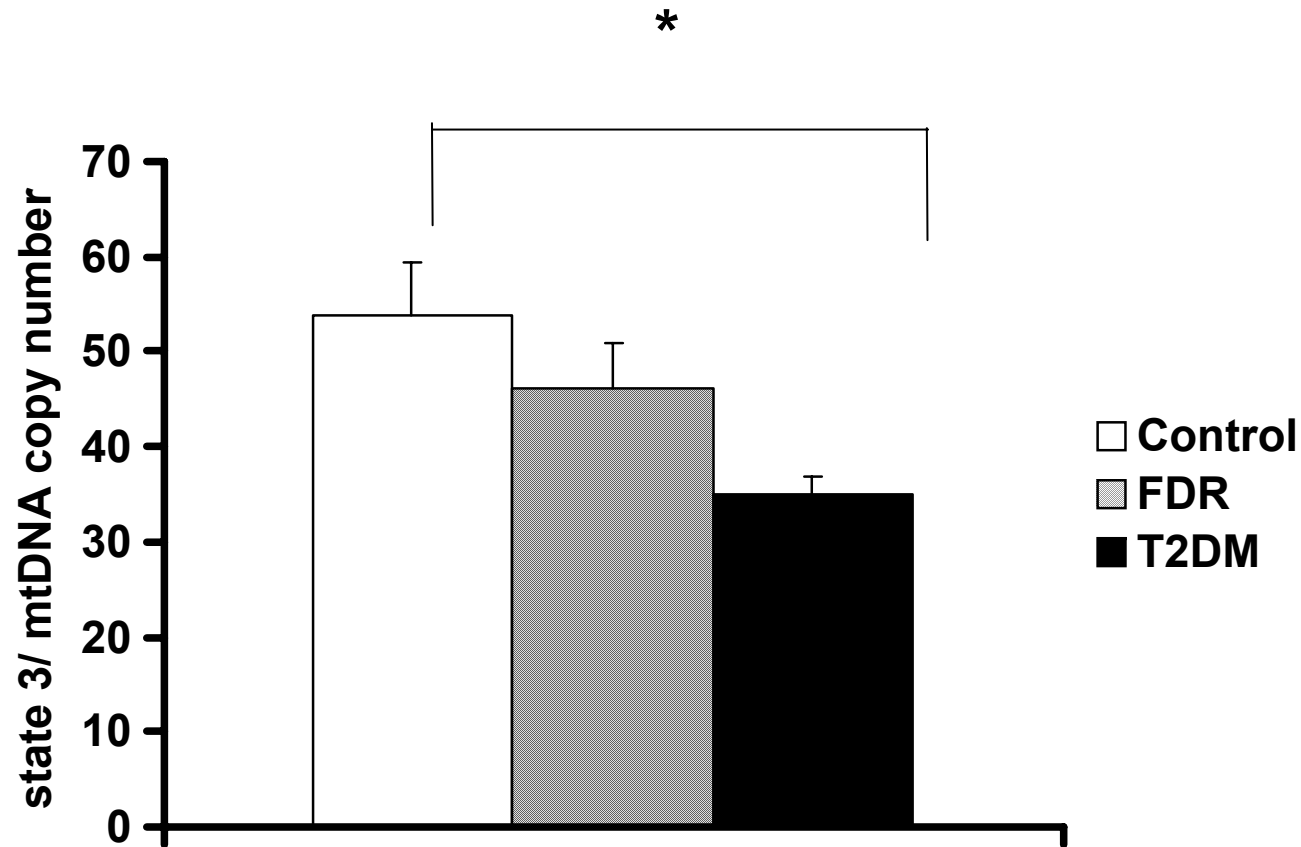
Intrinsic mitochondrial function *ex vivo* using high-resolution respirometry



Pre-diabetic subject Characteristics

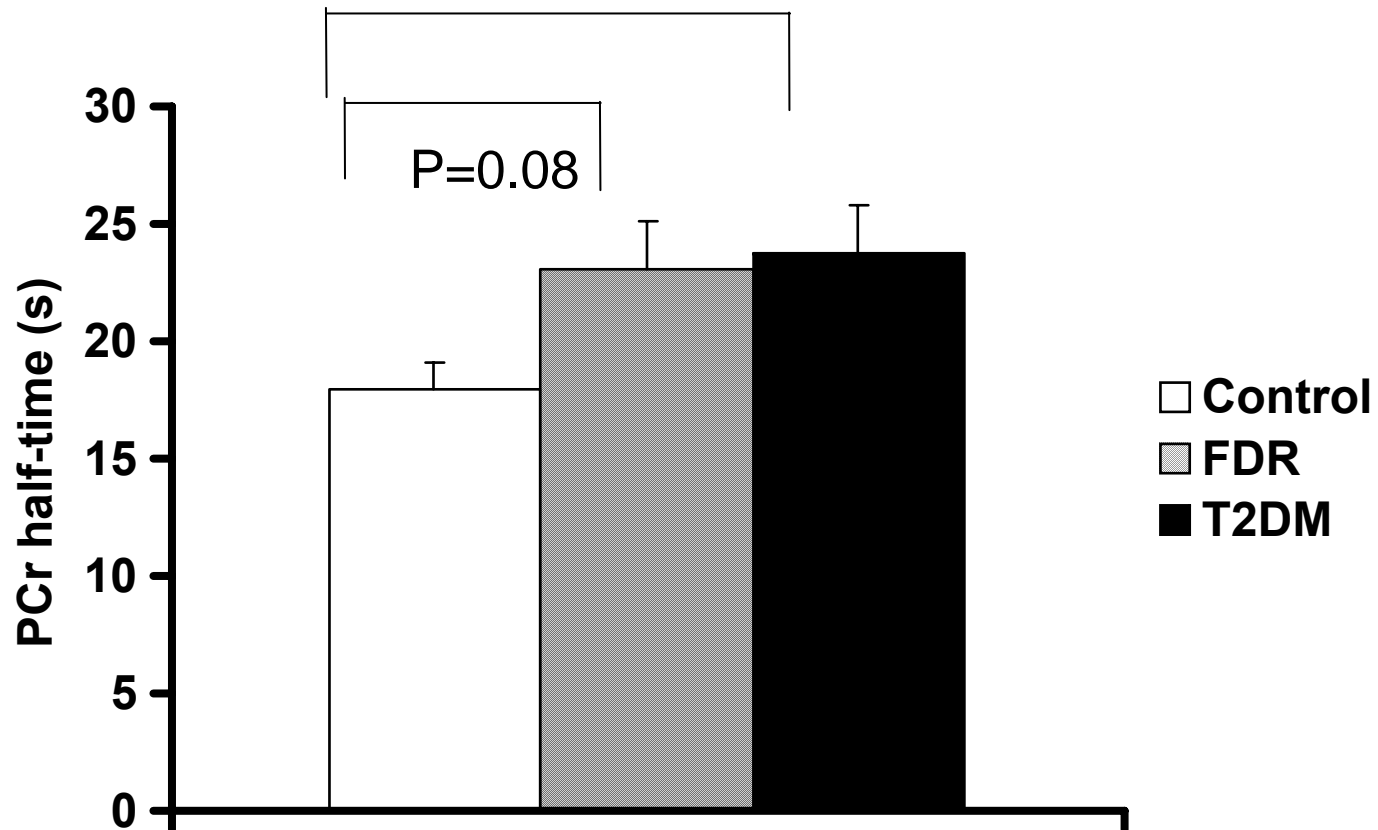
	Controls N=16	FDR N=12	T2DM N=10	
Age (y)	59.2 ± 0.7	60.1 ± 0.9	61.4 ± 1.6	n.s.
BMI (kg/m²)	29.1 ± 0.7	30.1 ± 1.2	28.9 ± 0.7	n.s.
VO₂max (ml/kg/min)	31.2. ± 1.6	32.6 ± 9.6	28.2 ± 2.3	n.s.
Ins-stim R_d (umol/kg/min)	28.9 ± 3.7	22.1 ± 3.4	11.2 ± 2.8	* p<0.05

Lower *ex vivo* mitochondrial function in T2DM irrespective of mitochondrial density (mtDNA)



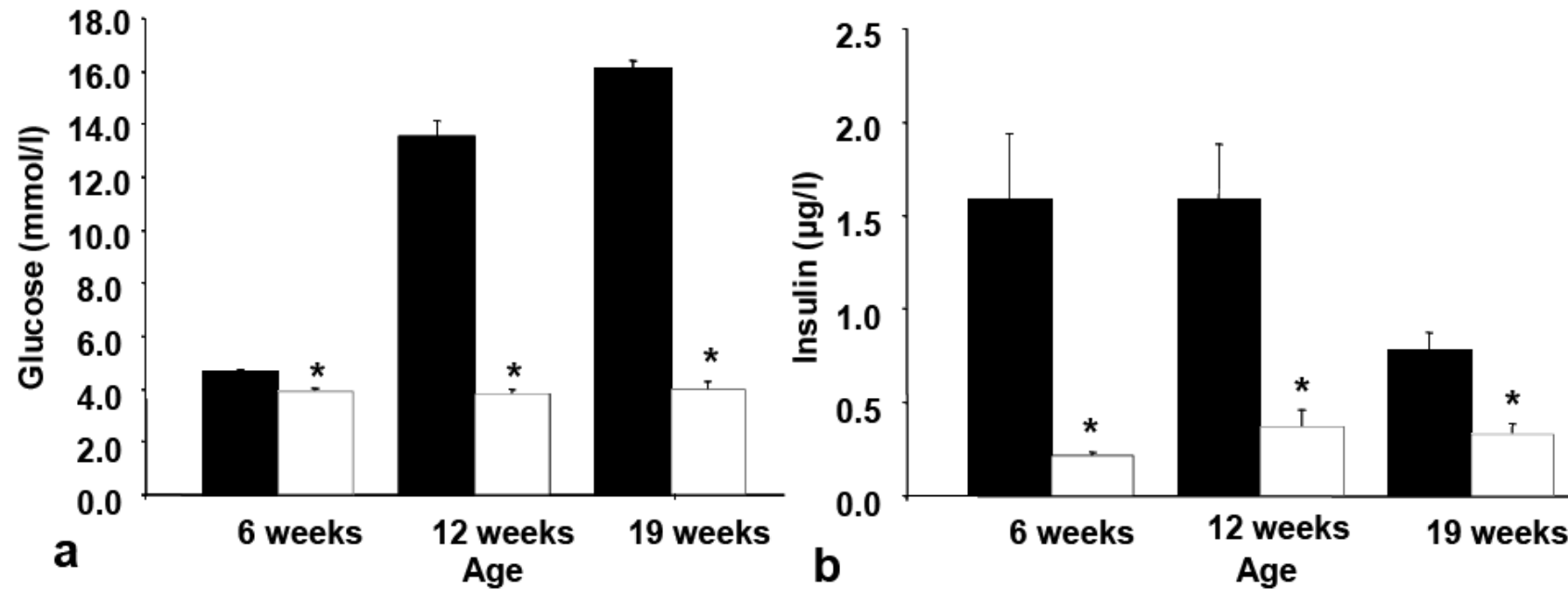
Lower *in vivo* mitochondrial function in T2DM and first-degree relatives

