Breathtaking organelles in vital bodies; *in vivo* measures of mitochondrial function

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Mitochondrial function defined

- The ability of a mitochondrion to adjust oxidative degradation of lipids and carbohydrates to a variety in energy demand and supply

In vivo methods defined

Measuring mitochondrial function non-invasively

- Methods based on breath gas analyses (whole body VO_2 & VCO_2)
  - Respiration Chambers
  - Ventilated hood
  - Face mask/mouth piece based systems
  - Any of the above combined with (^{13}C) tracer analysis

- Methods based on non-invasively measuring mitochondrial metabolites or indirect markers thereof
  - NMR based systems (ATP saturation transfer, ^{31}PCr resynthesis rate)
  - *PET Scan*
  - *Thermal recorders*

Methods based on breath gas analyses

common denominator

- Measuring ambient air conditions
  - % O_2
  - % CO_2
  - T
  - P
  - Humidity
- Measuring exhaled air conditions
  - % O_2
  - % CO_2
  - *T*
  - *P*
  - *Humidity*

Methods based on breath gas analyses
common denominator

- Convert to Standard Temperature Pressure Dry (STPD)
- Measuring flow (face and mouth masks) or Create standard flow (ventilated hood and respiration chambers)
- Compute whole body O_2 uptake and CO_2 production

$VO_2 = V(e) \times (FiO_2 - FeO_2)$

So, for example if $V(e) = 24.5$ liters/min
$FeO_2 = 0.1602$
$FiO_2 = 0.2093$ (20.93%)
$VO_2 = 24.5 \times (0.2093 - .1602) = 1.20$ liters of O_2 per minute

$VCO_2 = V(e) \times (FeCO_2 – FiCO_2)$

So, for example if $V(e) = 24.5$ liters/min
$FeCO_2 = 0.0388$
$FiCO_2 = 0.0003$ (0.03%)
$VCO_2 = 24.5 \times (0.0388 – 0.0003) = 0.94$ liters of CO_2 per minute

Methods based on breath gas analyses

post-processing the data

- Compute Respiratory Exchange Ratio (RER) $\approx$ Respiratory Quotient (RQ)

$$VCO_2/VO_2$$

RQ Glucose $C_6H_{12}O_6 + 6O_2 \Rightarrow 6CO_2 + 6H_20$

$$RQ = 6/6 = 1.00$$

RQ Palmitate $C_{16}H_{32}O_2 + 23O_2 \Rightarrow 16CO_2 + 16H_2O$

$$RQ = 16/23 = 0{,}696$$

Under aerobic conditions

$$0.696 > RQ < 1.00$$

Methods based on breath gas analyses

post-processing the data

	Energy provided	O_2 required	kJ/l O_2
1 g Glucose	16 kJ	0,75 l	22
1 g Fat (depends on C)	41 kJ	2,00 l	20

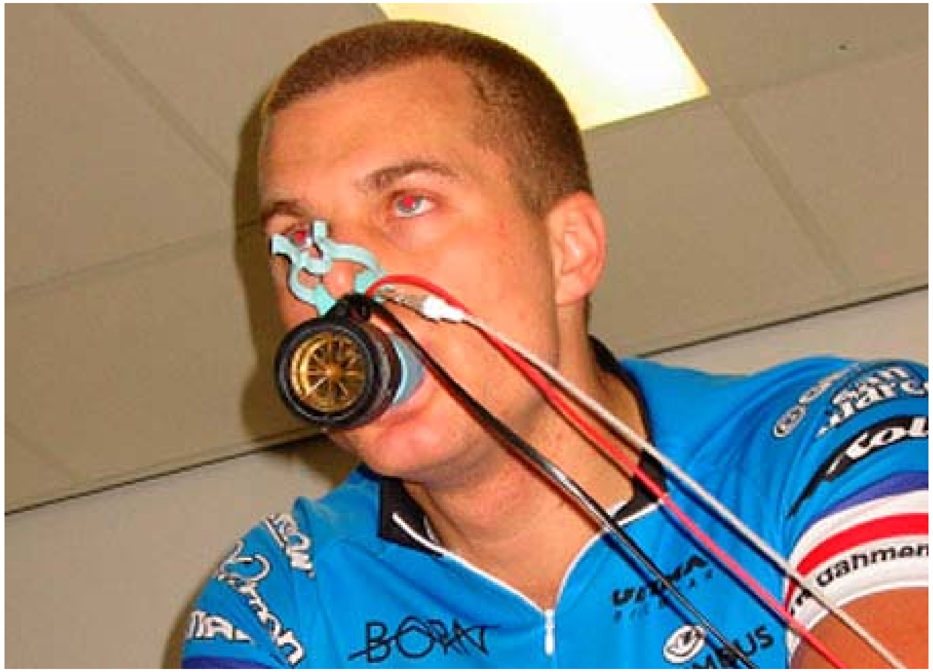
$$\text{CHO oxidation} = 4{,}585 * VCO_2 - 3{,}226 * VO_2$$

$$\text{Fat oxidation} = 1{,}695 * VCO_2 - 1{,}701 * VO_2$$

Valid for steady state conditions and RQ<1.00

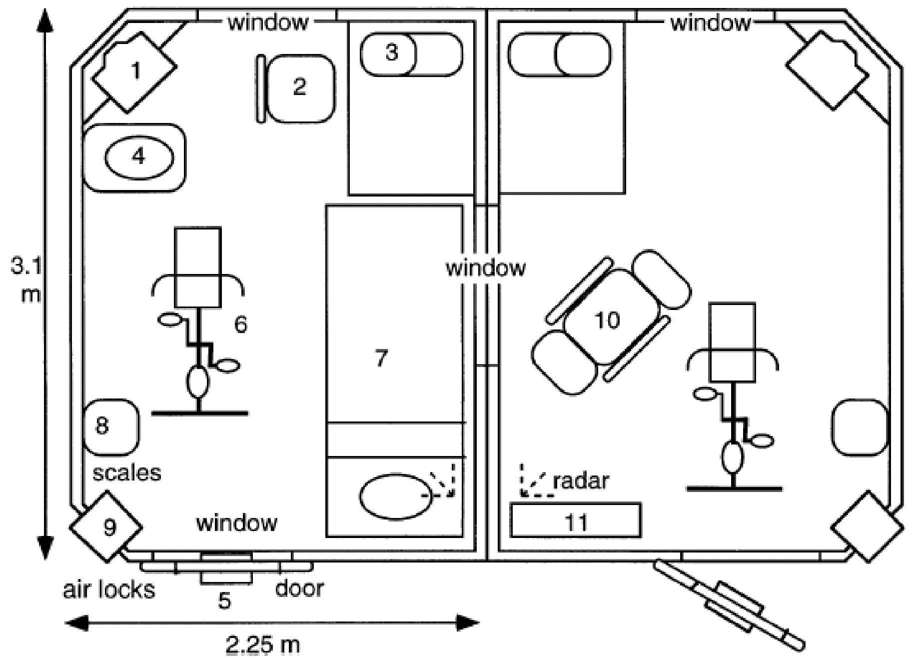
Weir, J. B. New methods for calculating metabolic rate with special reference to predicting protein metabolism. *J. Physiol. (Lond.)* 109: 1–9, 1949.

Breath gas analyses
mouth piece



- Measuring flow by turbidometry
- Breath-by-breath analyses possible
- Measuring ventilatory rate and volume
- Suitable for exercise, not (hardly) for resting conditions
- Allows analyses of substrate selection during exercise

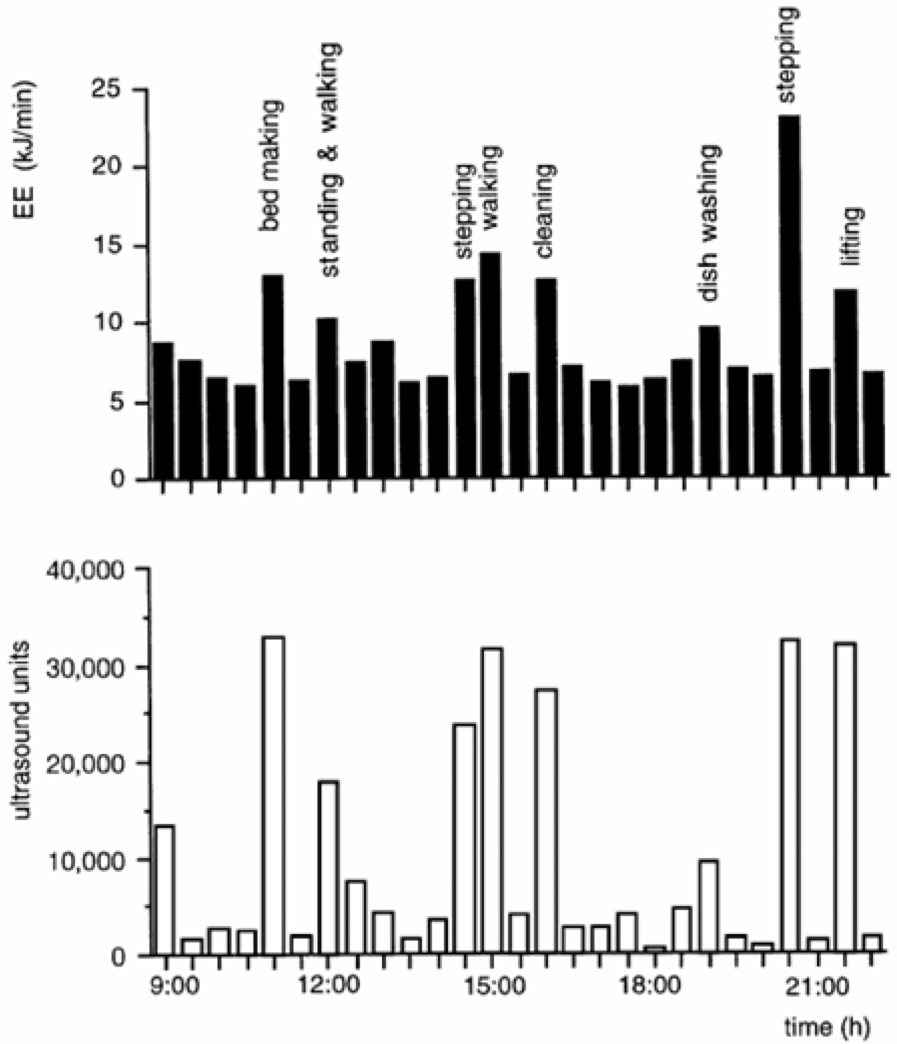
Breath gas analyses
Respiration chamber



- Flow through chamber pre-set, but adjustable
- No measures of ventilatory rate and volume
- Suitable for assessing RMR & SMR as well as exercise with very accurate measures of substrate selection
- All other conditions (diet, mobility, faeces, urine samples) controlled or collected

