

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
 - % O₂
 - % CO₂
 - T
 - P
 - Humidity
- Measuring exhaled air conditions
 - % O₂
 - % CO₂
 - *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 O₂ uptake and CO₂ production

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

So, for example if $V(e) = 24.5$ liters/min

$$FeO_2 = 0.1602$$

$$FiO_2 = 0.2093 \text{ (20.93\%)}$$

$$VO_2 = 24.5 \times (0.2093 - 0.1602) = 1.20 \text{ liters of O}_2 \text{ per minute}$$

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

So, for example if $V(e) = 24.5$ liters/min

$$FeCO_2 = 0.0388$$

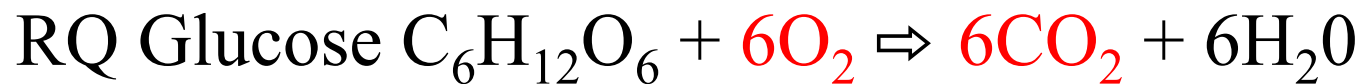
$$FiCO_2 = 0.0003 \text{ (0.03\%)}$$

$$VCO_2 = 24.5 \times (0.0388 - 0.0003) = 0.94 \text{ liters of CO}_2 \text{ per minute}$$

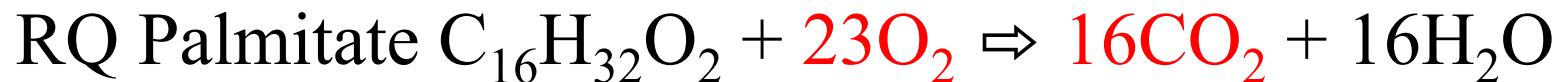
Methods based on breath gas analyses post-processing the data

- Compute Respiratory Exchange Ratio (RER)
≈ Respiratory Quotient (RQ)

$$VCO_2/VO_2$$



$$RQ = 6/6 = 1.00$$



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

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

$$\text{CHO oxidation} = 4,585 \cdot \text{VCO}_2 - 3,226 \cdot \text{VO}_2$$

$$\text{Fat oxidation} = 1,695 \cdot \text{VCO}_2 - 1,701 \cdot \text{VO}_2$$

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.