*

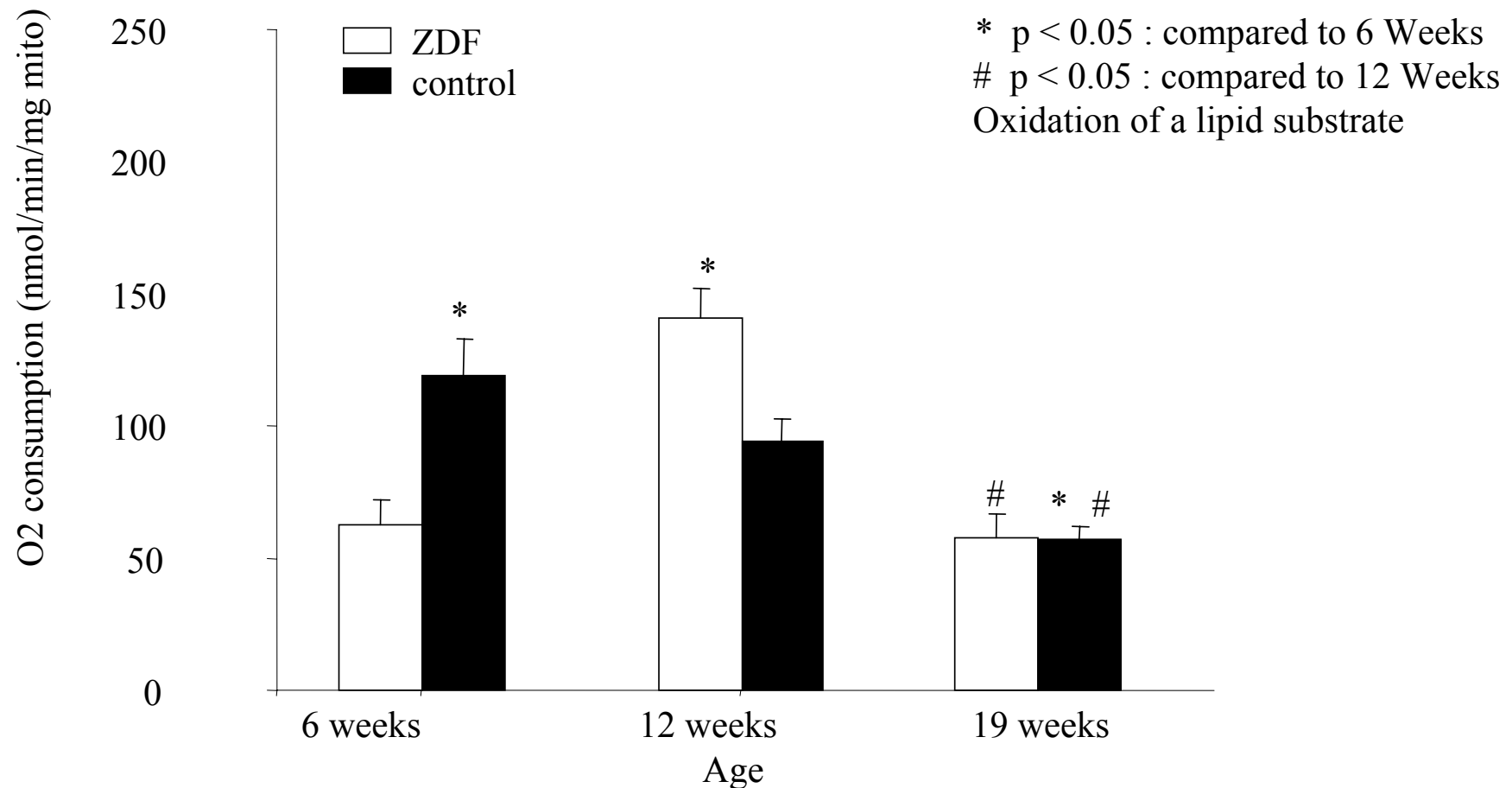


Q: Is compromised mitochondrial function in the pre-diabetic state required to develop insulin resistance?

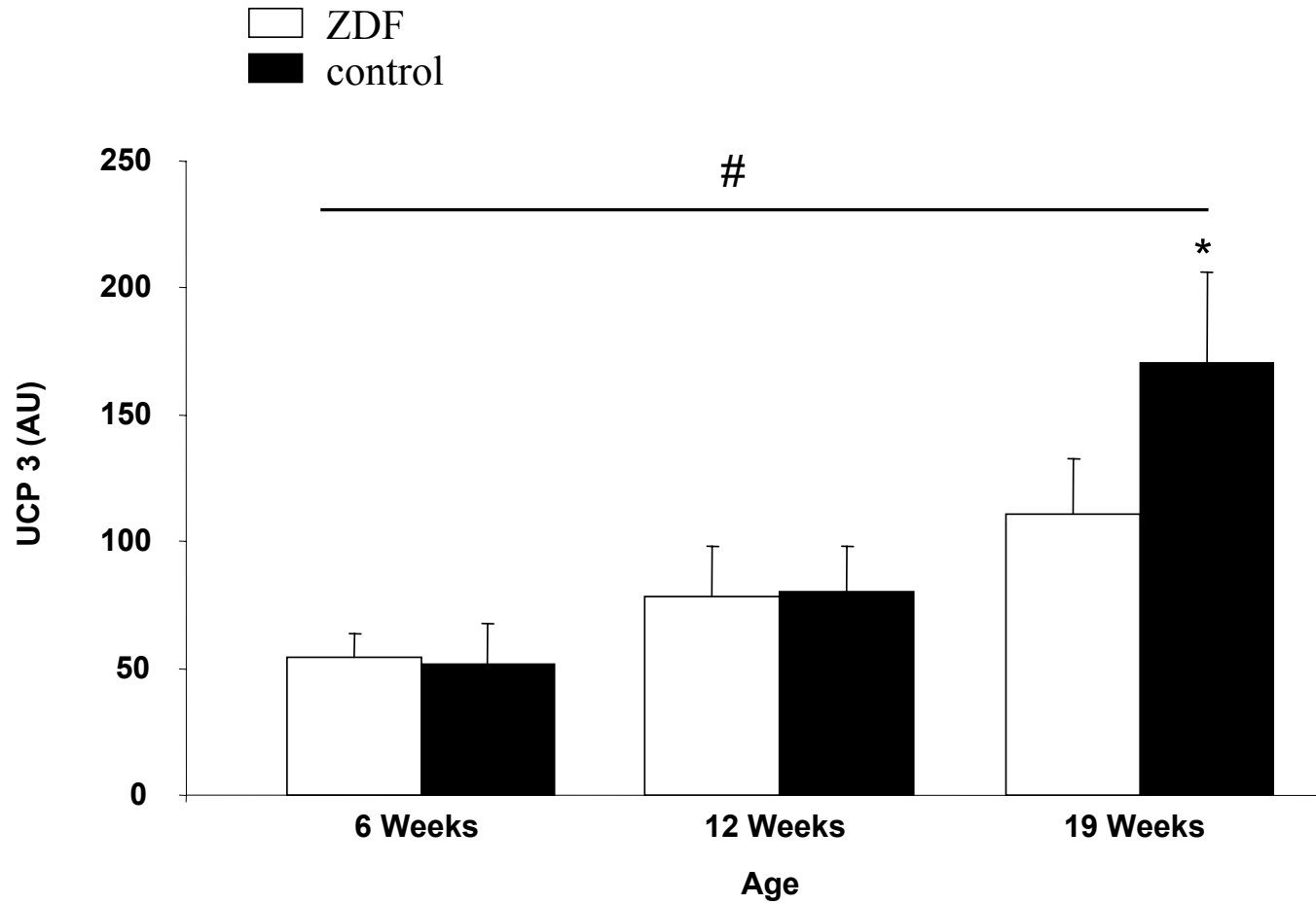
Transition of pre-diabetes to T2D during maturation of ZDF rats



No mitochondrial dysfunction during the development of T2D in ZDF rats



Increased UCP3 protein blunted in ZDF rats



Uncoupling protein 3 (UCP3)