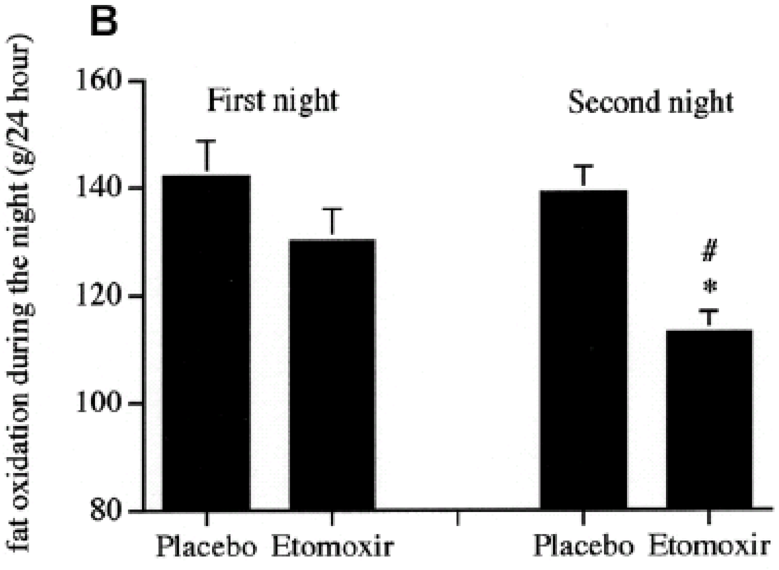
Schoffelen et al., JAP 1997

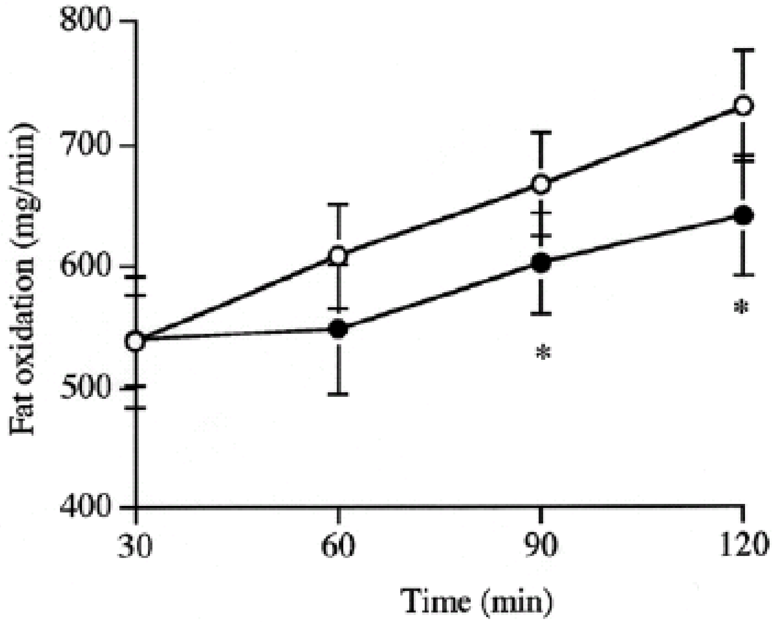
Respiration chamber
to measure energy expenditure



Schoffelen et al., JAP 1997

Respiration chamber
to measure substrate selection





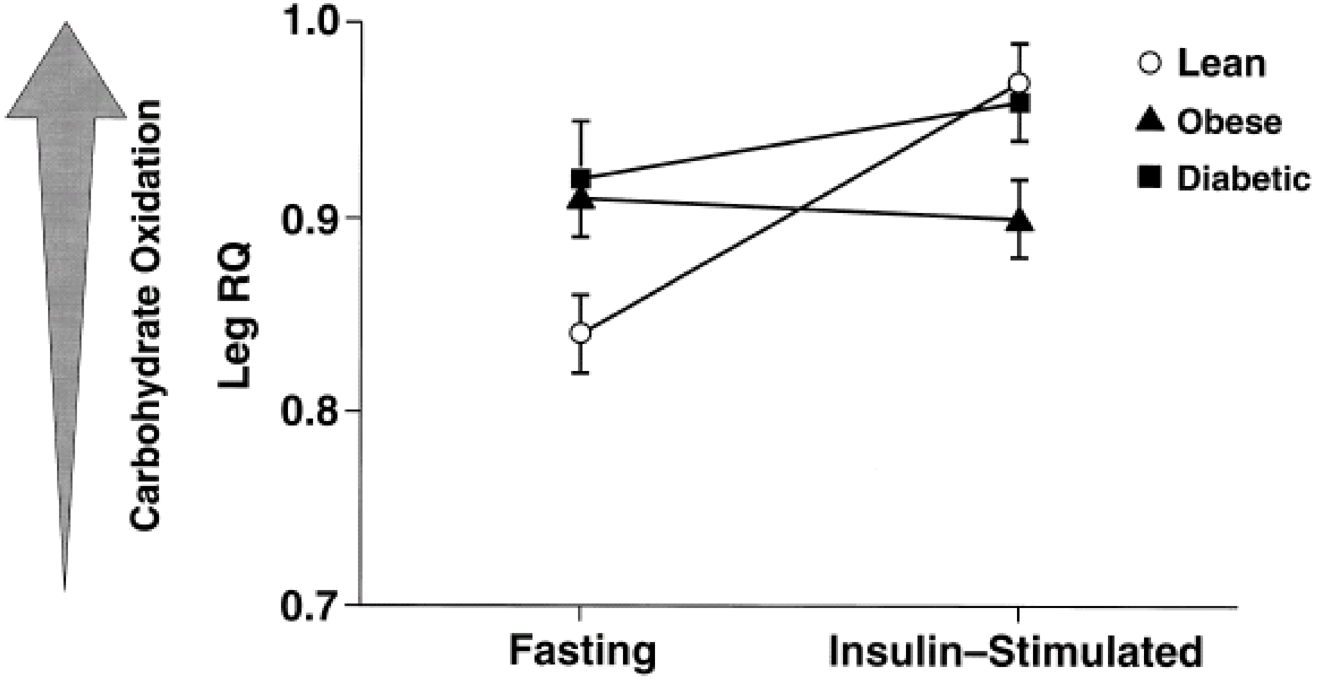
- Etomoxir blocks CPT1 (mitochondrial entry of long chain fatty acids)
- 36 Hours stay + exercise in the chamber (only water provided, black circles etomoxir),

Schrauwen et al., FASEB J 2002

Breath gas analyses
ventilated hood

- Flow through hood pre-set
- No measures of ventilatory rate and volume
- Suitable for assessing RMR
- Allows analyses of substrate selection during interventions

Ventilated hood to measure ‘metabolic flexibility’



- Insulin-mediated increase in CHO oxidation blunted in obese and T2D
- Leg RQ extended to whole body
- Mitochondrial phenomenon?

Kelley et al., Diabetes 2000

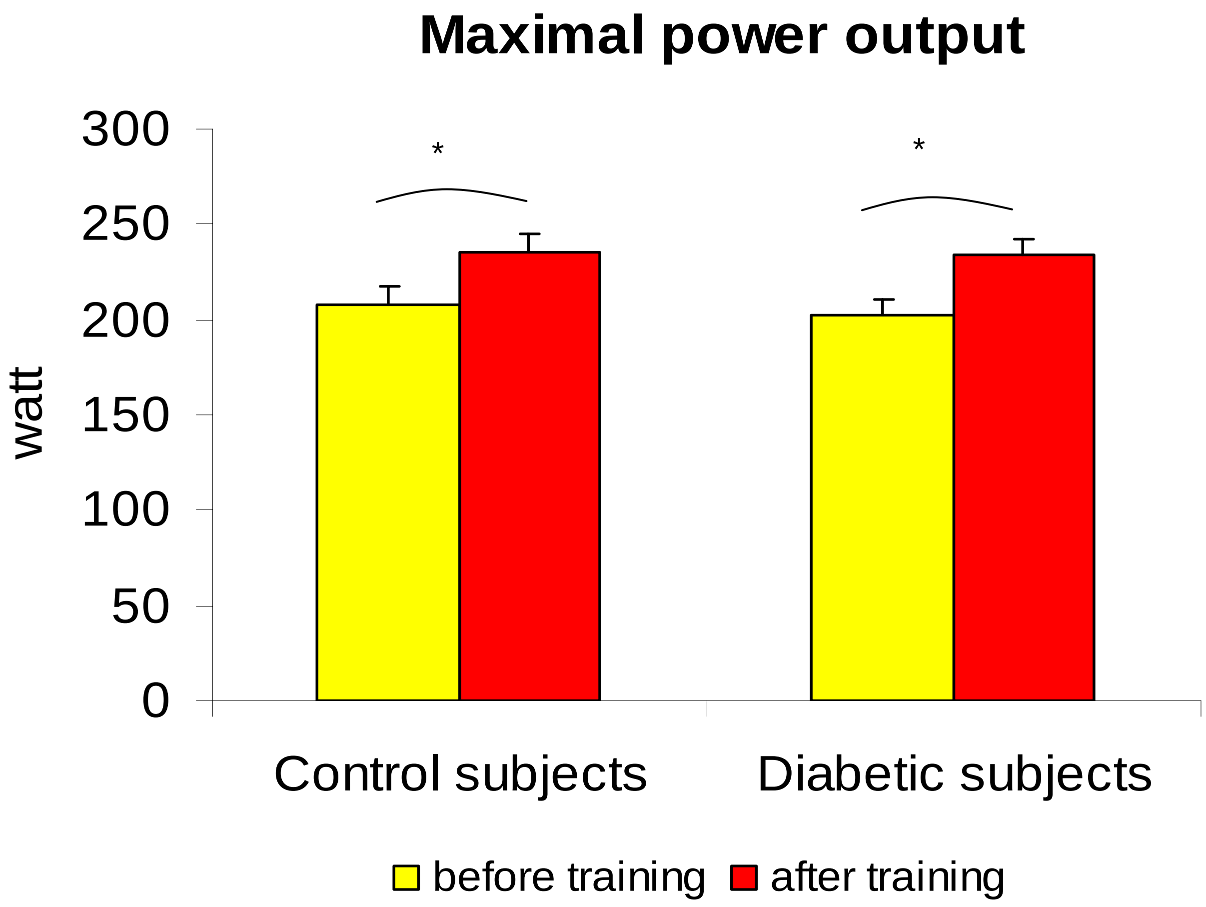
Ventilated hood based metabolic flexibility
effect of exercise training T2D

	Controls (n = 20)	T2D (n = 18)
Age (Years)	59 ± 1	59 ± 1
Weight (kg)	95 ± 3	94 ± 3
Length (cm)	179 ± 1	177 ± 1
BMI (kg/m^2)	30 ± 1	30 ± 1

Meex et al. in progress

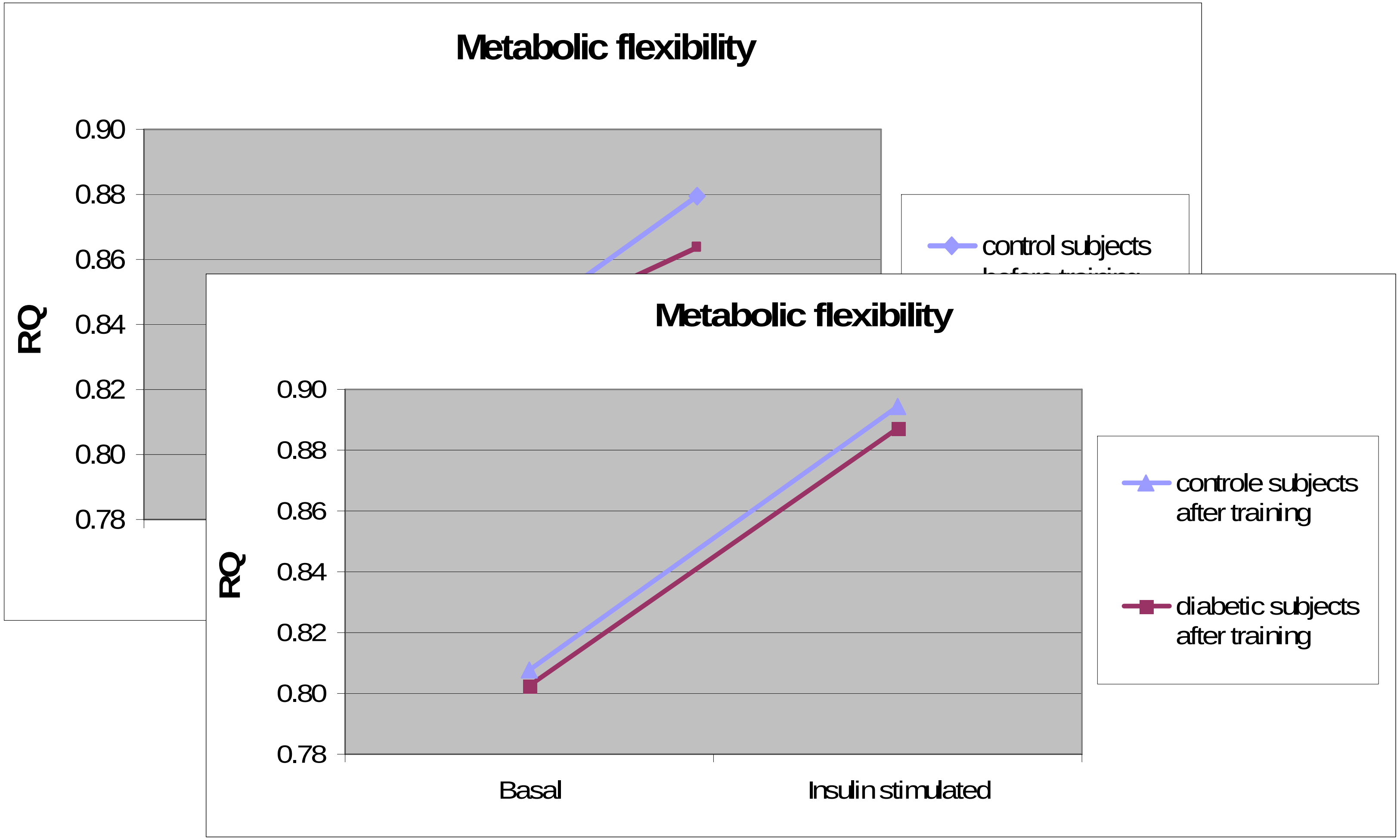
Ventilated hood based metabolic flexibility