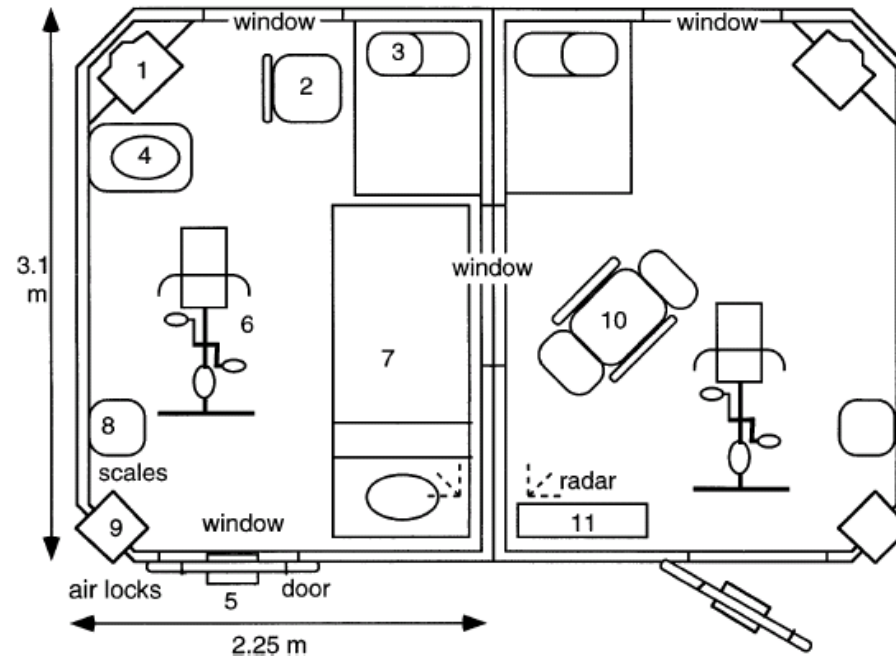
Breath gas analyses mouth piece



- 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

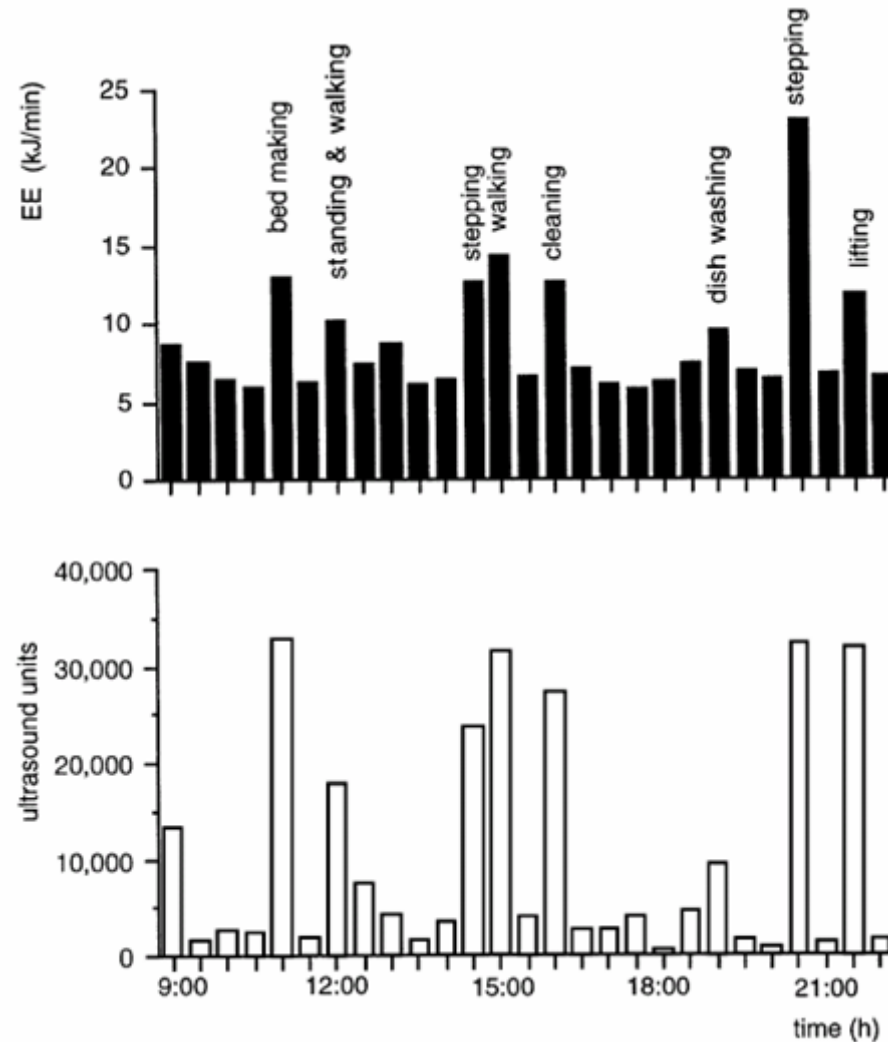
Breath gas analyses

Respiration chamber

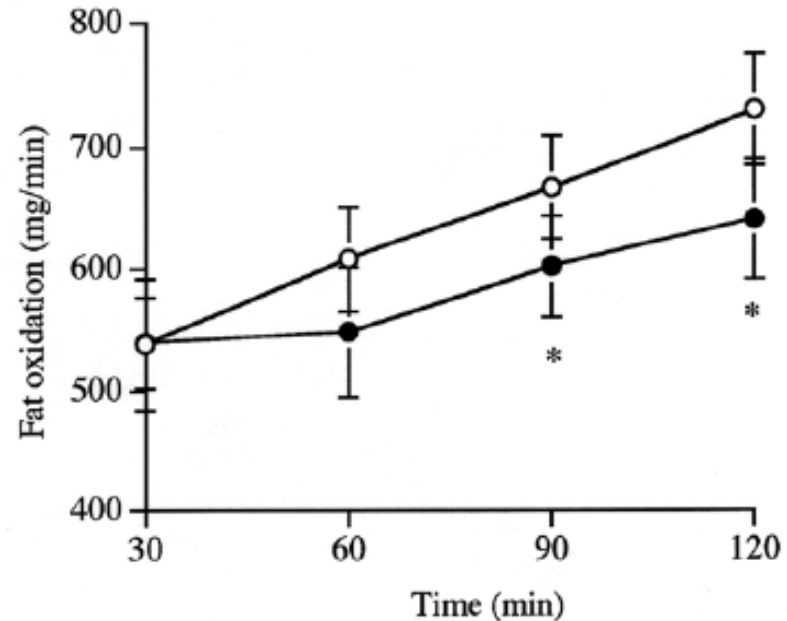
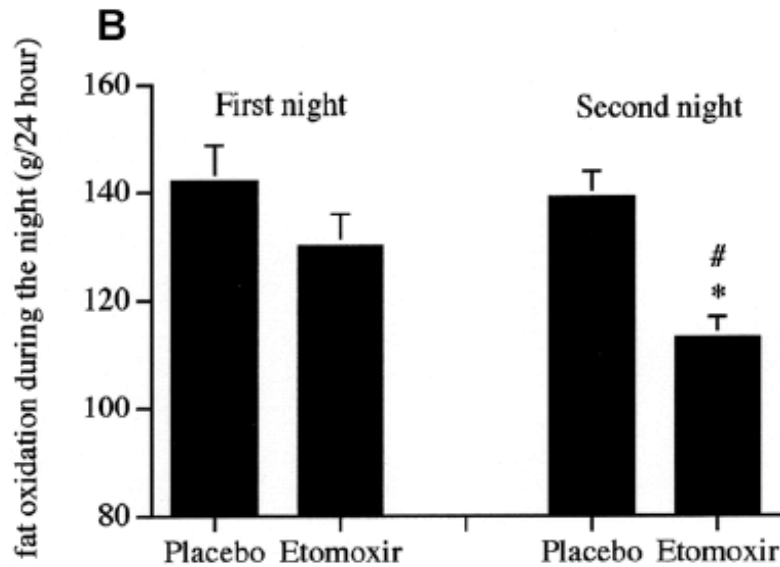


- Flow through chamber pre-set, but adjustable
- No measures of ventilatory rate and volume
- Suitable for assessing RMR & SMR as well as exercise with very accurate measures of substrate selection
- All other conditions (diet, mobility, faeces, urine samples) controlled or collected

Respiration chamber to measure energy expenditure



Respiration chamber to measure substrate selection



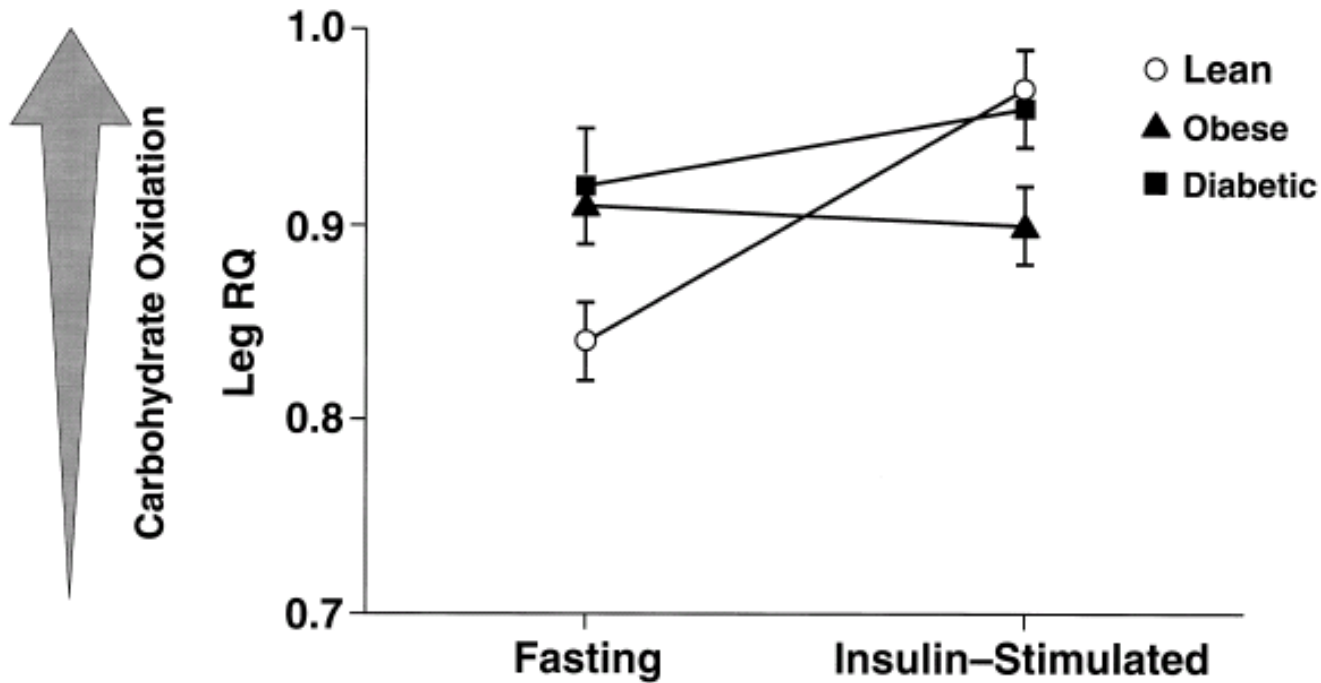
- Etomoxir blocks CPT1 (mitochondrial entry of long chain fatty acids)
- 36 Hours stay + exercise in the chamber (only water provided, black circles etomoxir),