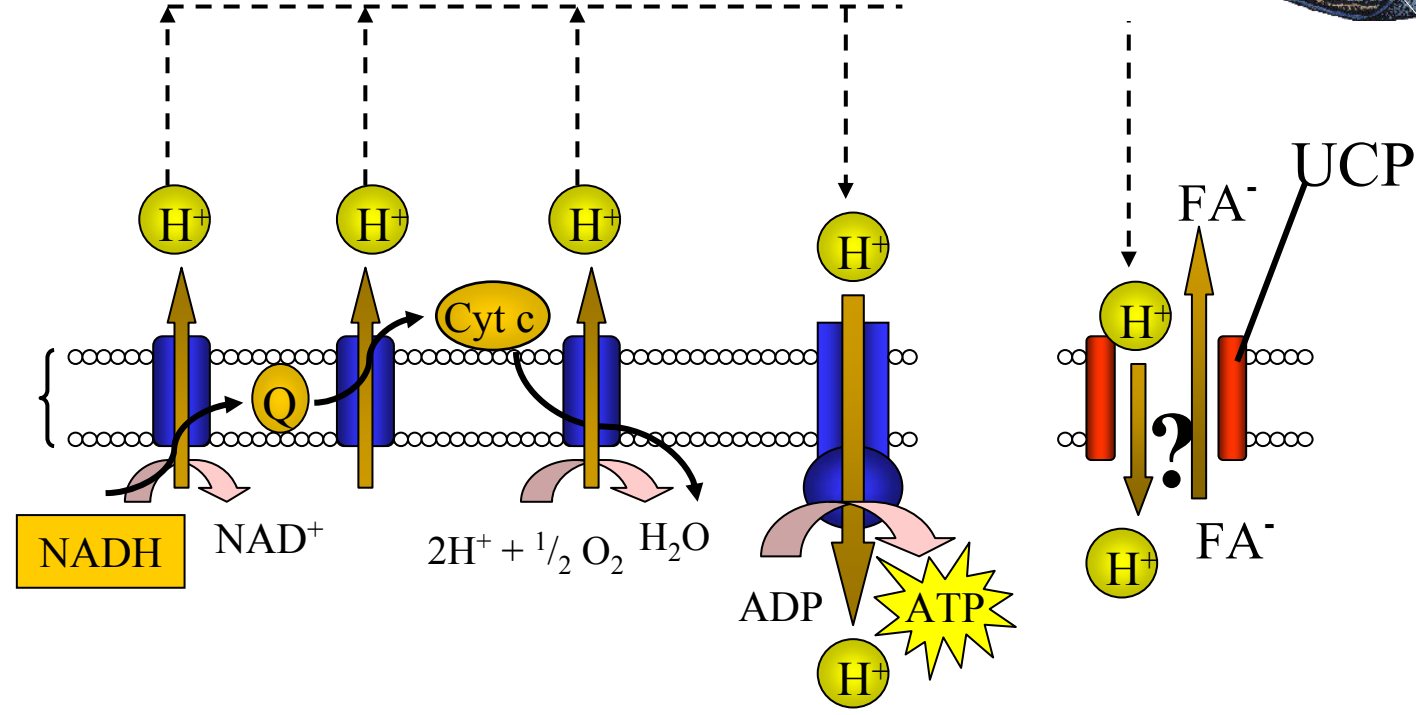
- ~55% homology to UCP1 which is responsible for the thermogenesis in of brown adipose tissue
- Primarily expressed in skeletal muscle and heart
- UCP3 is able to lower the mitochondrial proton gradient - upon activation by fatty acids - by transporting H^+ or FA^-