effect of exercise training T2D



Meex et al., in progress

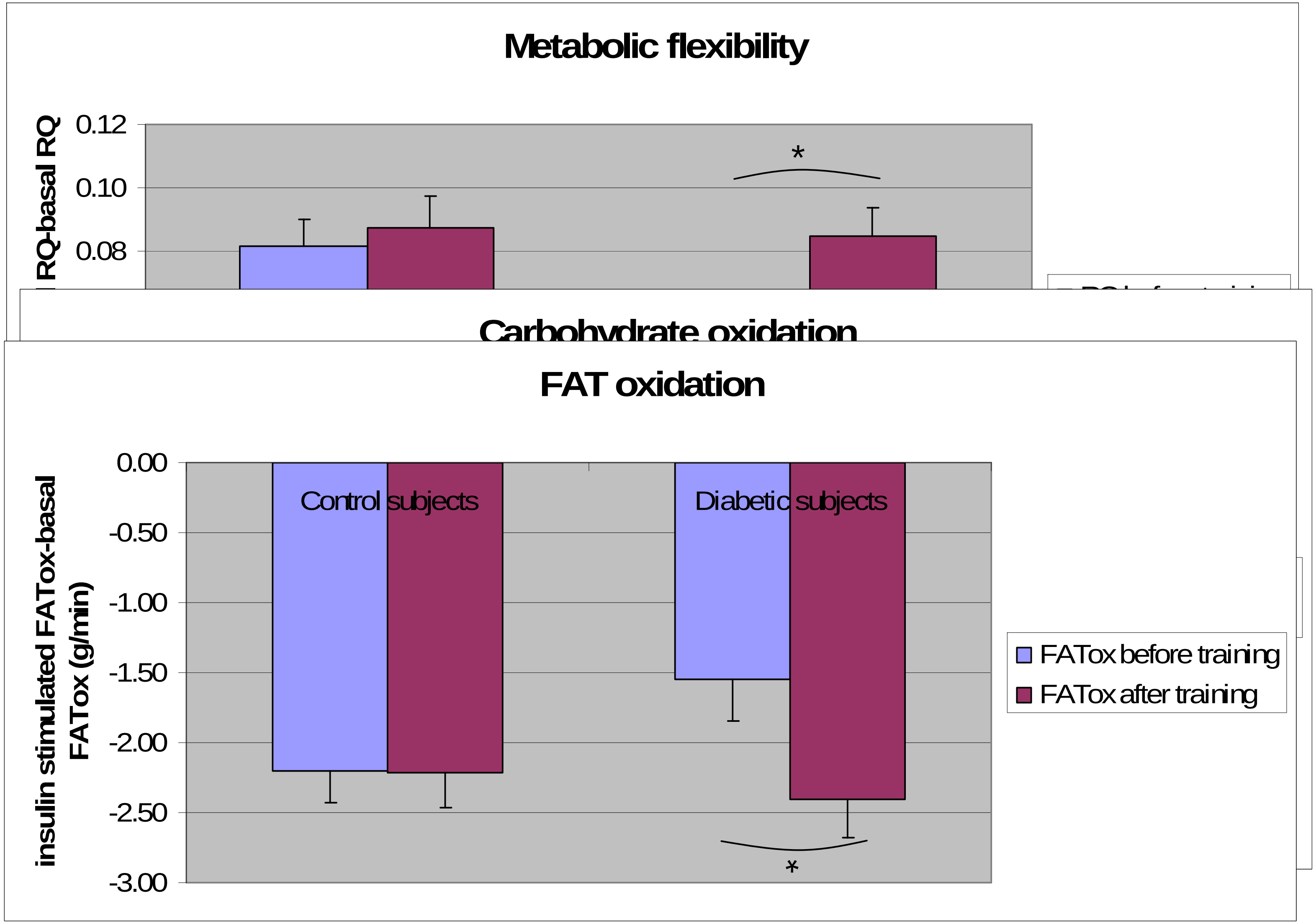
Ventilated hood based metabolic flexibility
effect of exercise training T2D



Meex et al., in progress

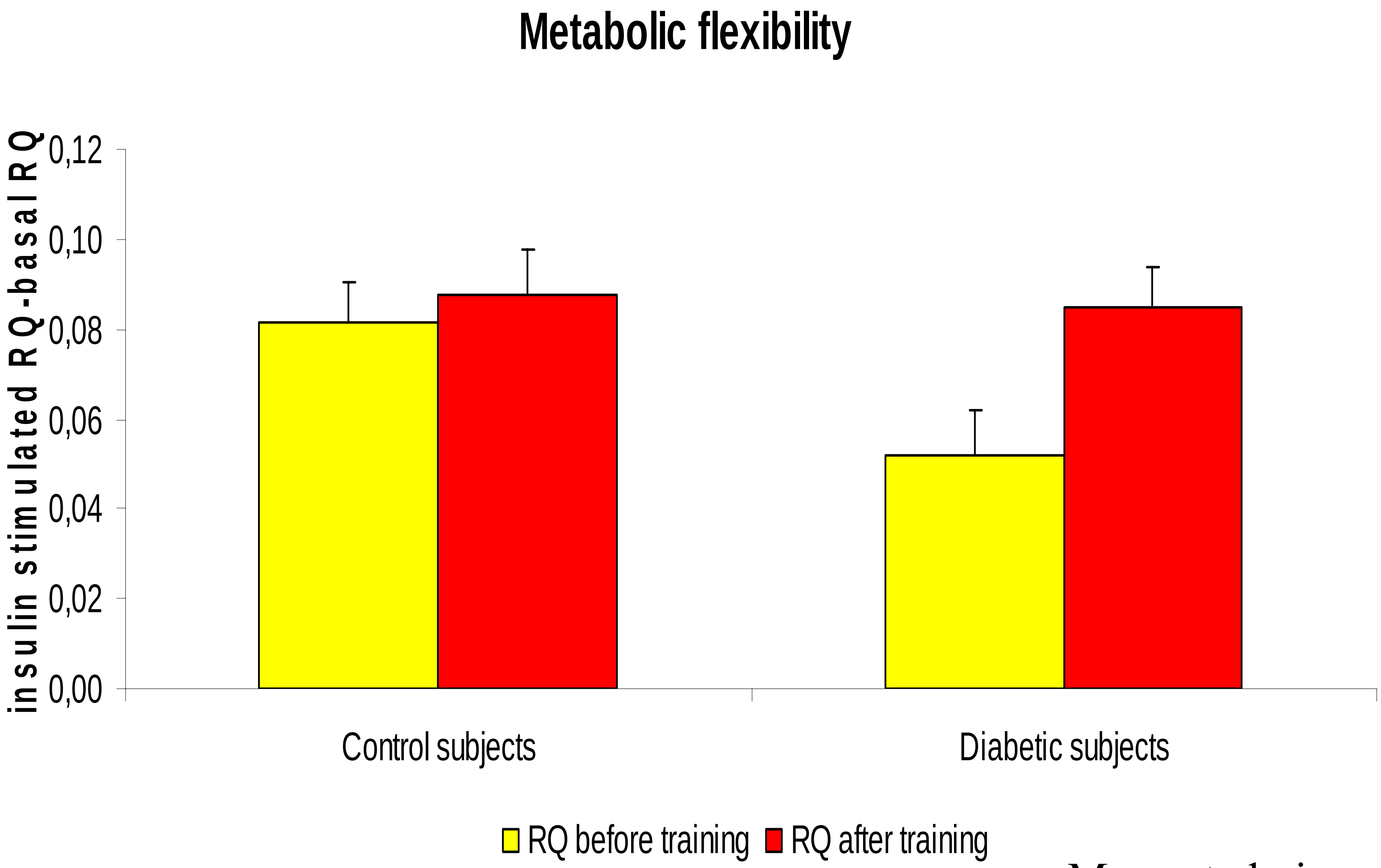
Ventilated hood based metabolic flexibility

effect of exercise training T2D



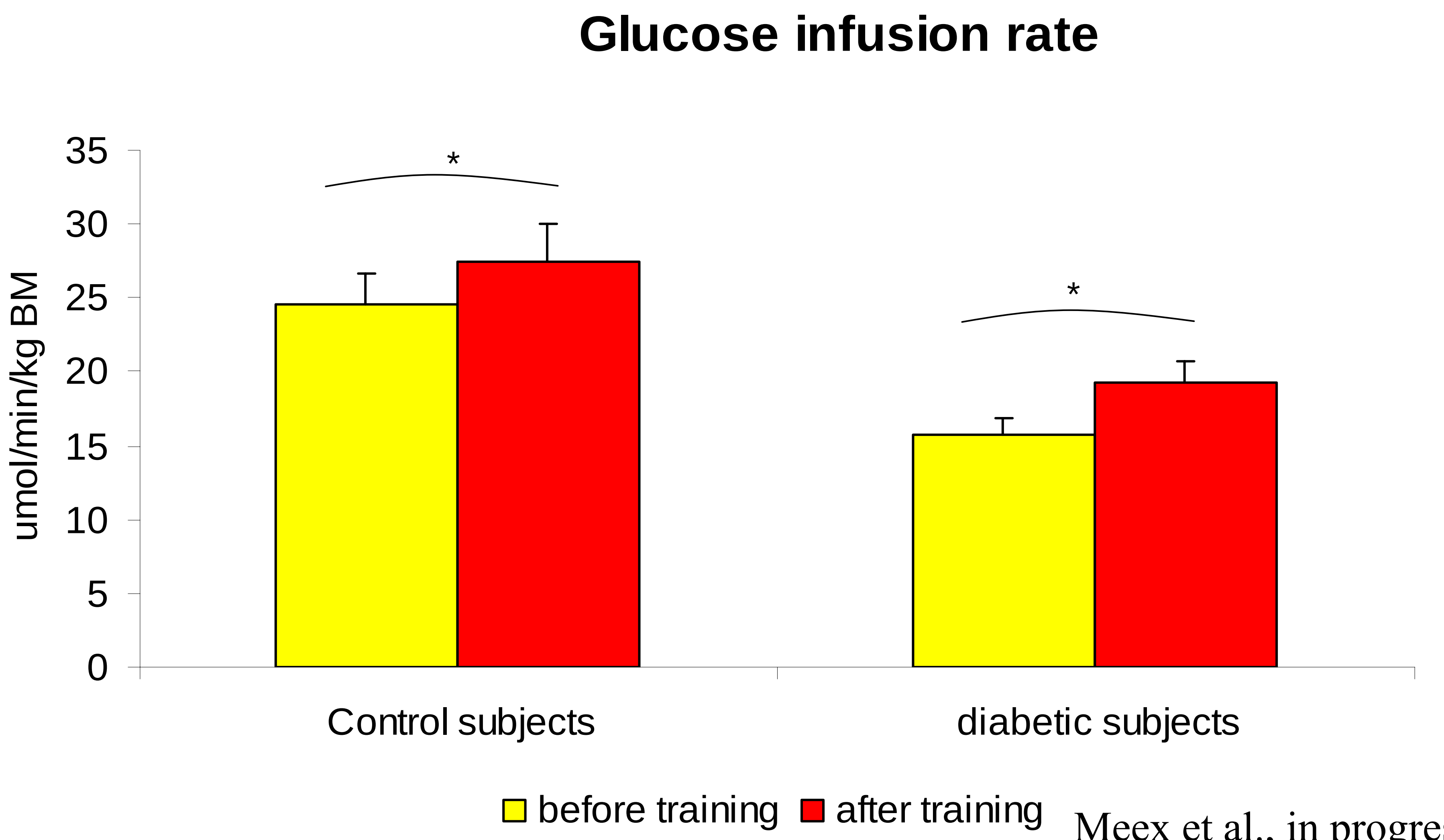
Q: Does metabolic inflexibility reflect insulin resistance?