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'



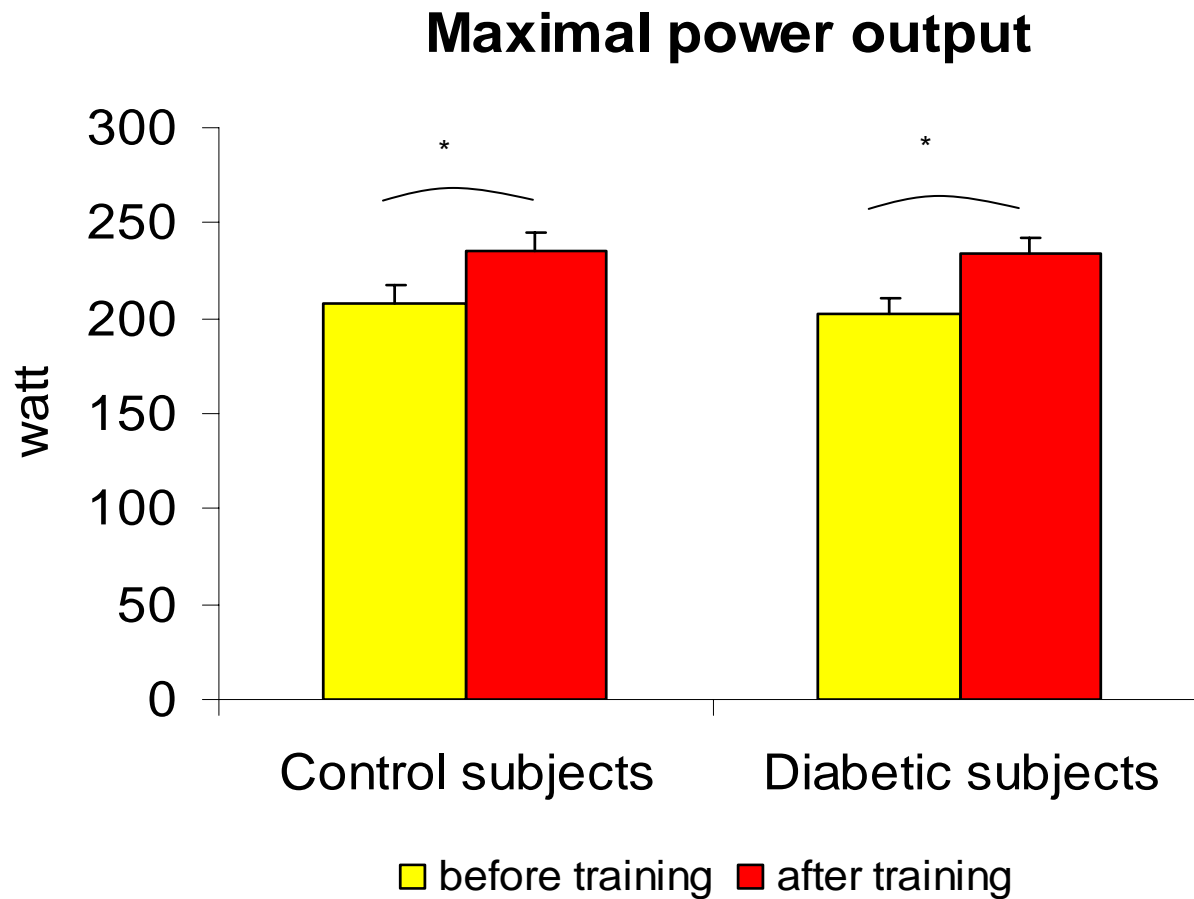
- Insulin-mediated increase in CHO oxidation blunted in obese and T2D
- Leg RQ extended to whole body
- Mitochondrial phenomenon?