Intermembrane space

Inner mitochondrial membrane

Mitochondrial matrix

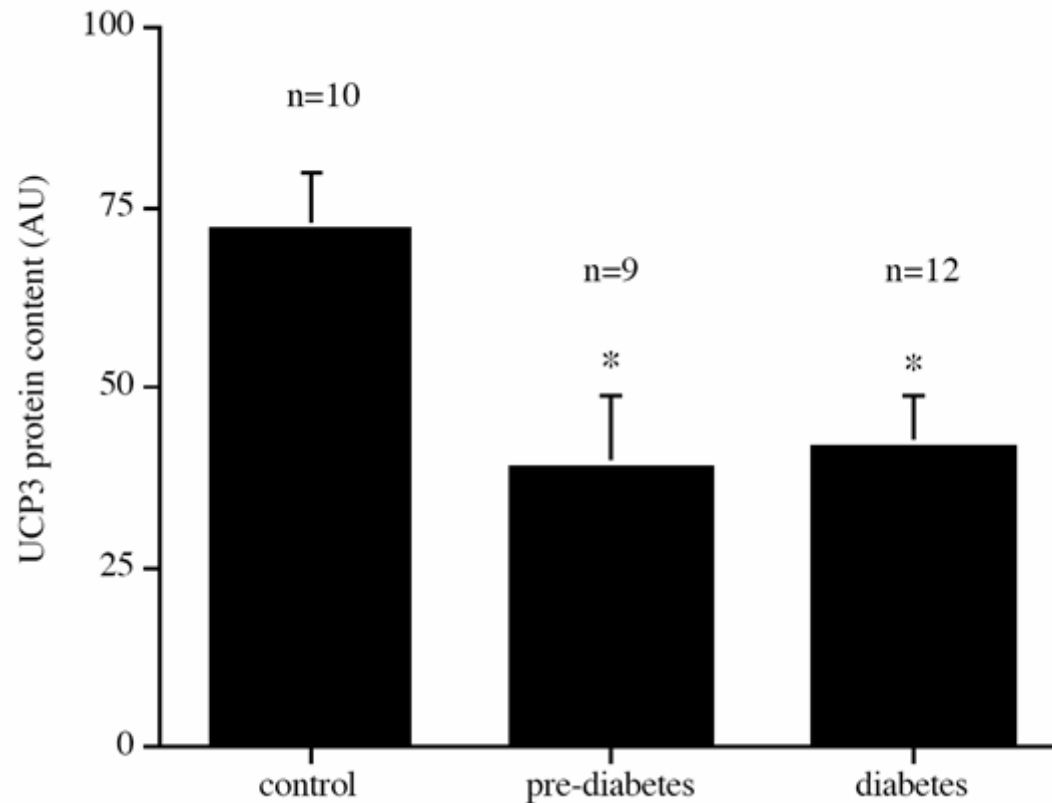


Electron transport chain

ATP synthase

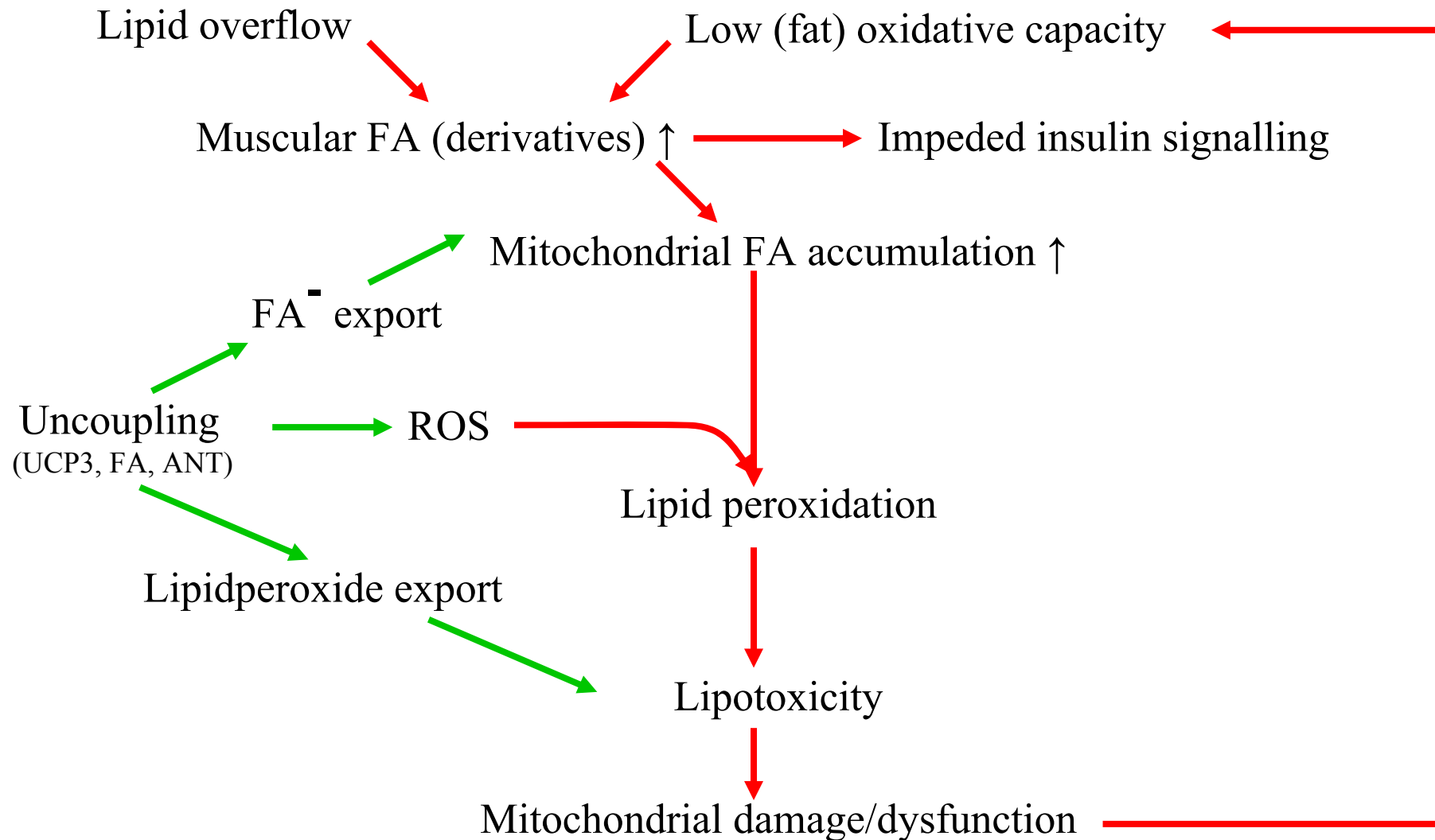
UCP leak

UCP3 is reduced in (pre)diabetic patients



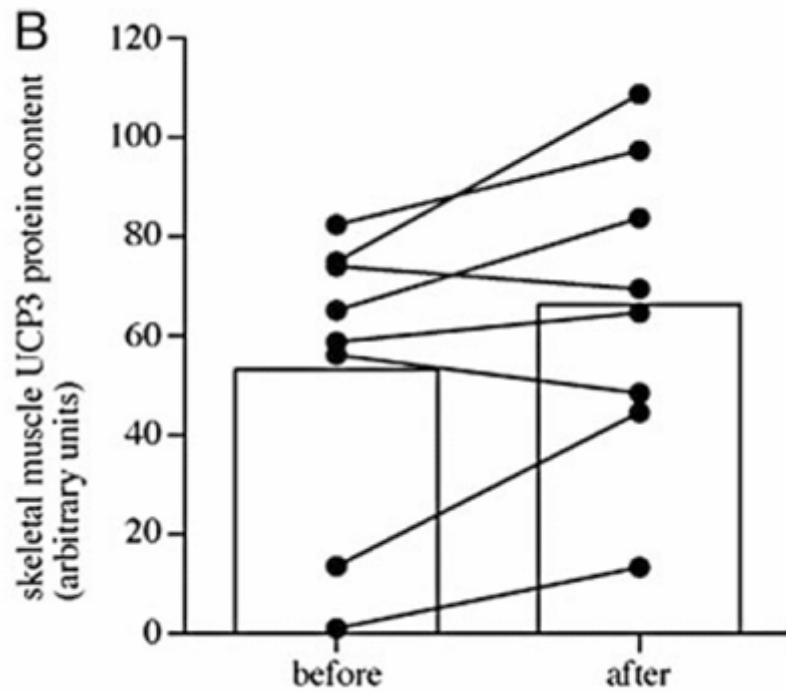
Q: Is UCP3 involved in the development of mitochondrial dysfunction in T2D?

Lipotoxicity, mitochondrial function and insulin resistance

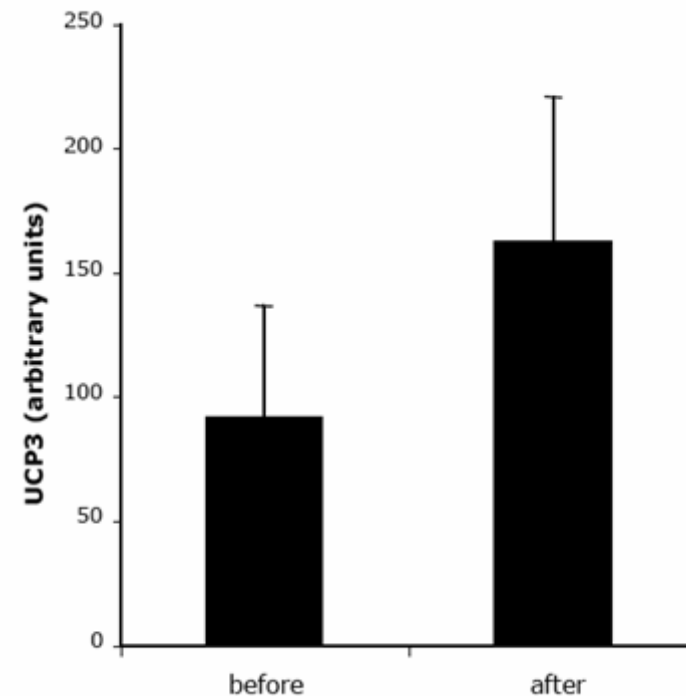


Q: Can insulin sensitizing by exercise training restore mitochondrial aberrations observed in T2D?

Insulin sensitizing by Rosiglitazone restores UCP3 protein content in T2D...