Training T2D restores metabolic flexibility



Meex et al., in progress

Exercise training in T2D and controls improves, not restores, insulin sensitivity



Meex et al., in progress

Q: Do the many faces of insulin require the same concentration of insulin?

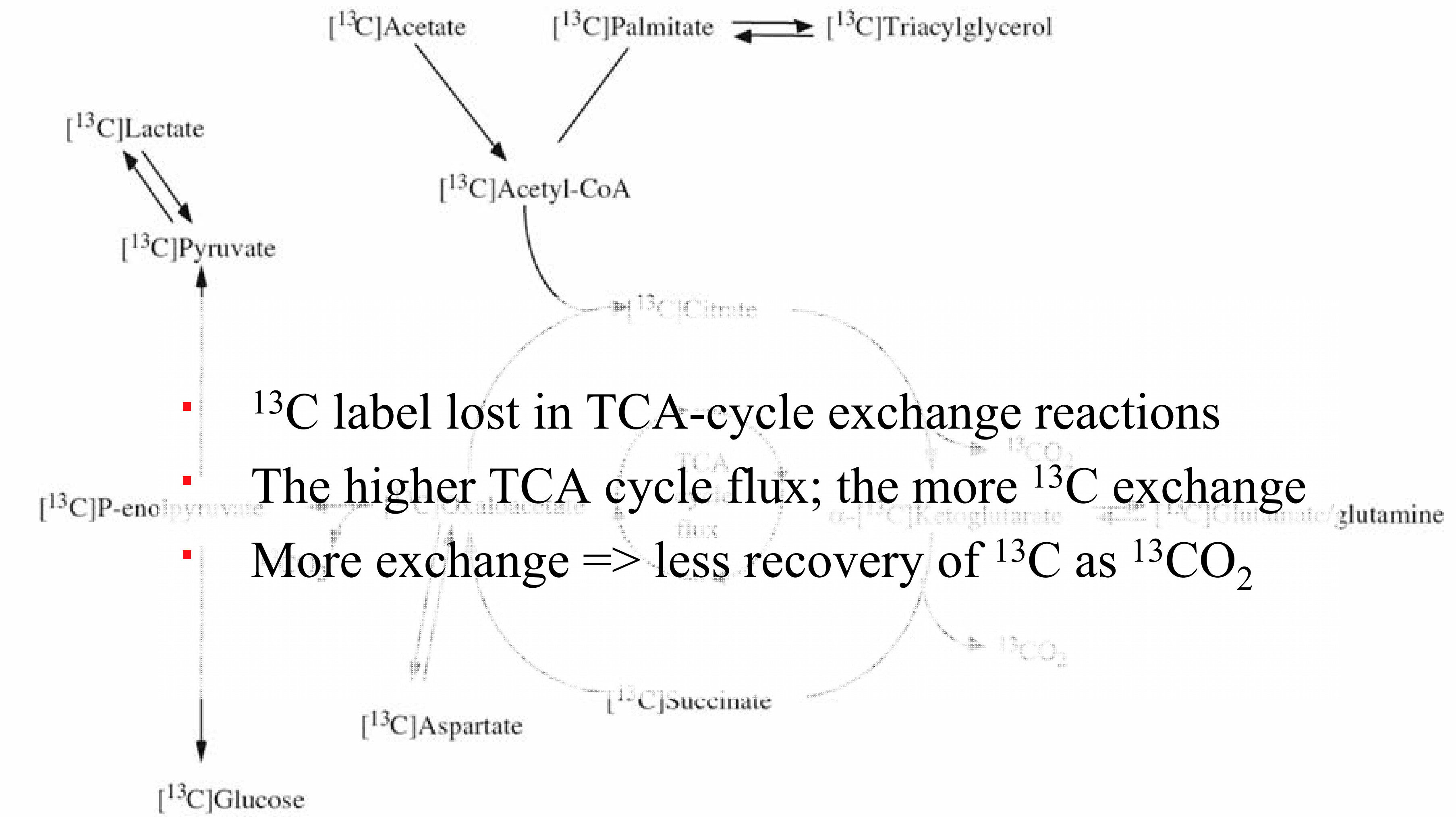
Faces of insulin and concentrations (μU/ml) for computed ½ max effect

FAT	LIPOLYSIS	20
Na-K-ATP-ase	POTASSIUM	25
GLUCOSE	OXIDATION	28
GLUCOSE	PRODUCTION	29
GLUCOSE	UPTAKE	65
GLUCOSE	GLYCOGEN SYNTHESIS	109
PROTEIN	PROTEOLYSIS	32

Data compiled by Prof H. P. Sauerwein

1,2-^{13}C acetate to measure TCA cycle activity

breath gas analyses after venous infusion



- ^{13}C label lost in TCA-cycle exchange reactions
- The higher TCA cycle flux; the more ^{13}C exchange
- More exchange => less recovery of ^{13}C as $^{13}CO_2$

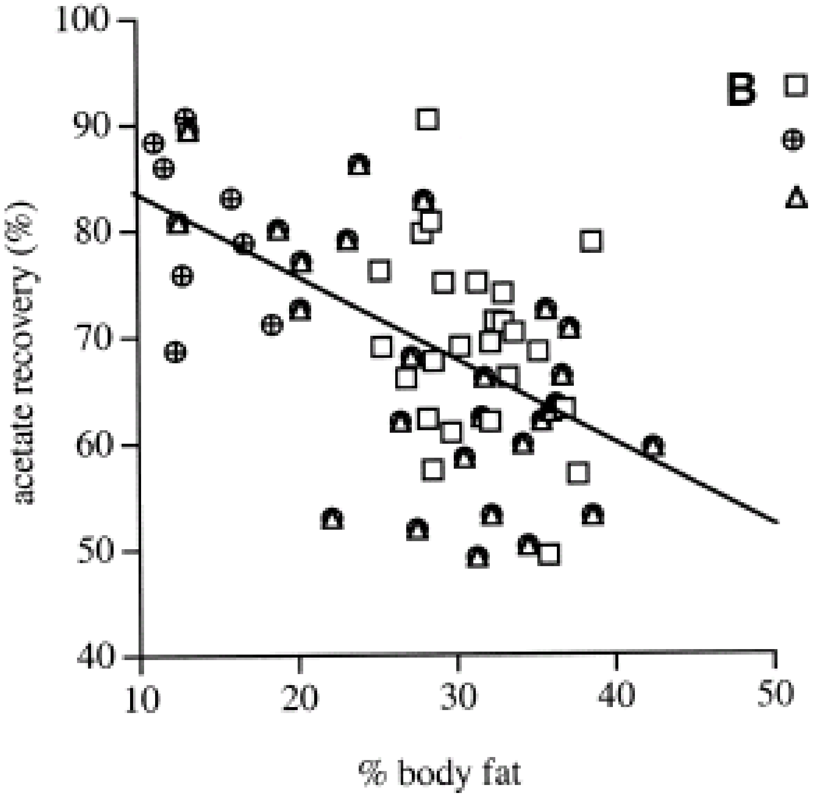
Schrauwen & Hesselink, Diabetologia, 2008

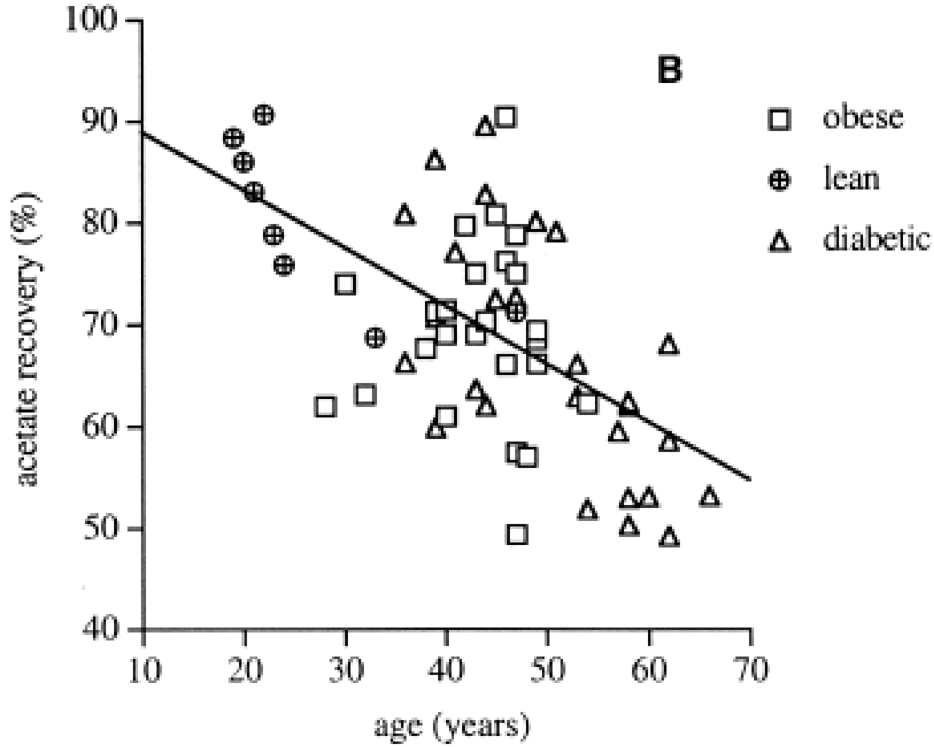
1,2-^{13}C acetate to measure TCA cycle activity