Ventilated hood based metabolic flexibility

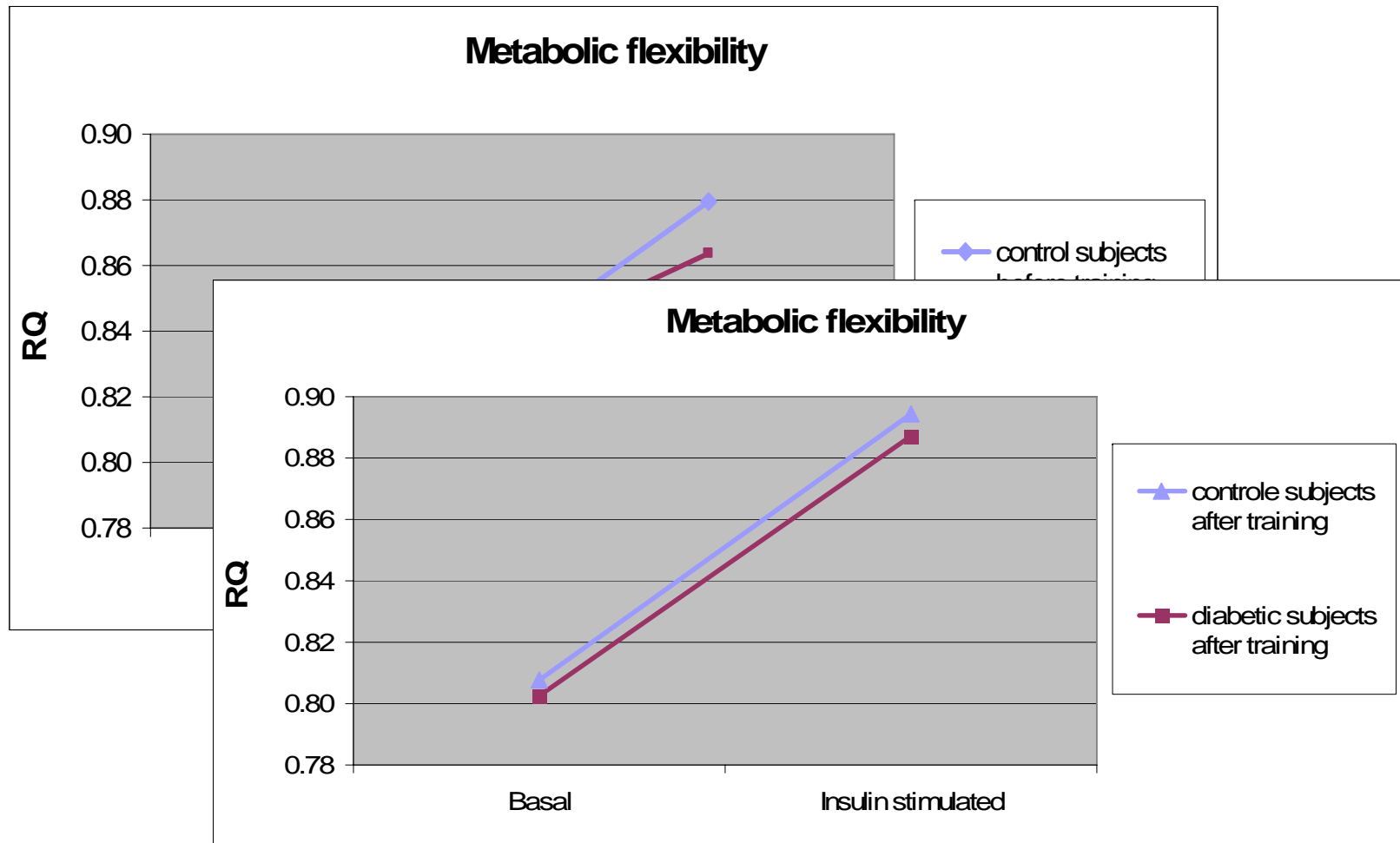
effect of exercise training T2D

	Controls	T2D
	(n = 20)	(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

Ventilated hood based metabolic flexibility effect of exercise training T2D

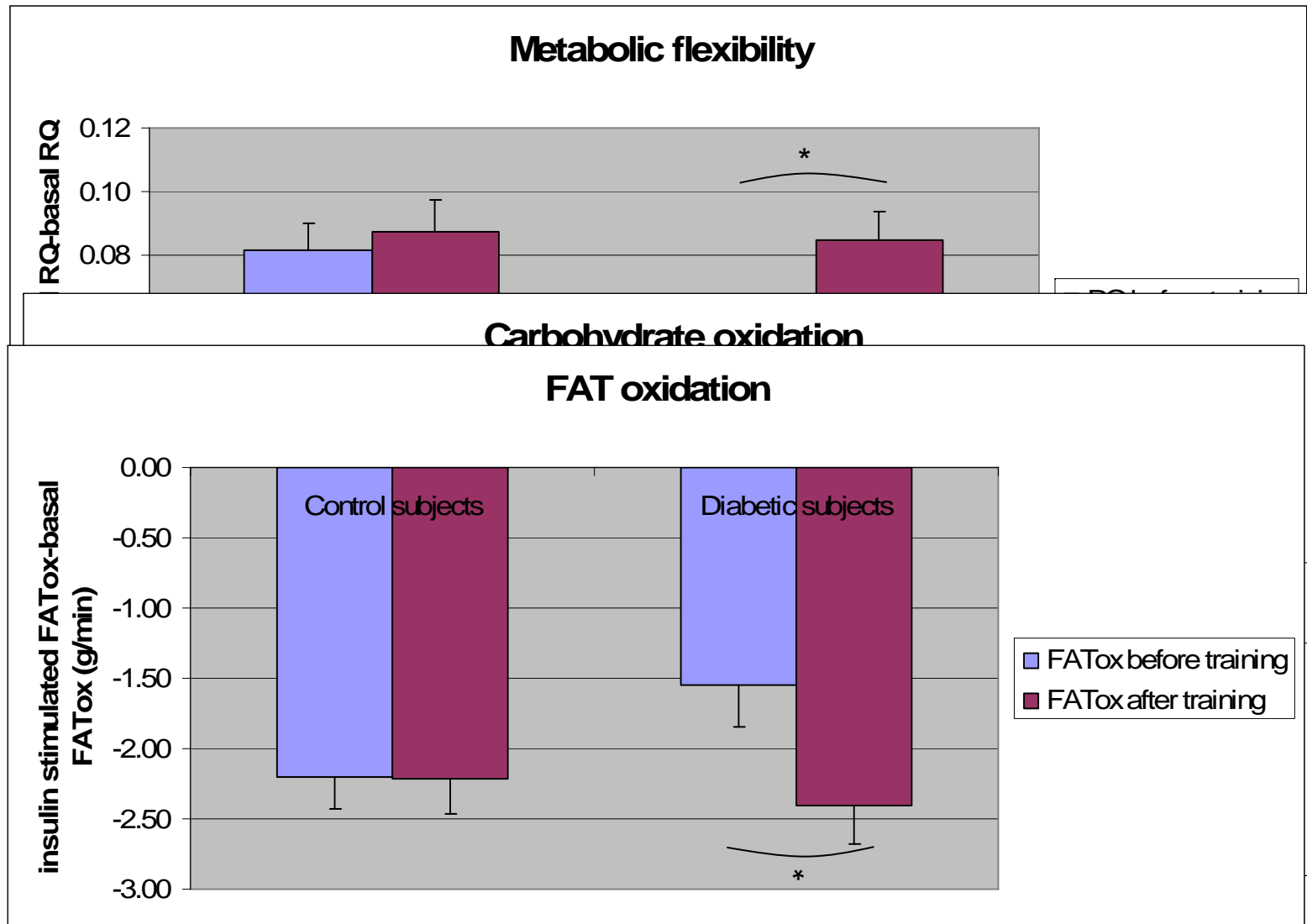