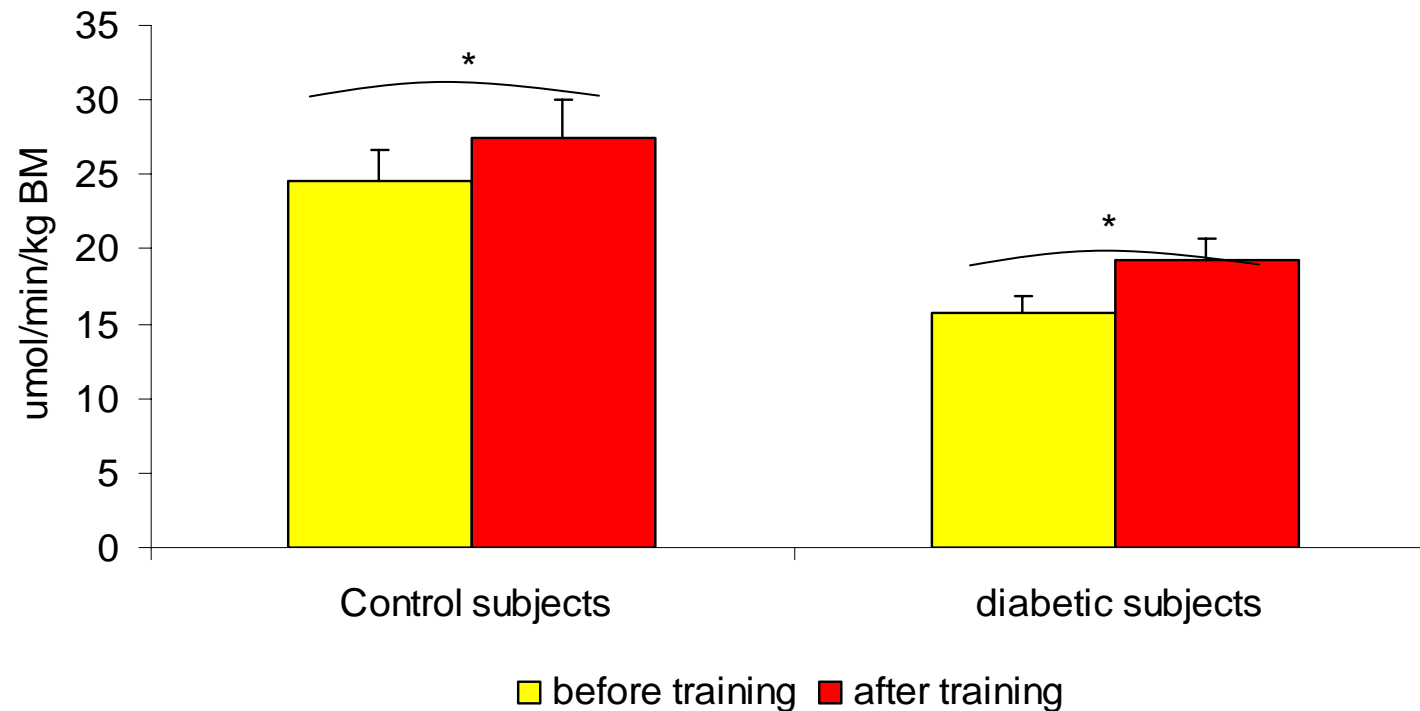
Schrauwen et al, JCEM 2006



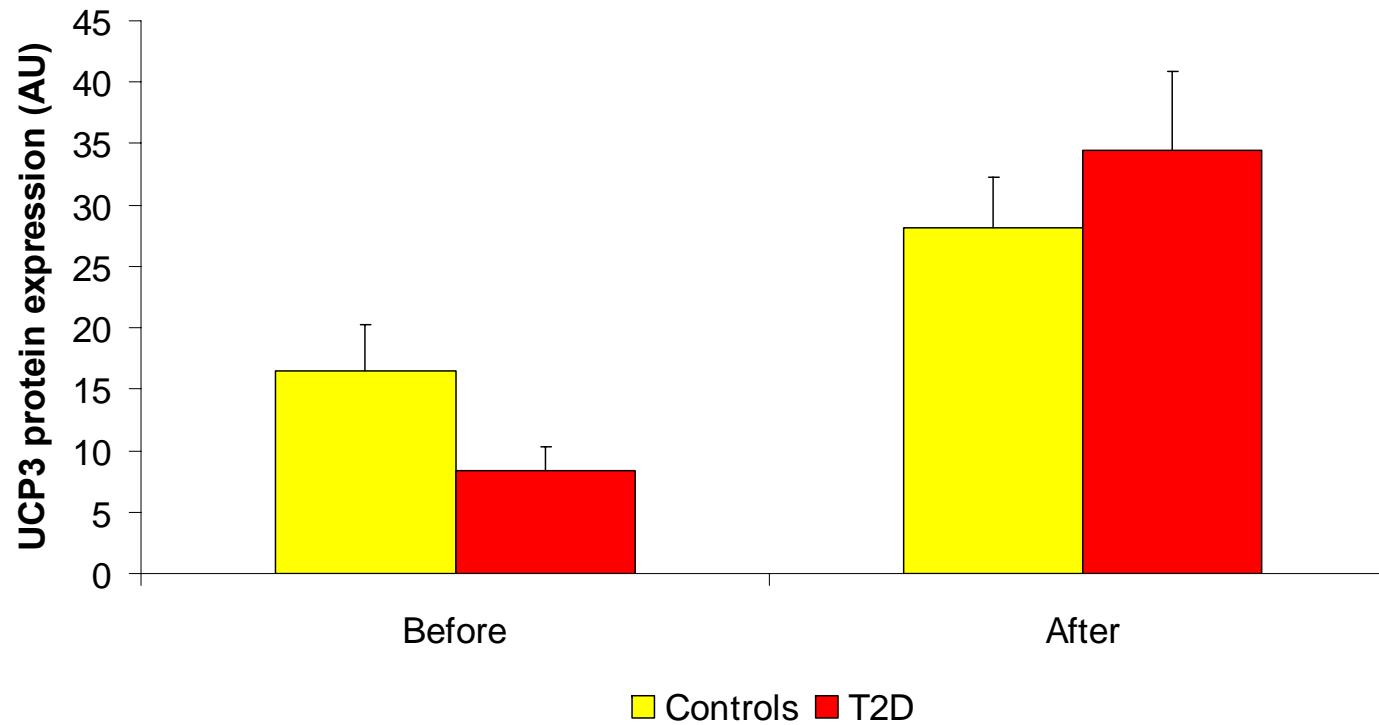
Mensink et al, Diab Obes Met 2006

Exercise training in T2D and controls improves insulin sensitivity

Glucose infusion rate

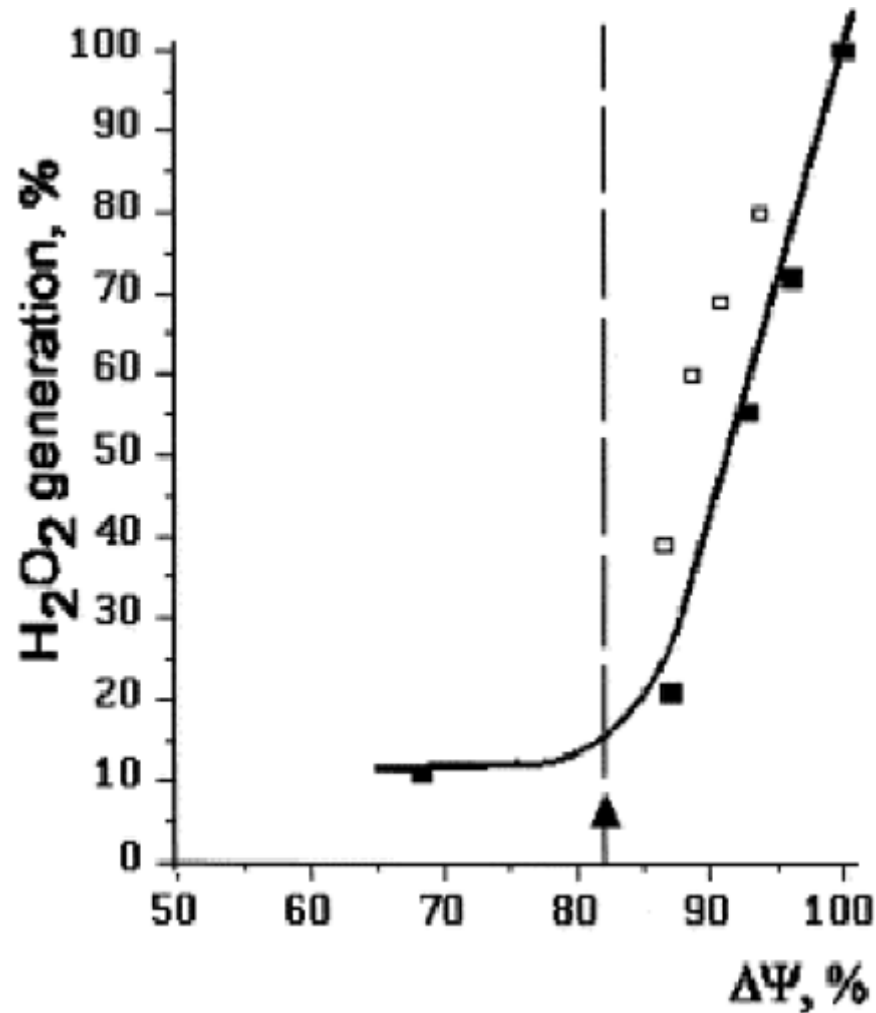


Exercise training in T2D restores UCP3 content

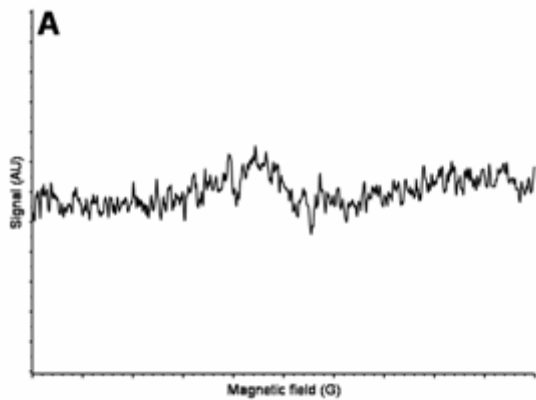


Q: Can mitochondrial uncoupling reduce superoxide production in diabetogenic conditions?

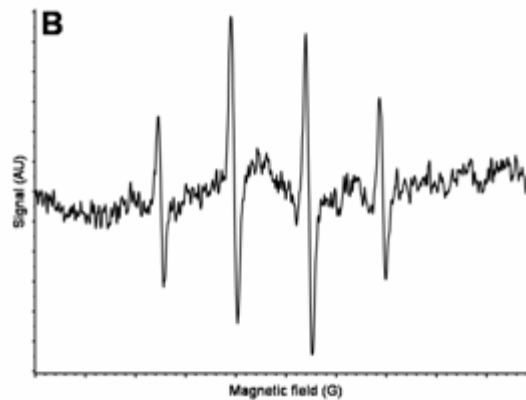
Mitochondrial proton gradient and ROS production



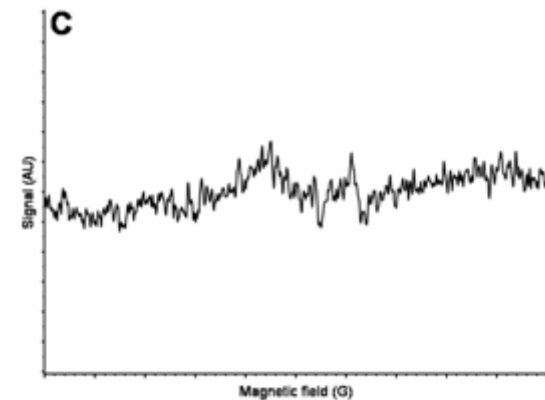
Measuring superoxide production by electron spin resonance in isolated mitos



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

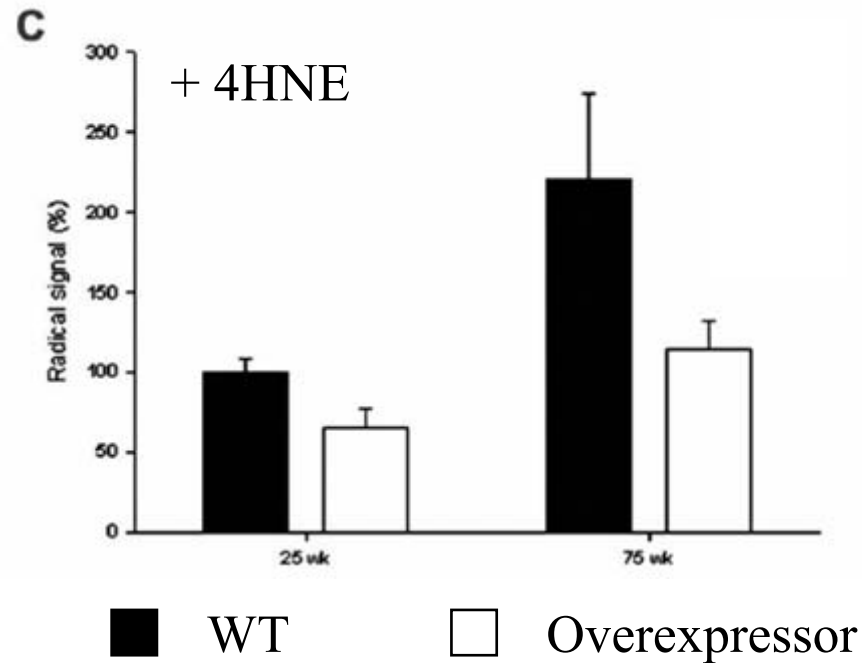
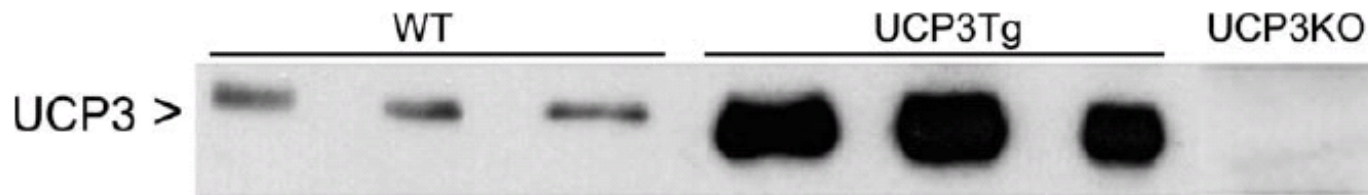


muscle mito 0.2 mg/ml
5 min 37° C
100 mM DMPO
10 mM glutamate
10 mM succinaat
3 mM malate