breath gas analyses after venous infusion

Schrauwen & Hesselink, Diabetologia, 2008

1,2-^{13}C acetate lower with incremental body fat and ageing

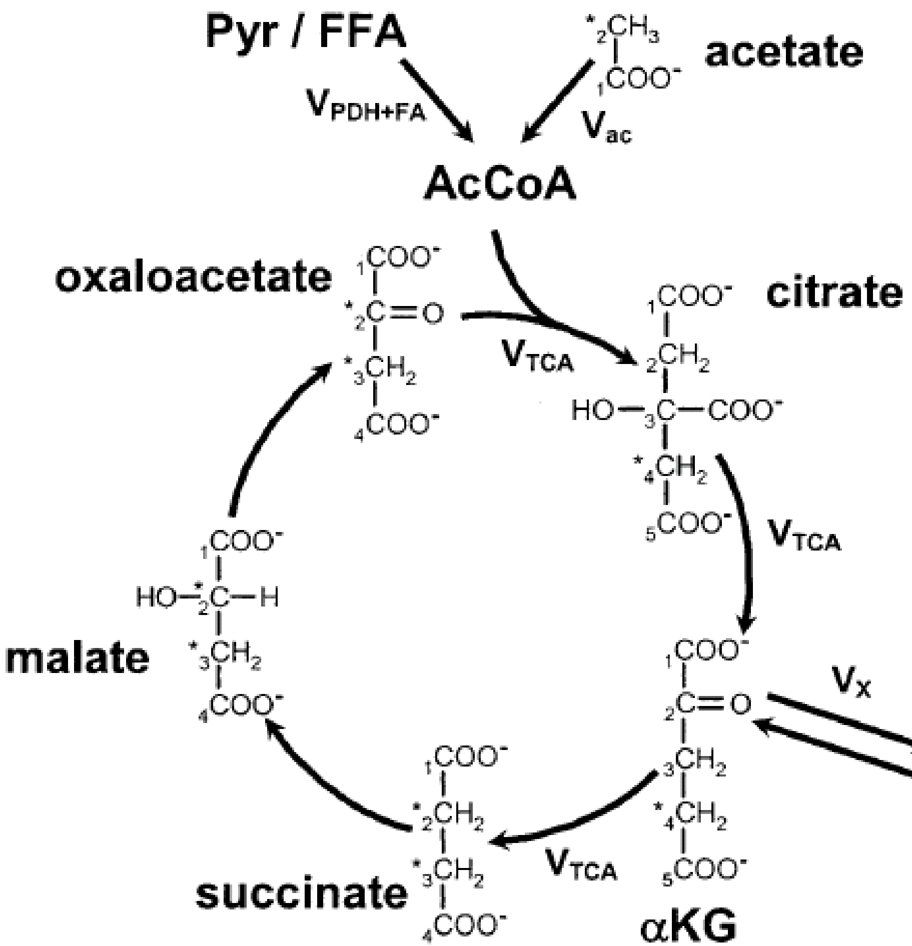


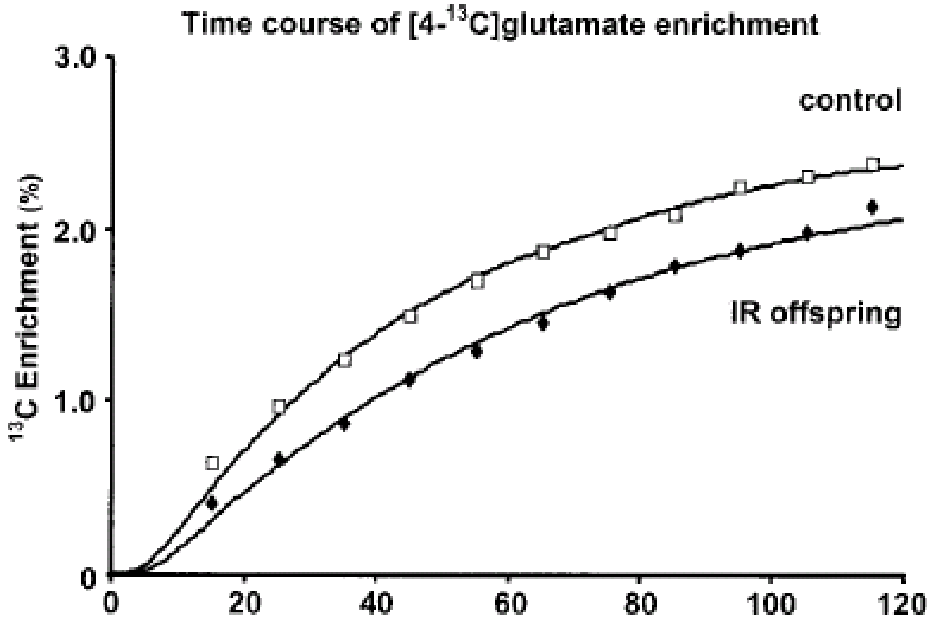


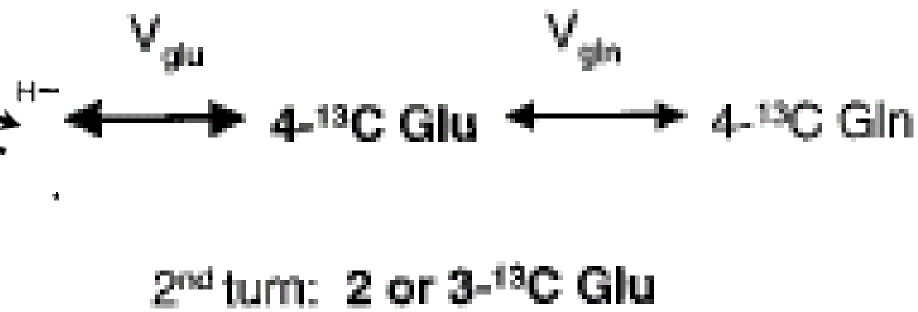
Schrauwen et al., Clin Sci 2000

NMR-based methods

2-^{13}C-acetate incorporation in 4-^{13}C-glu for TCA cycle flux



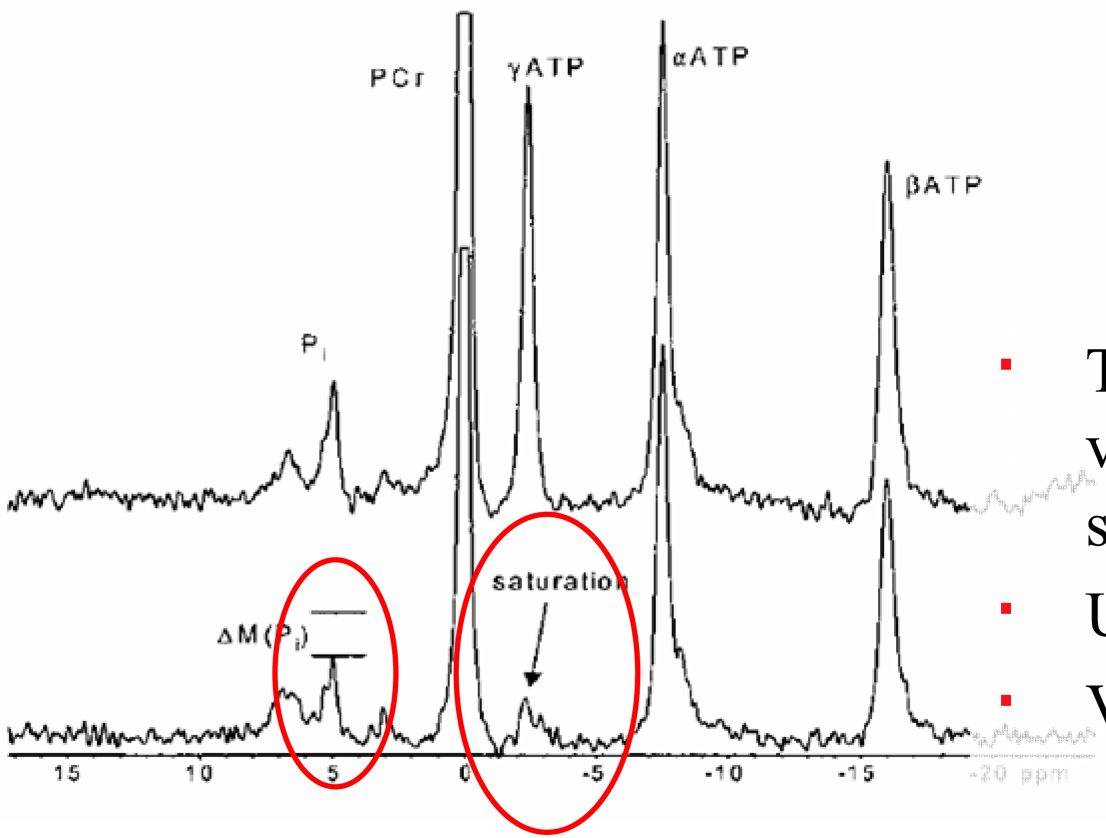




Jucker et al., PNAS 2000

NMR-based methods

^{31}P saturation transfer: unidirectional ATP synthase flux



- Transfer of saturated γ-ATP, via PCr results in decline in saturated P_i (ΔM)
- Unidirectional
- Very low signal

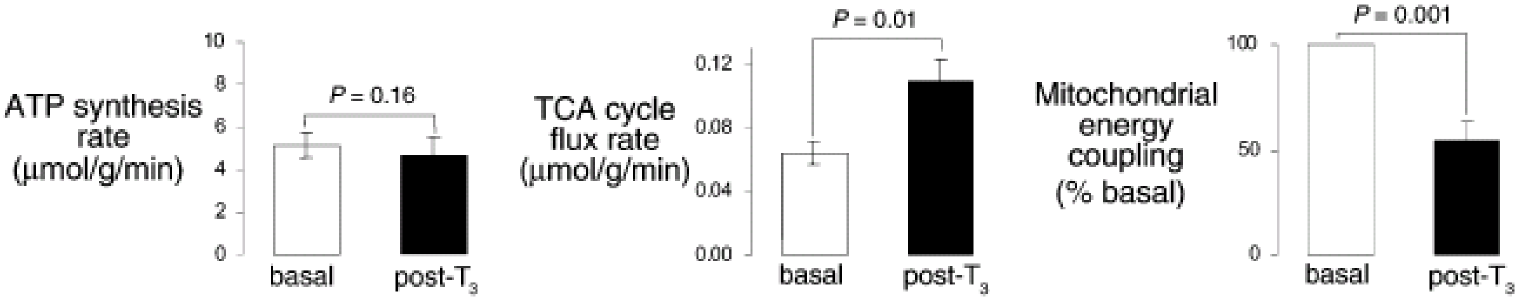
(ΔM)

Pi + CR ⇄ PCr^{2-} + $MgADP^{-}$ + H^{+} ⇄ Cr + $MgATP^{2-}$

Jucker et al., PNAS 2000

NMR-based methods

^{31}P saturation transfer combined with ^{13}C acetate



Lebon et al., JCI 2001

- T3 treatment reduces mitochondrial coupling

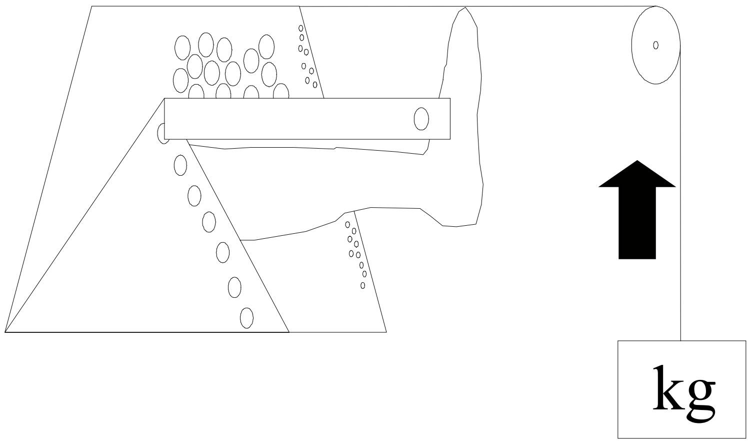
	Basal rates of glucose production (mg/kg of LBM/min)	Clamp peripheral glucose metabolism rate (mg/kg of LBM/min)	Intramyocellular lipid content (%)	Intrahepatic lipid content (%)	Mitochondrial TCA flux rate (nmol/g of muscle/min)	Mitochondrial ATP synthesis rate (µmol/g of muscle/min)
Young	2.3 ± 0.1	6.2 ± 0.6	0.96 ± 0.08	0.49 ± 0.10	96 ± 10	7.50 ± 0.77
Elderly	2.4 ± 0.1	4.0 ± 0.4	1.39 ± 0.15	1.61 ± 0.38	62 ± 5	4.06 ± 0.65
P value	0.34	<0.002	0.035	0.036	<0.006	<0.004