Ventilated hood based metabolic flexibility effect of exercise training T2D



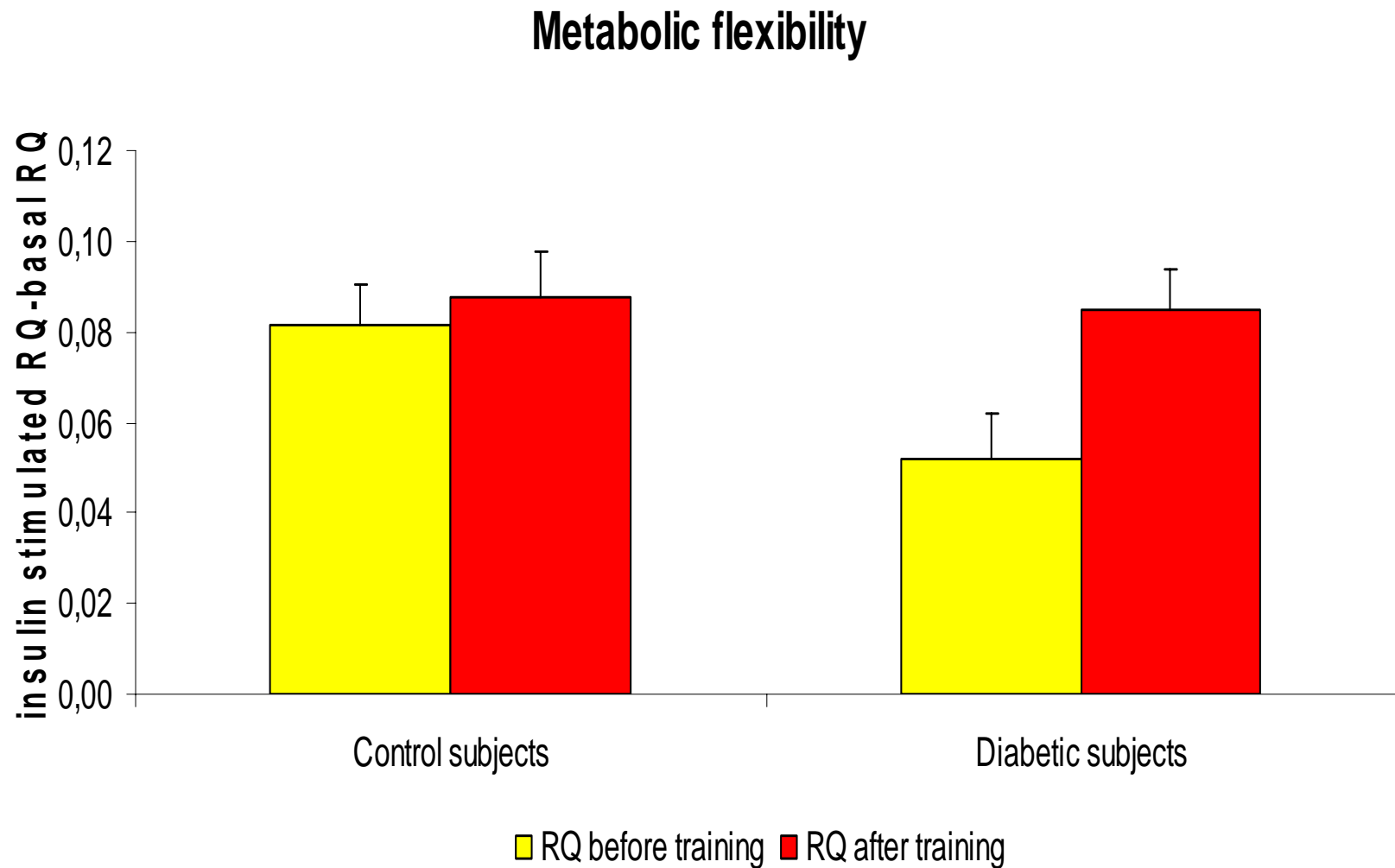
Meex et al., in progress

Ventilated hood based metabolic flexibility effect of exercise training T2D



Q: Does metabolic inflexibility reflect insulin resistance?