idem 2
500 U/ml SOD

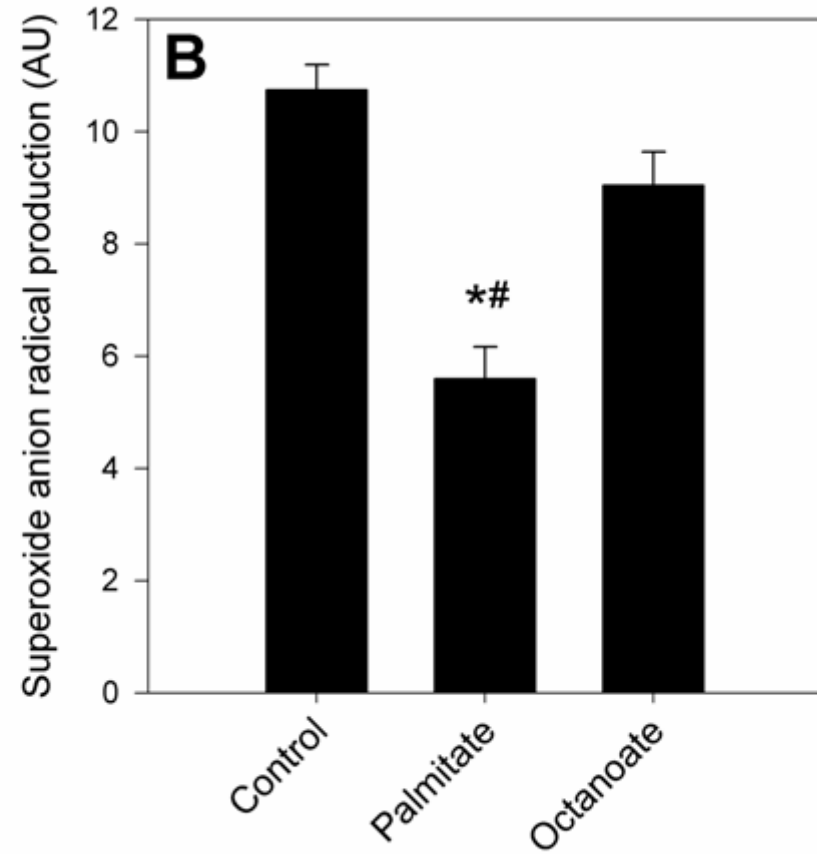
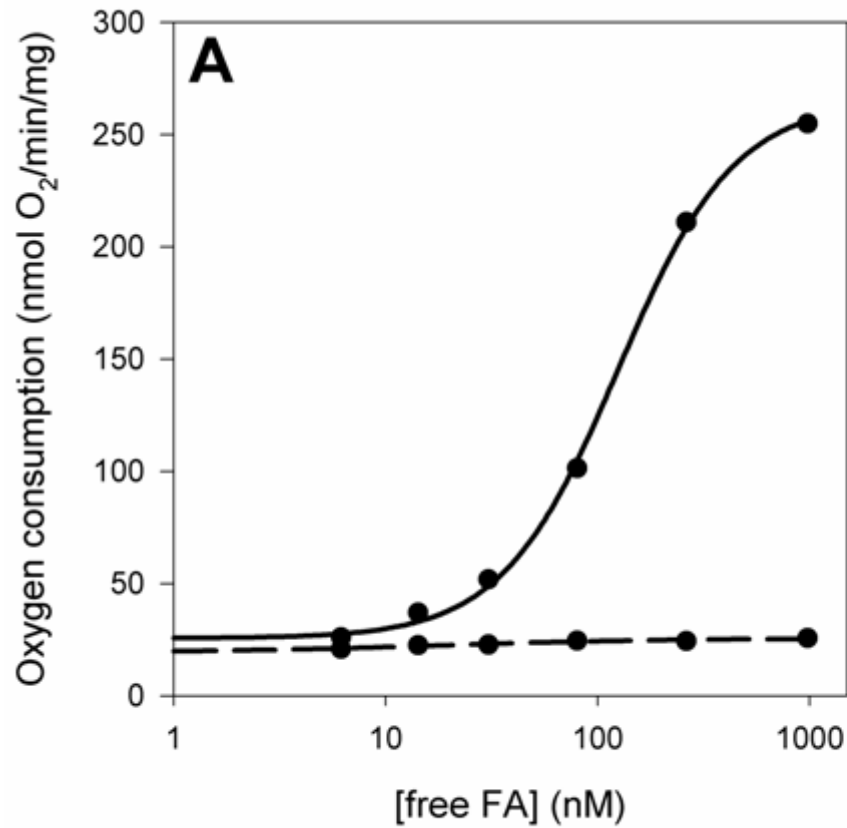
Aging related increased superoxide production is blunted in UCP3 tg mice



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

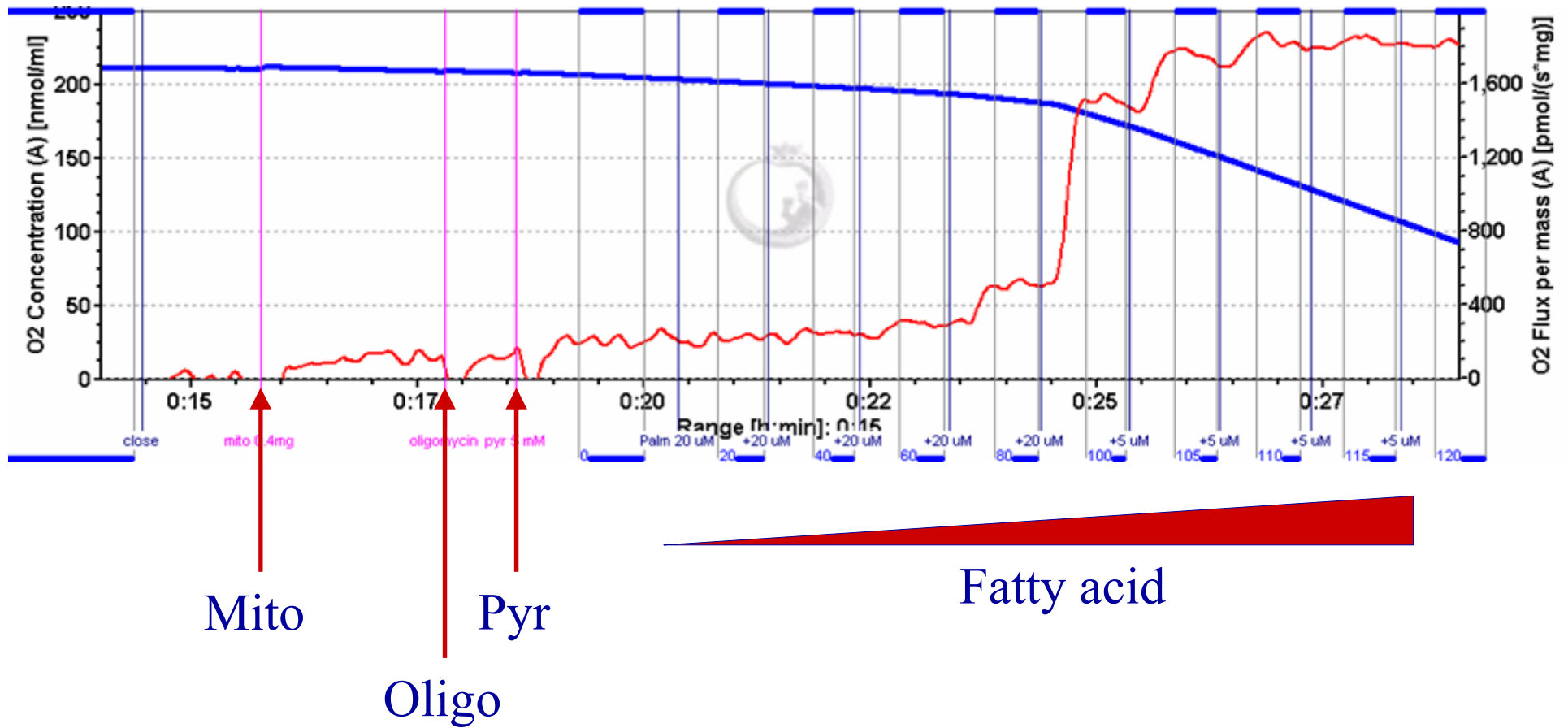
Hoeks et al., in progress

Hypotheses

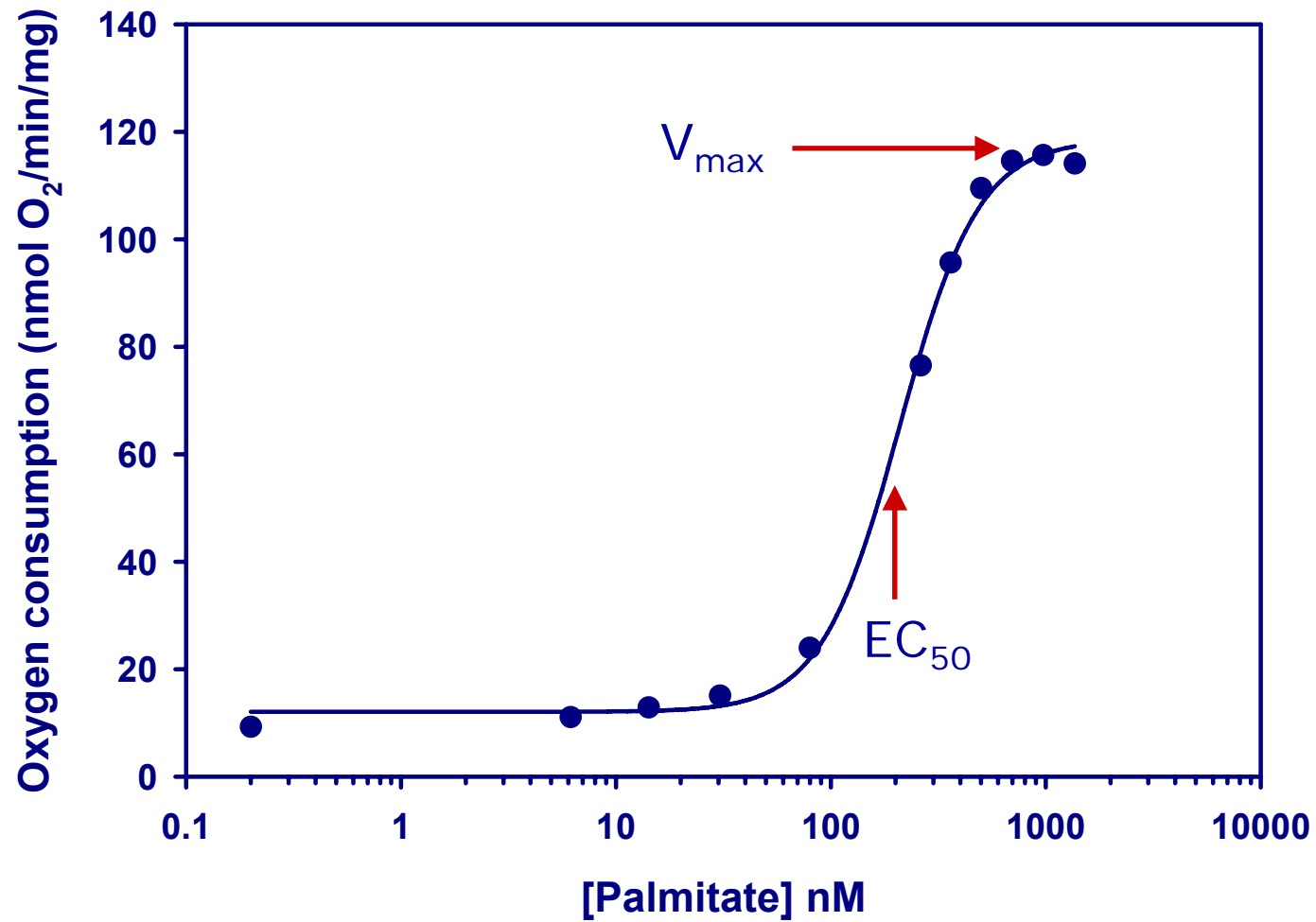
- FA-induced uncoupling controls mitochondrial ROS production when facing high intracellular fatty acid levels
- Reduced or defective FA-induced uncoupling contributes to the progression of type 2 diabetes