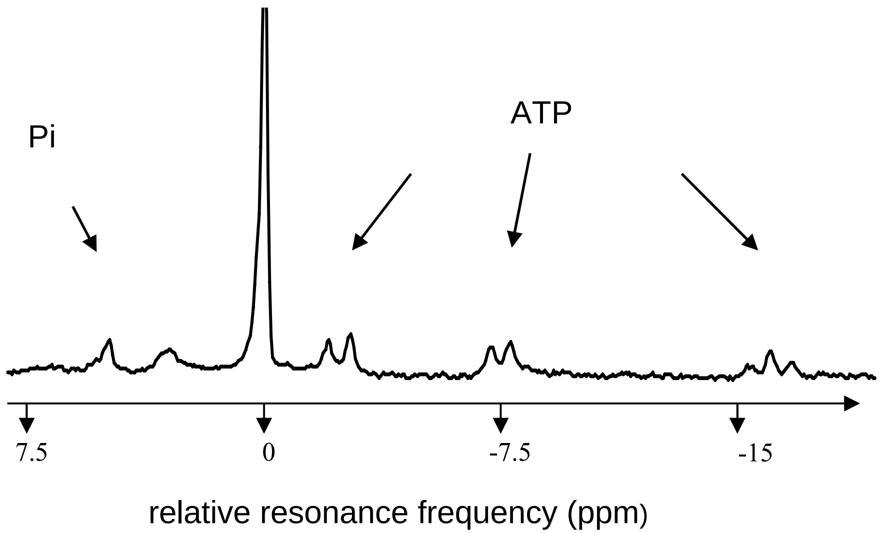
- TCA cycle flux and ATP synthesis declined in IR elderly

Petersen et al., Science 2003

NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate

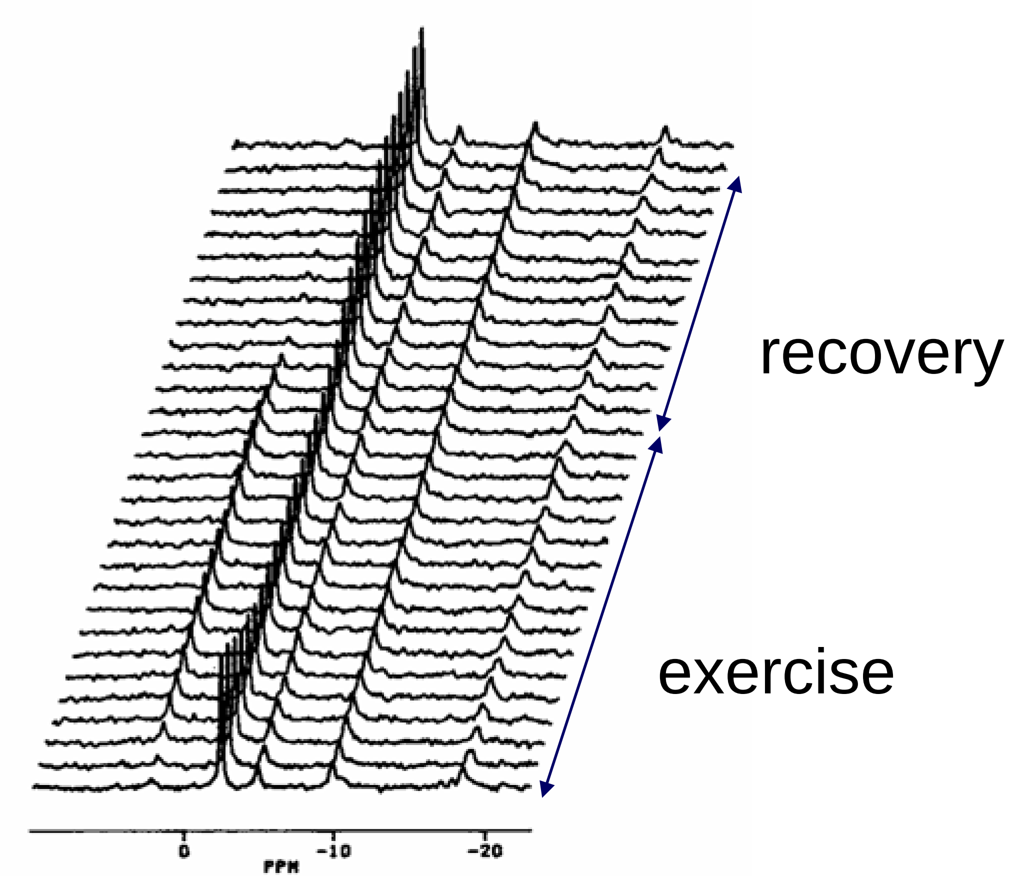




Schrauwen-Hiderling et al., Diabetologia 2007

NMR-based methods

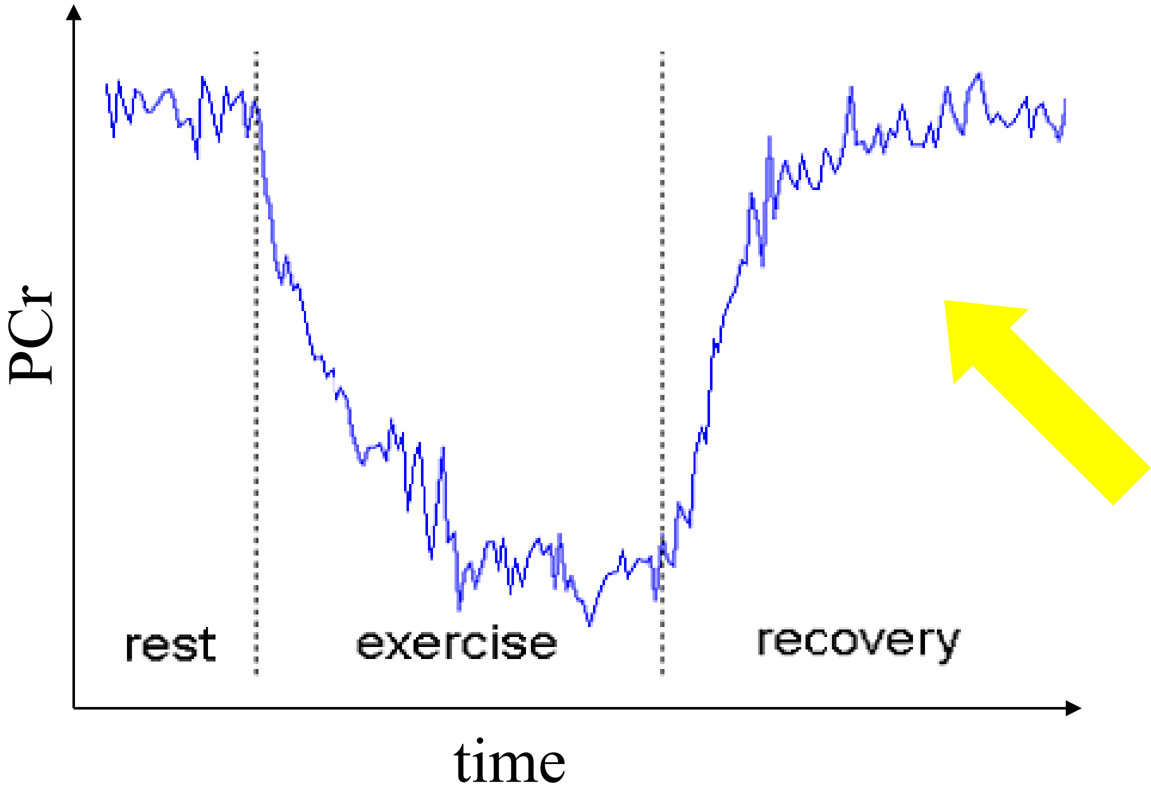
^{31}P-NMR post-exercise PCr resynthesis rate



Meyer et al., 1988

NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate



PCr resynthesis is almost purely aerobic

↓

PCr recovery half-time reflects oxidative capacity

NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate

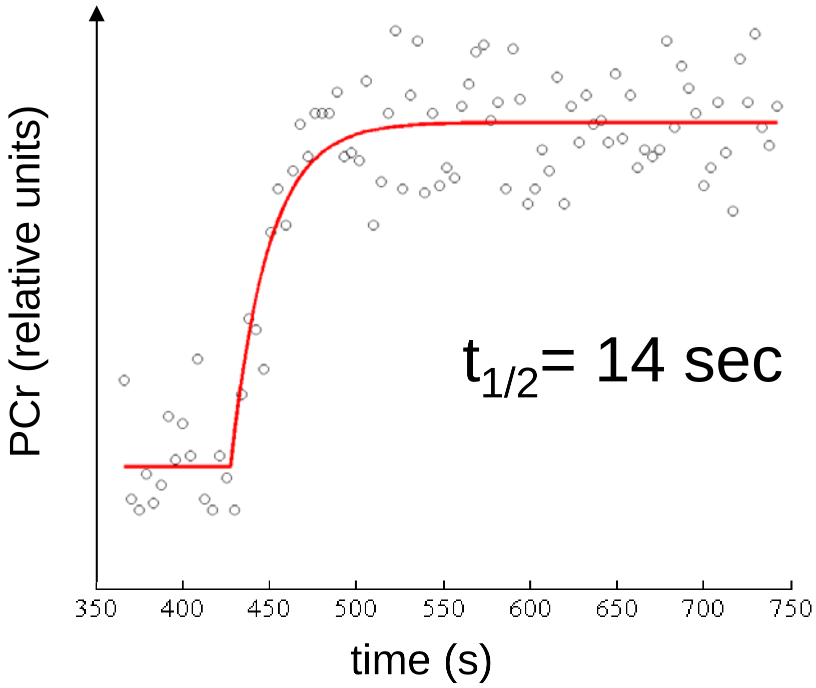
- Monoexponential curve for PCr recovery:
- $PCr(t) = [PCr]_{endex} + D * [1-e^{-(k * t)}]$

  k = timeconstant D = [PCr]rest- [PCr]endex
- Low k → slow repletion
- Time constant k (and $t_{1/2} = 0.693/k$); independent of work or force, used as indicator of mitochondrial function
- Prevent exercise-induced pH differences

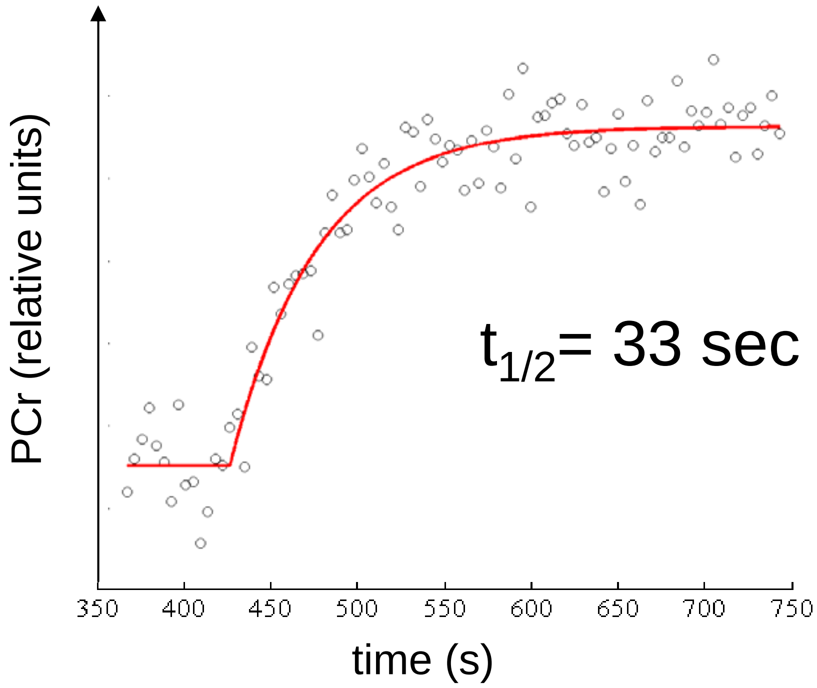
NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate