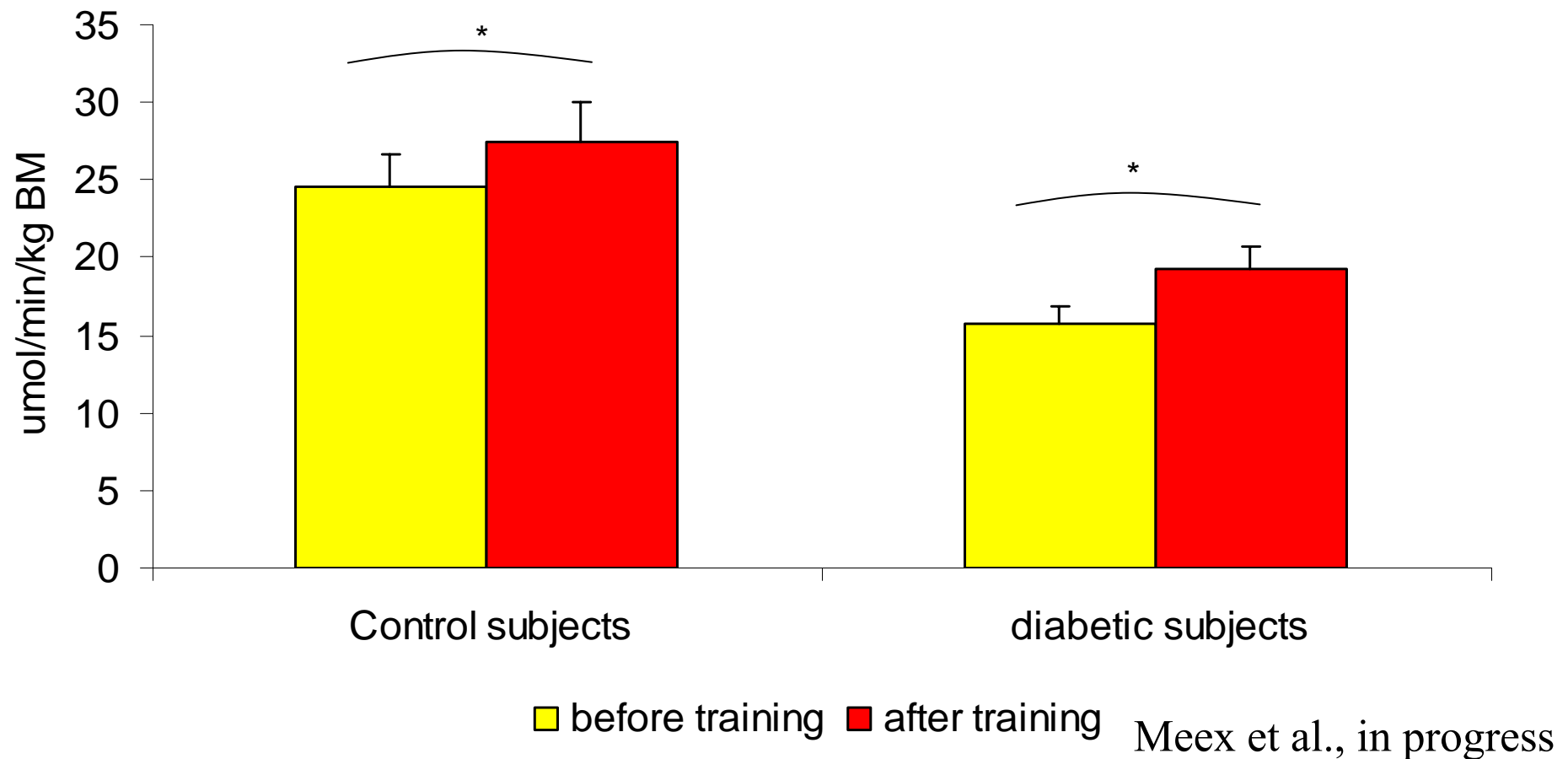
Training T2D restores metabolic flexibility



Meex et al., in progress

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

Glucose infusion rate



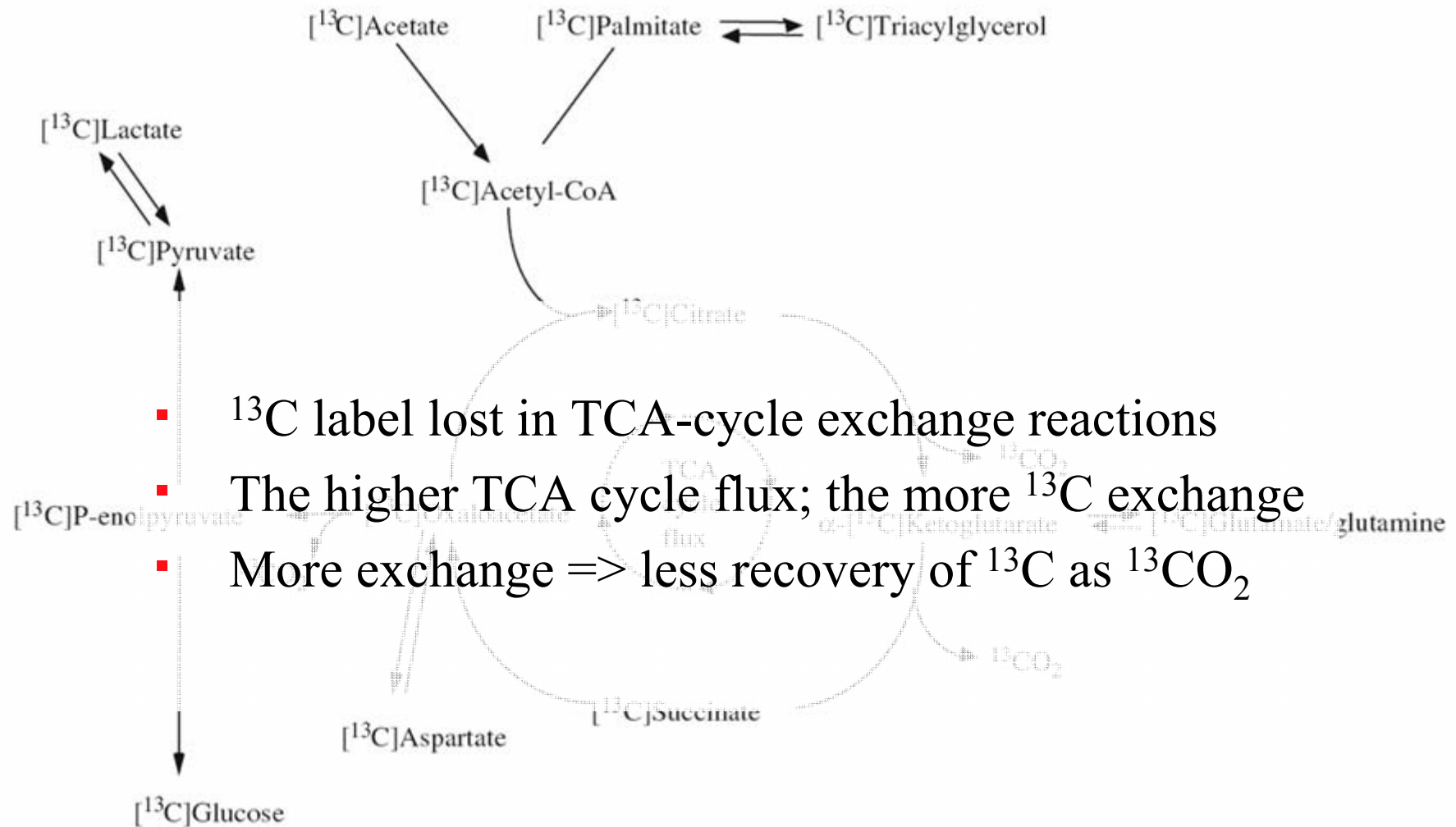
Q: Do the many faces of insulin require the same concentration of insulin?