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

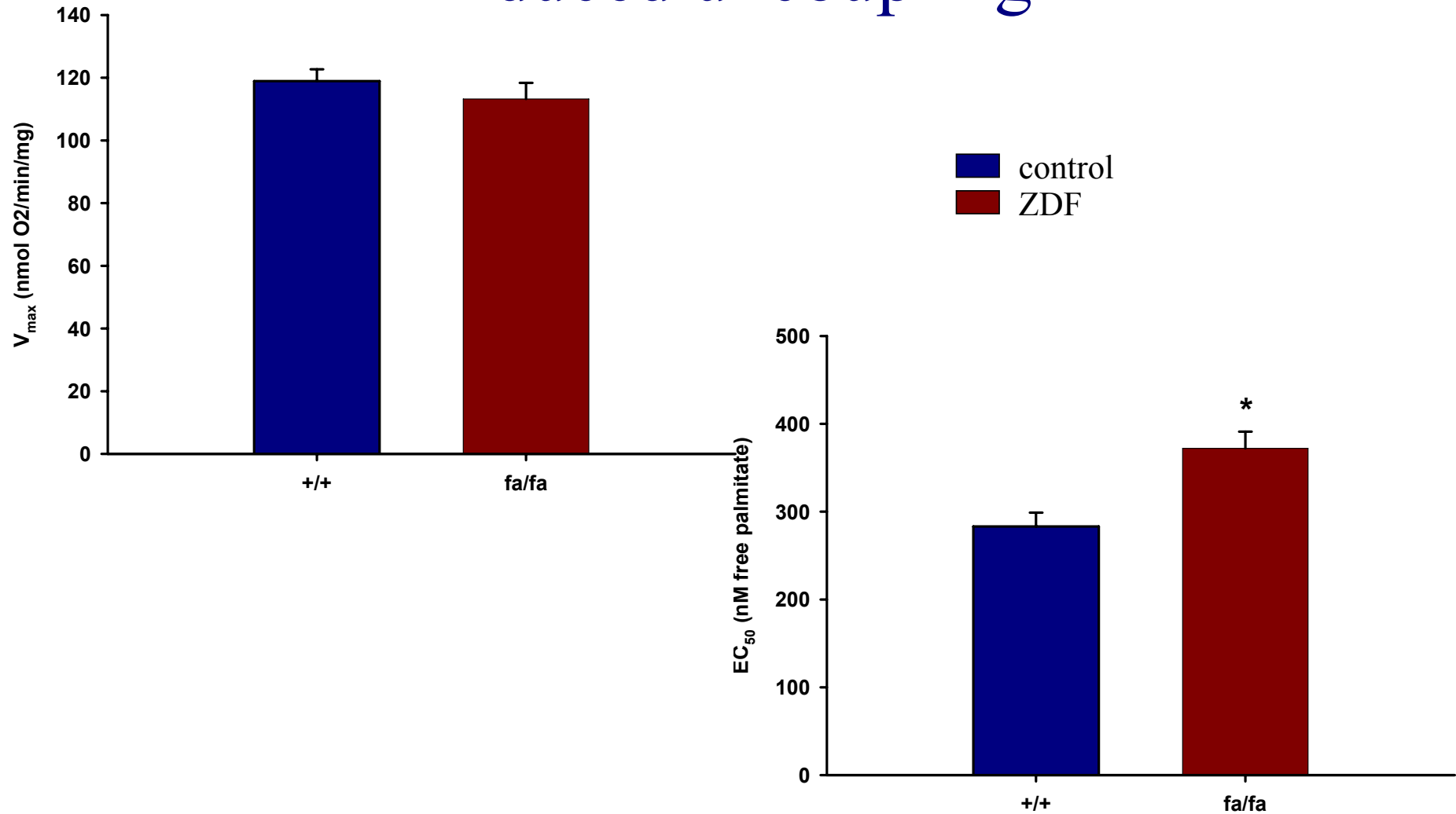
FA-induced uncoupling



FA-induced uncoupling



Diabetic ZDF rats less sensitive to FA-induced uncoupling



Hoeks et al., in progress

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

Mitochondrial uncoupling

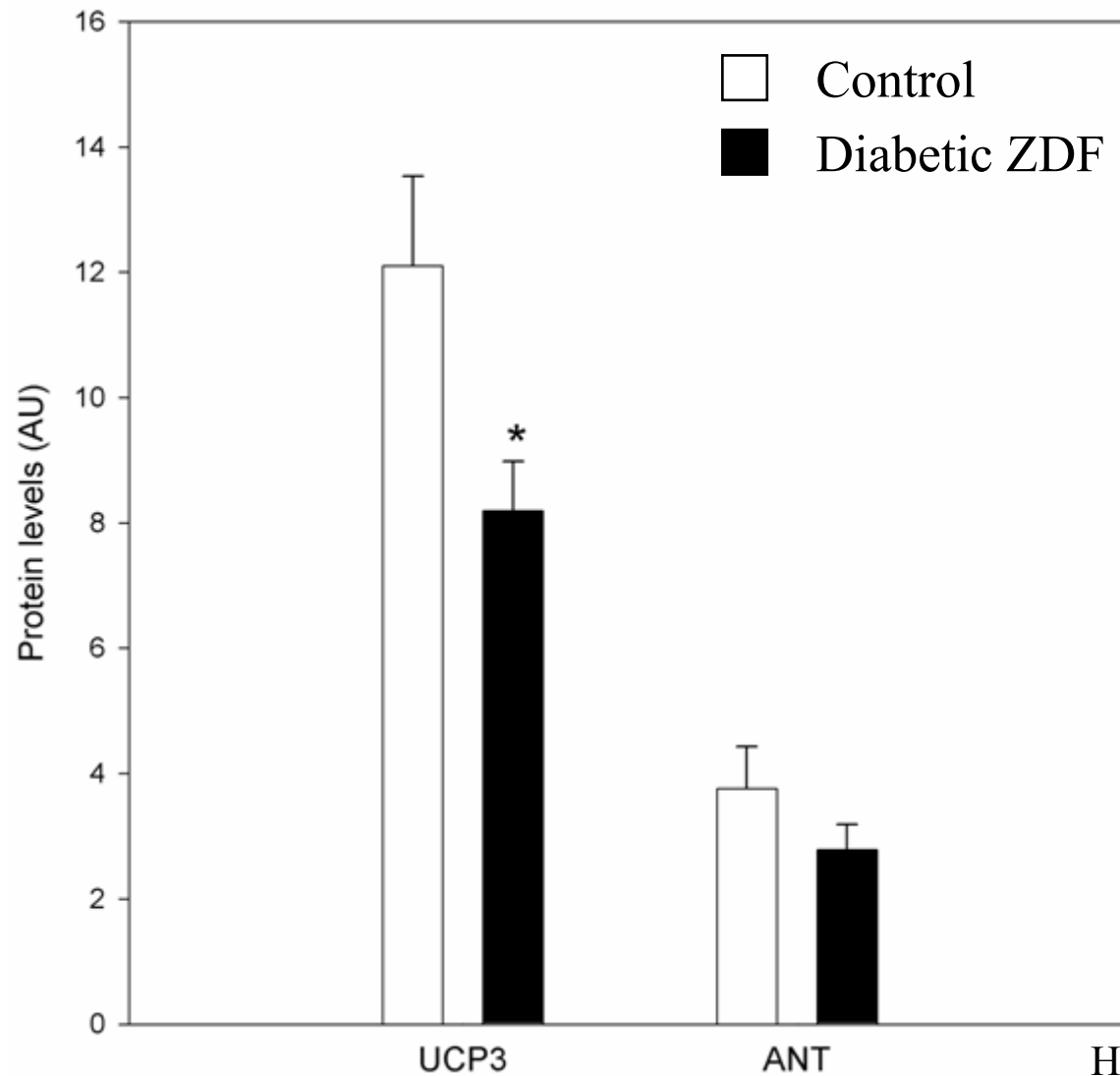
Uncoupling Protein 3 (UCP3)