fast recovery
‘good’ mito function

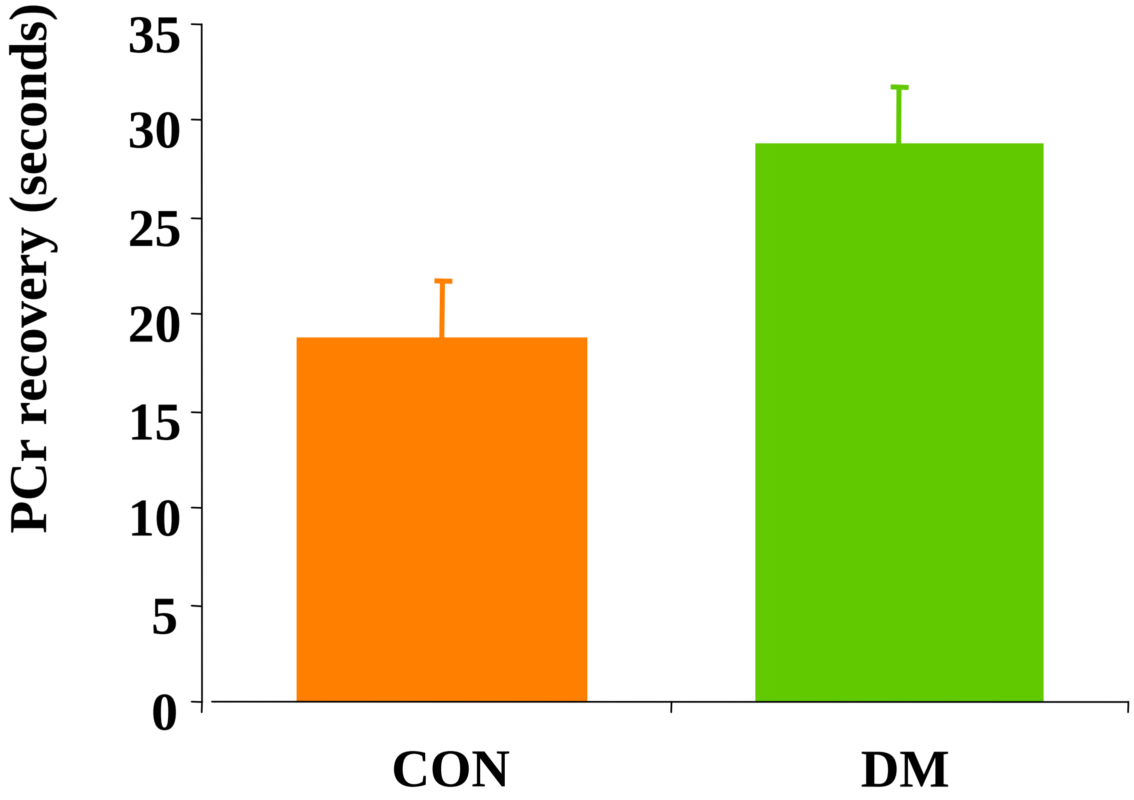


slow recovery
‘bad’ mito function



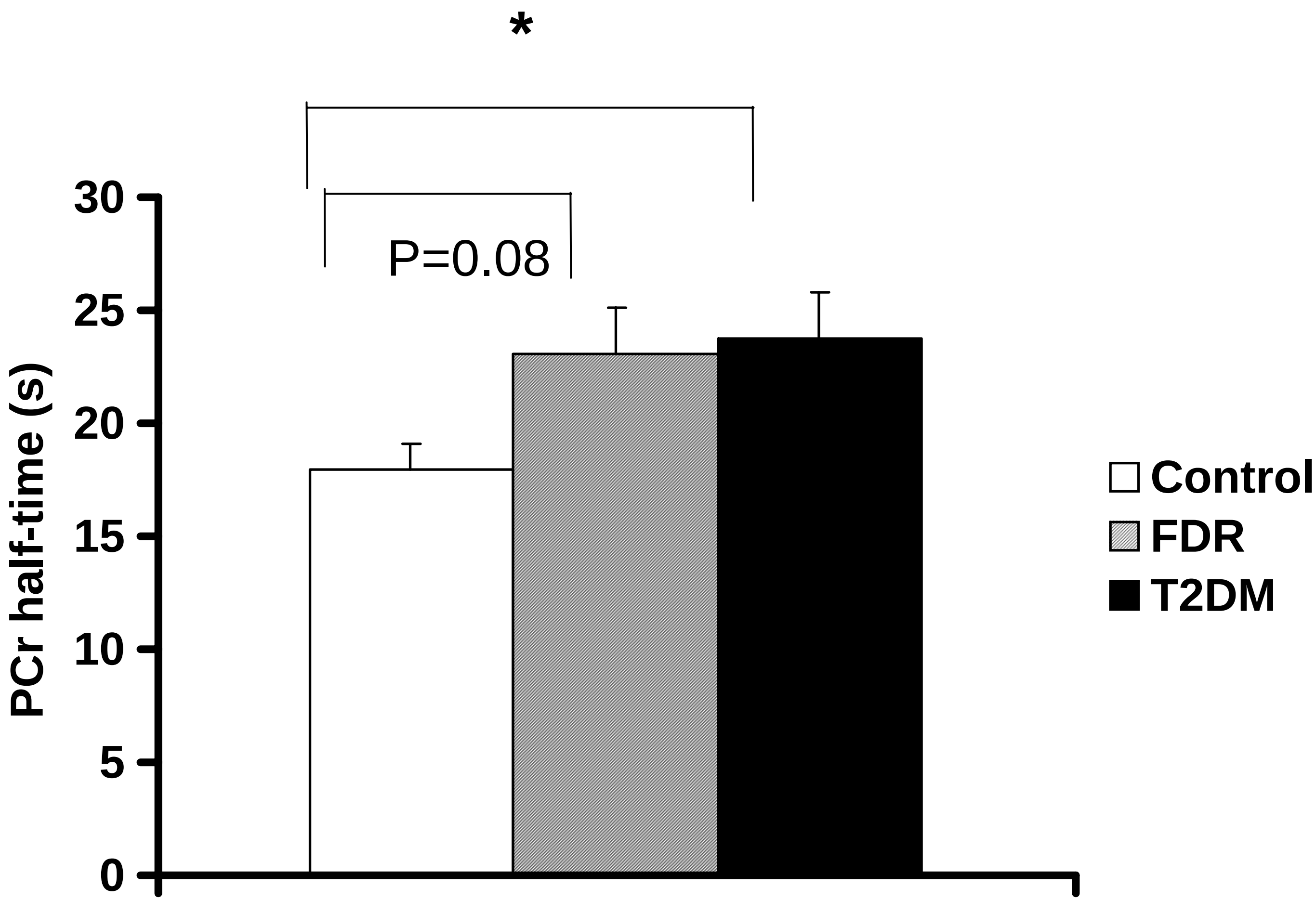
Schrauwen-Hiderling et al., Diabetologia 2007

In vivo mitochondrial function reduced in T2DM



Schrauwen-Hinderling et al., Diabetologia 2007

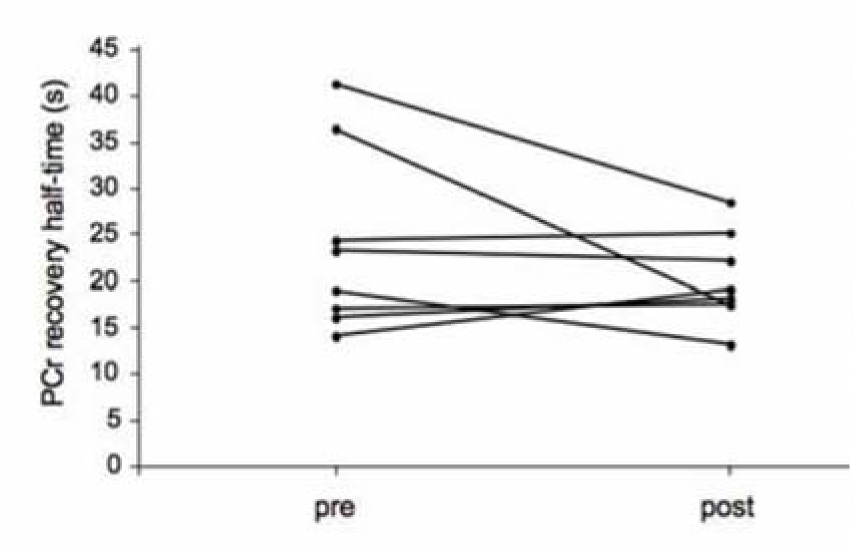
Lower *in vivo* mitochondrial function in T2DM and first-degree relatives