Faces of insulin and concentrations ($\mu\text{U}/\text{ml}$) for computed $\frac{1}{2}$ 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

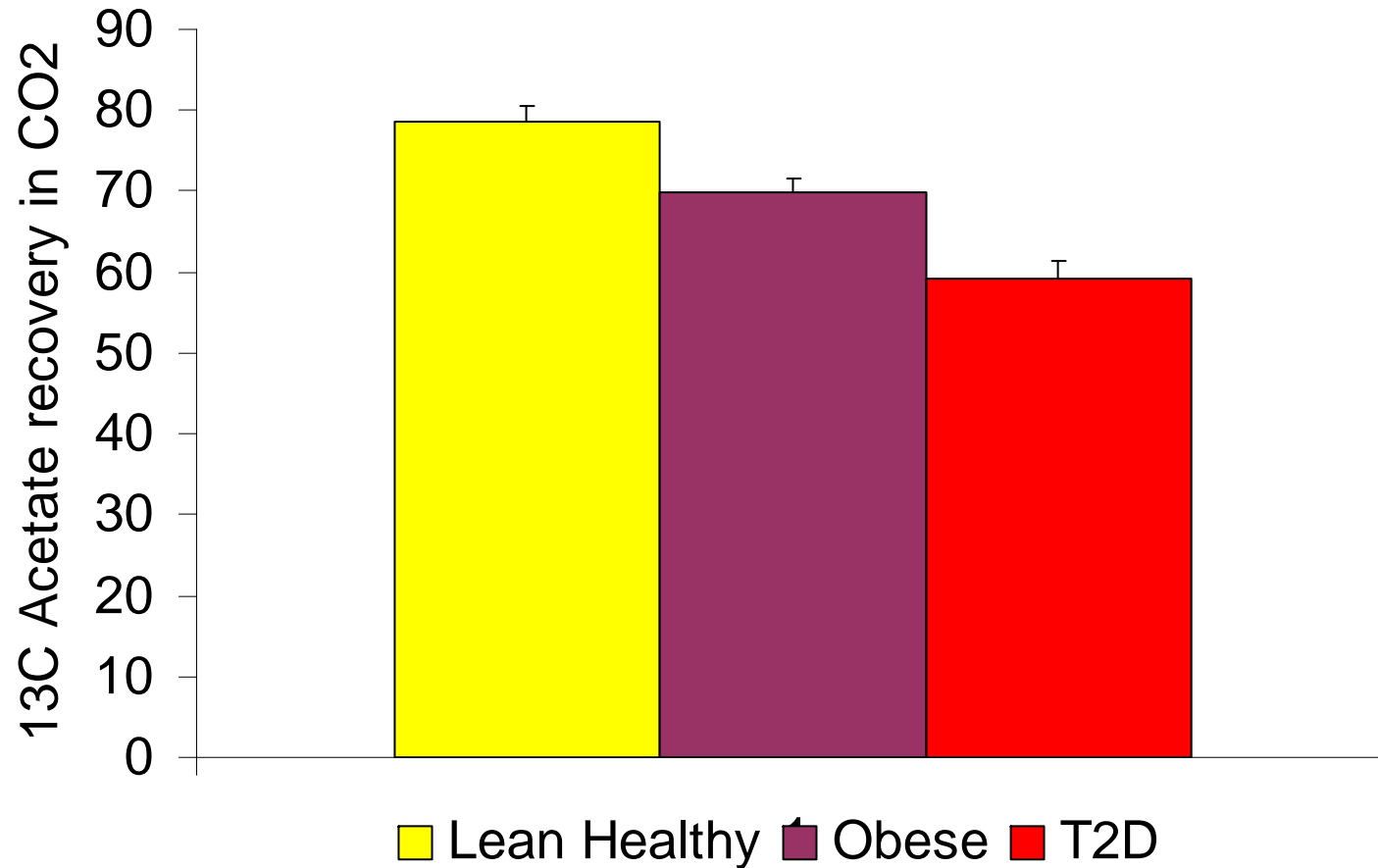
1,2-¹³C acetate to measure TCA cycle activity

breath gas analyses after venous infusion

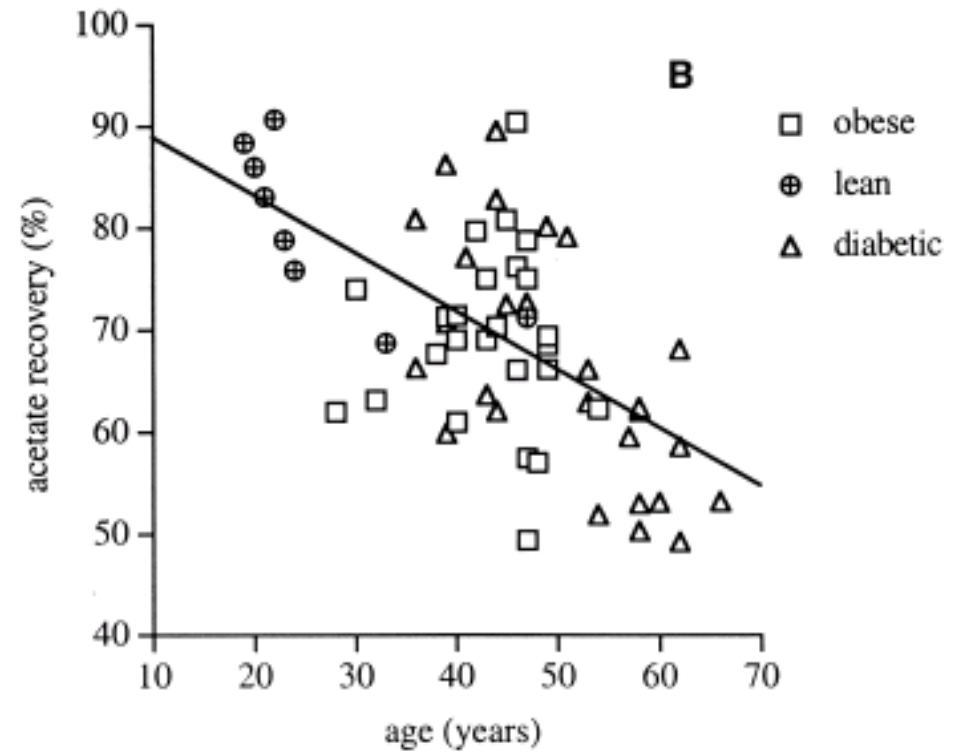
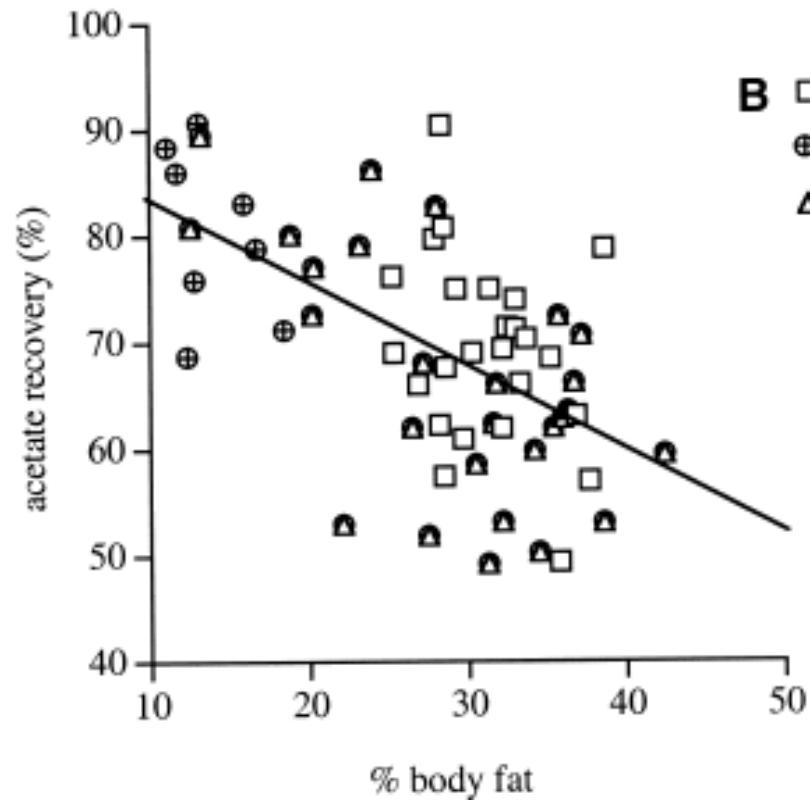