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

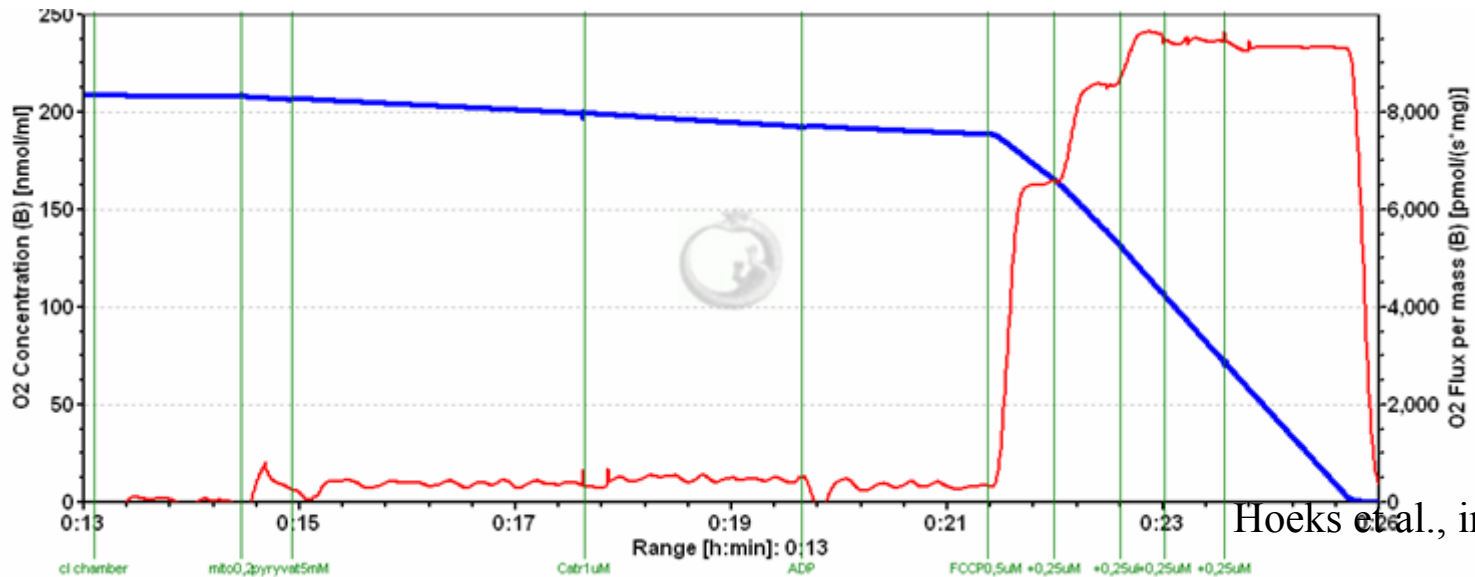
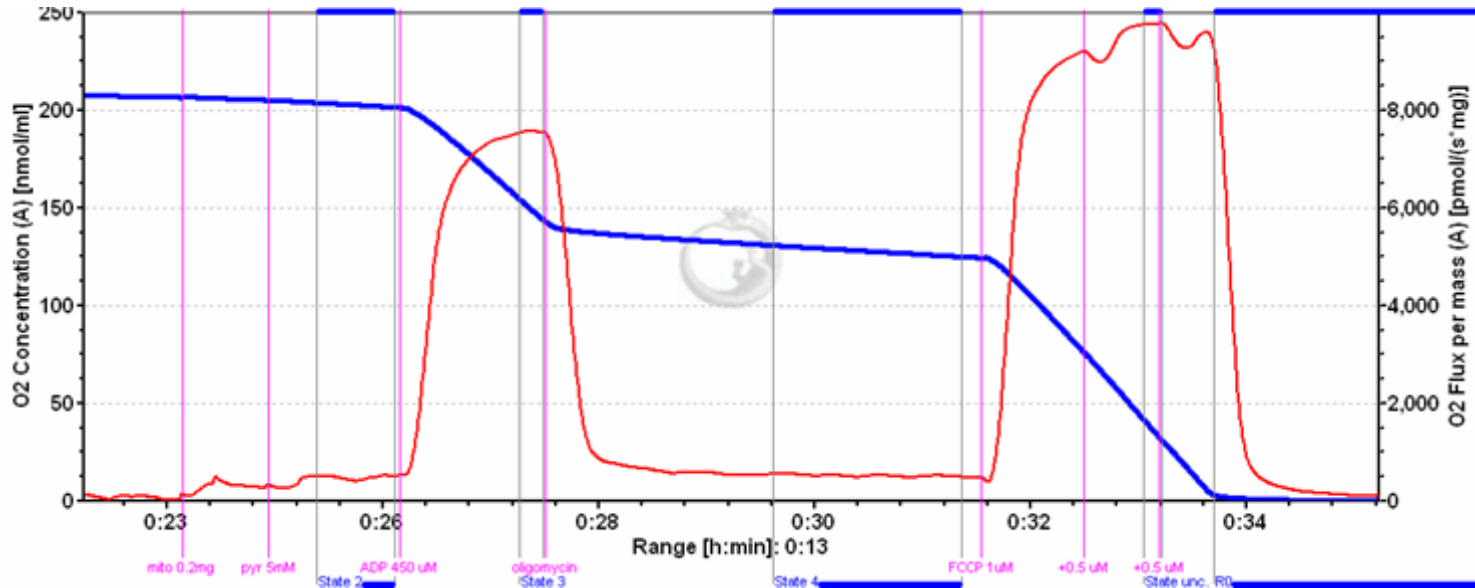
Lower UCP3 but comparable ANT levels in diabetic ZDF rat



Hoeks et al., in progress

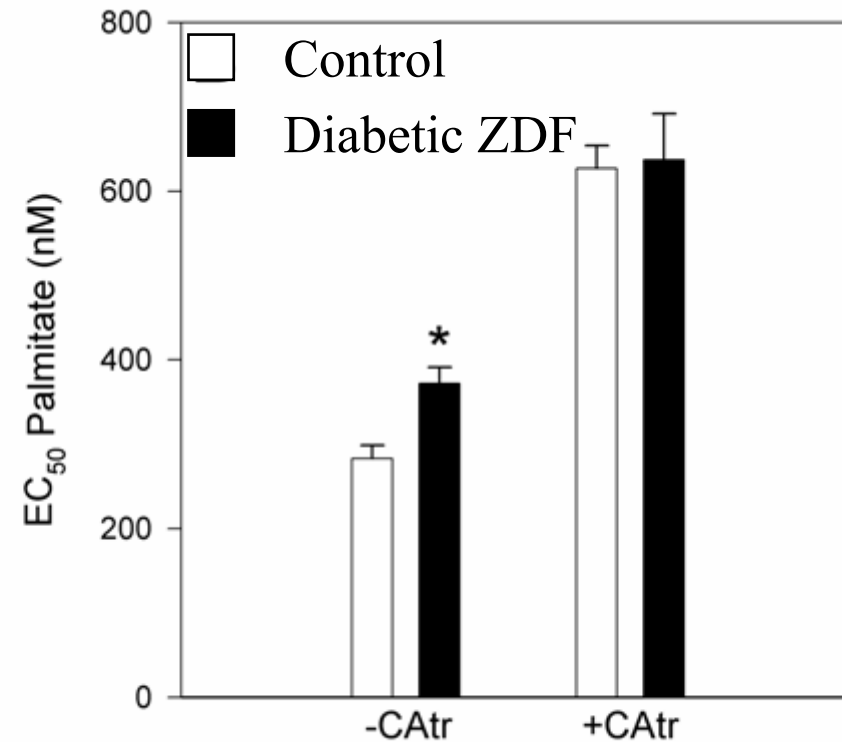
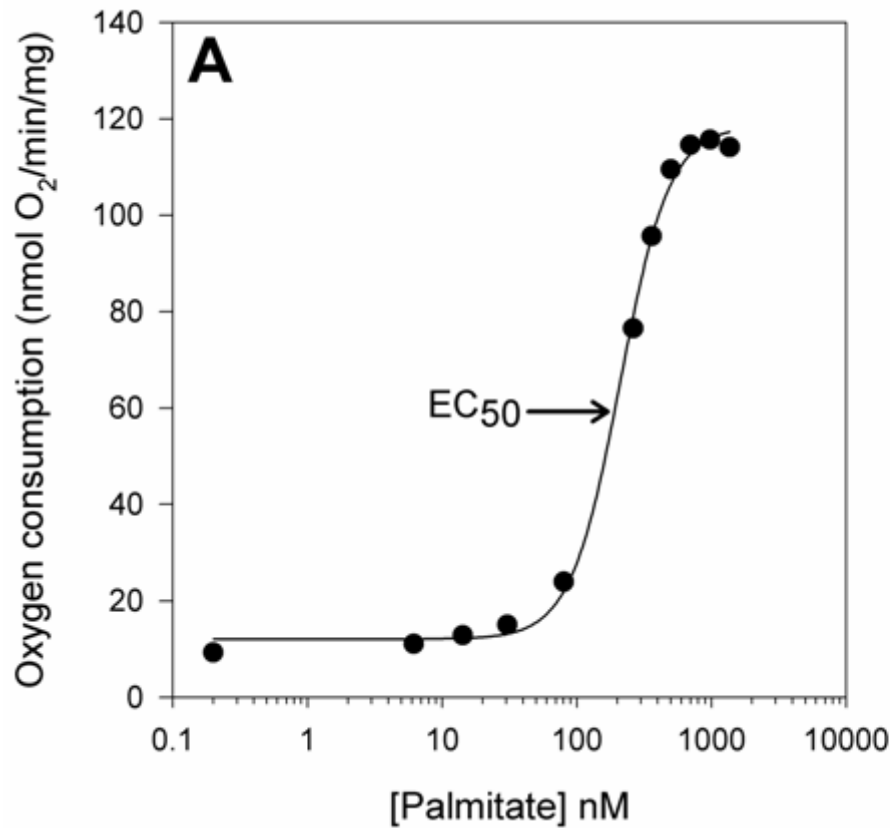
n=6

ANT inhibition: CarboxyAtractyloside



Hoeks et al., in progress

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



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



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