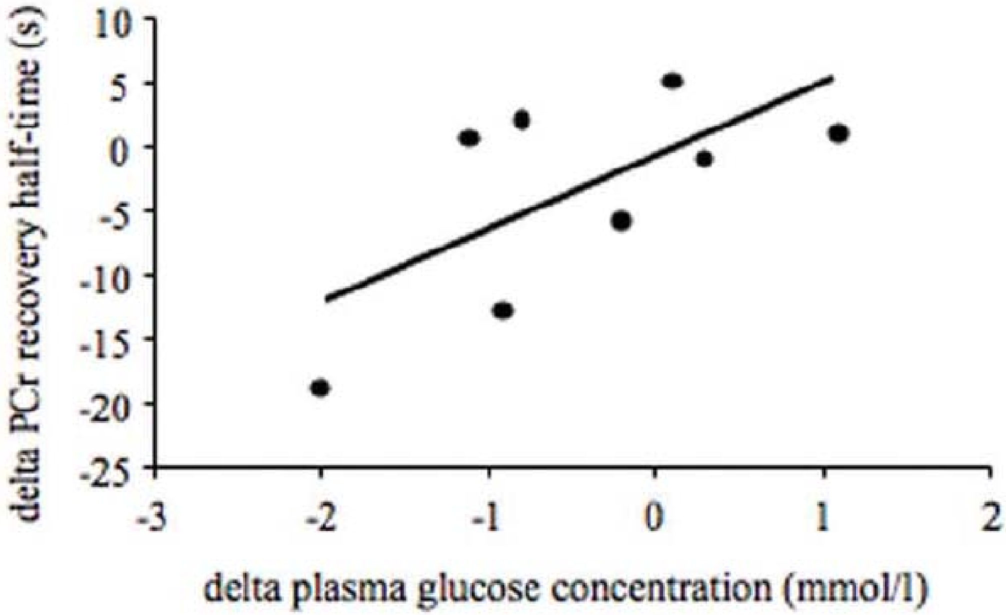


Phielix et al., Diabetes 2008

Insulin sensitizing by rosiglitazone

Effect on in vivo mitochondrial function in T2D

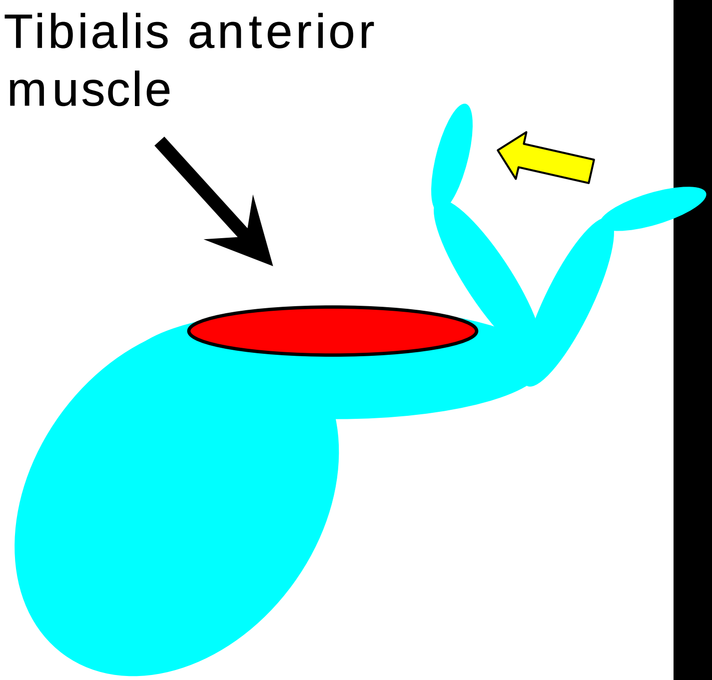


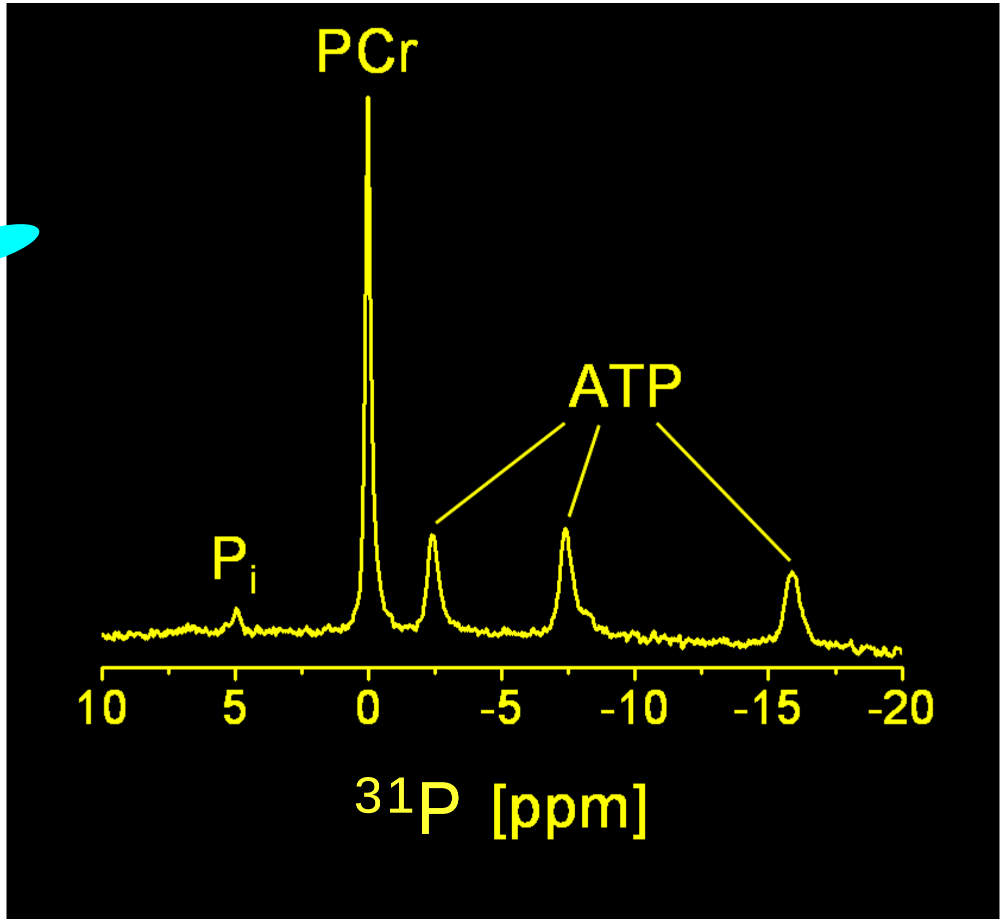


- Rosiglitazone treatment does not improve mitochondrial function
- Delta blood glucose correlates with delta mitochondrial function

NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate (rats)

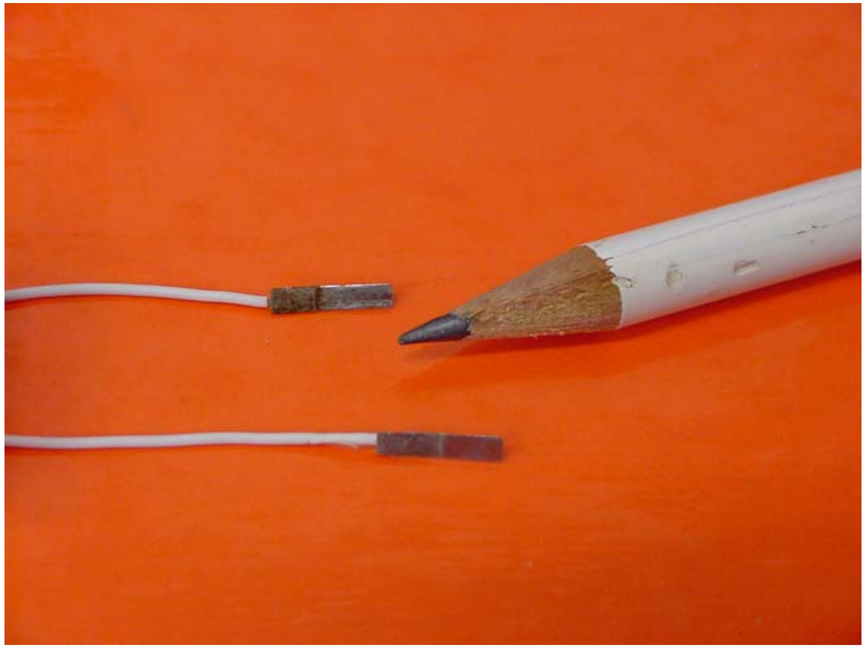


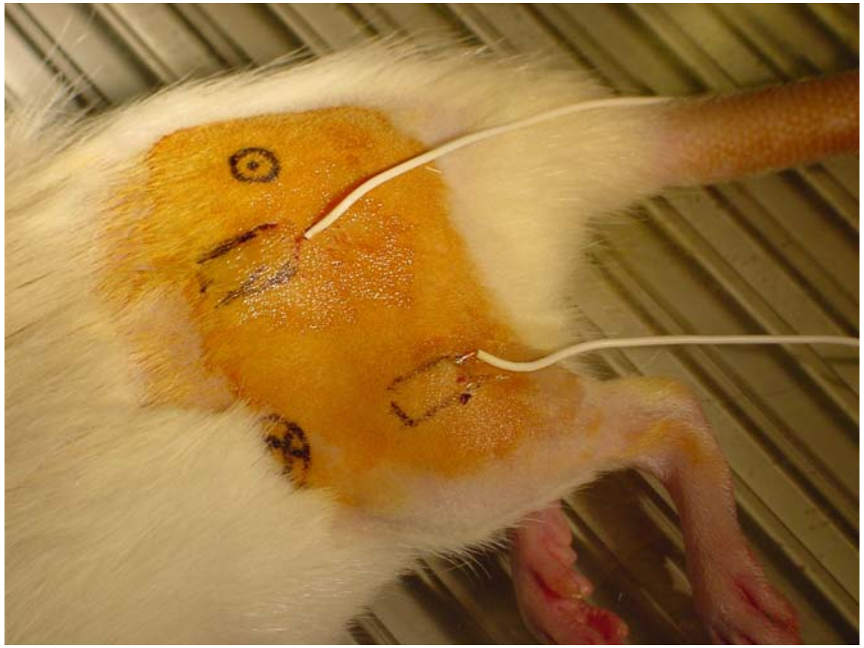


De Feyter et al., FASEB J 2008

NMR-based methods

^{31}P-NMR post-exercise PCr resynthesis rate (rats)





De Feyter et al., FASEB J 2008

NMR-based methods

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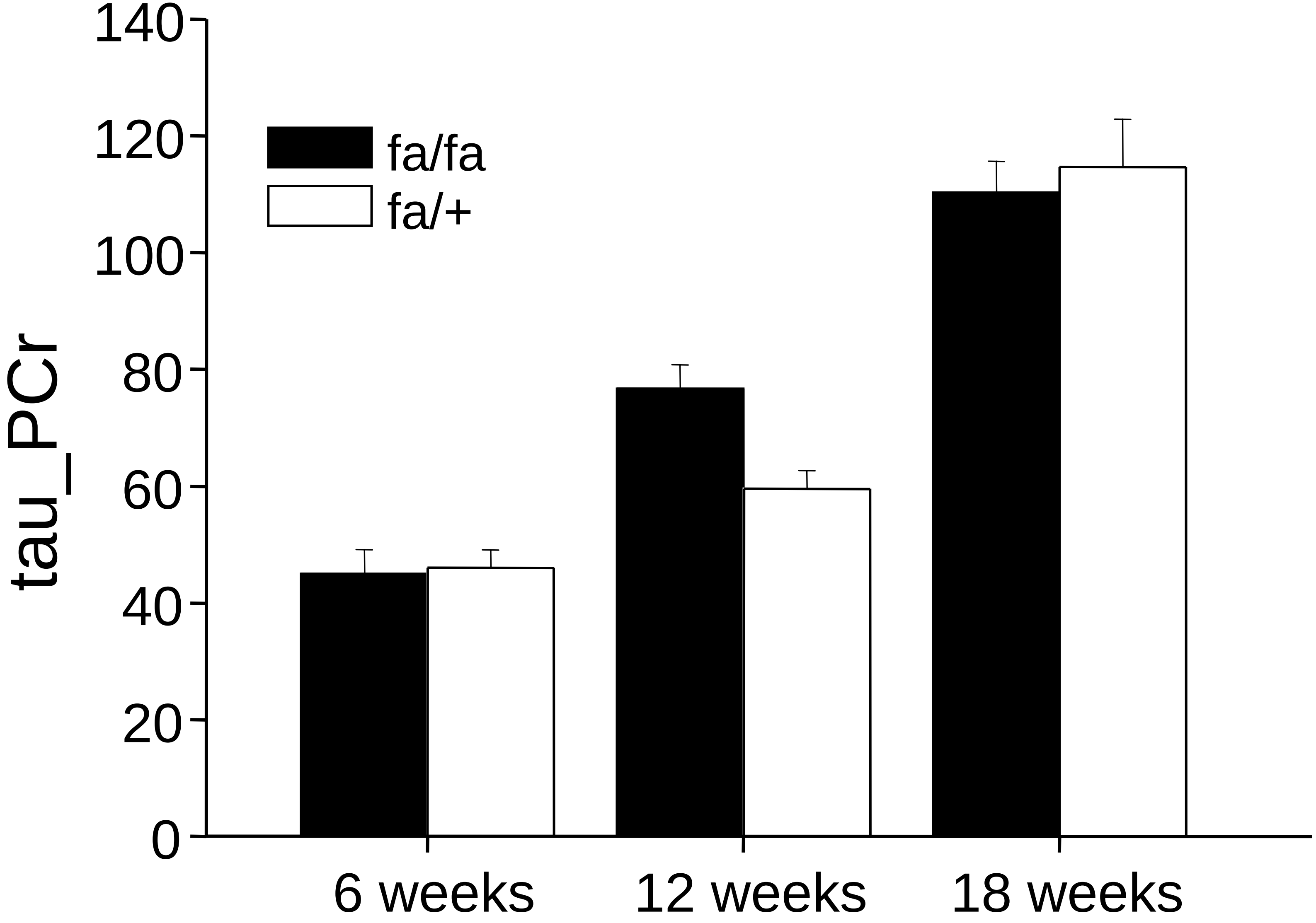

De Feyter et al., FASEB J 2008

Post-exercise pH similar

End exercise pH	6 weeks	12 weeks	18 weeks
fa/fa	6.86	6.98	6.95
fa/+	6.91	7.02	6.96

De Feyter et al., FASEB J 2008

PET scanning/thermal recorders

- PET allows measuring substrate uptake in cell/tissue regions
- Thermal recording reflects metabolic activity
- Combined PET and thermal recording allows examining metabolic consequences of activation of brown adipose tissue, also in humans

- Cold exposing humans activates subscapular BAT in humans and enhances F-DOG uptake (under review)

Thanks to…

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