1,2-¹³C acetate to measure TCA cycle activity

breath gas analyses after venous infusion

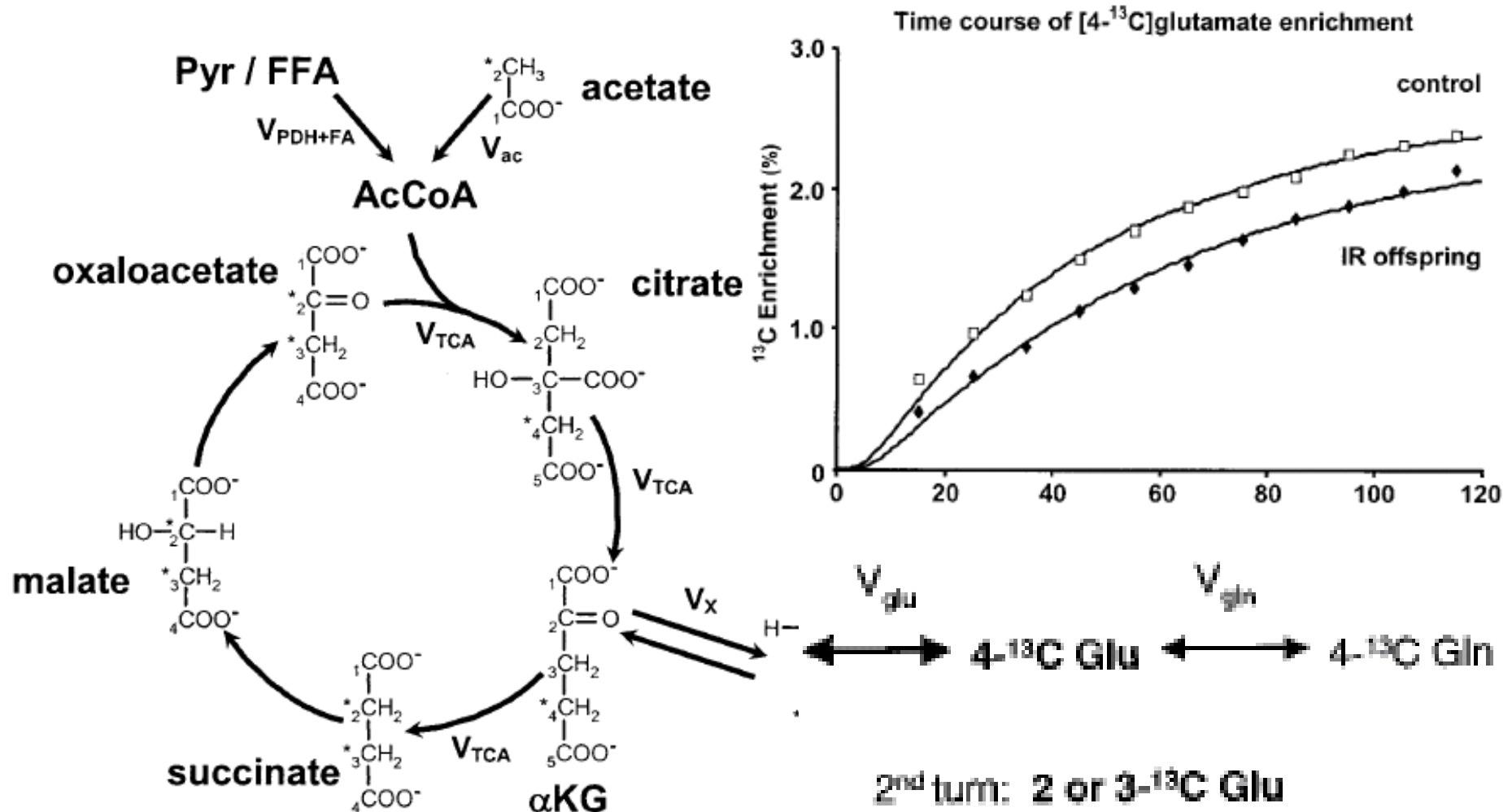


1,2-¹³C acetate lower with incremental body fat and ageing



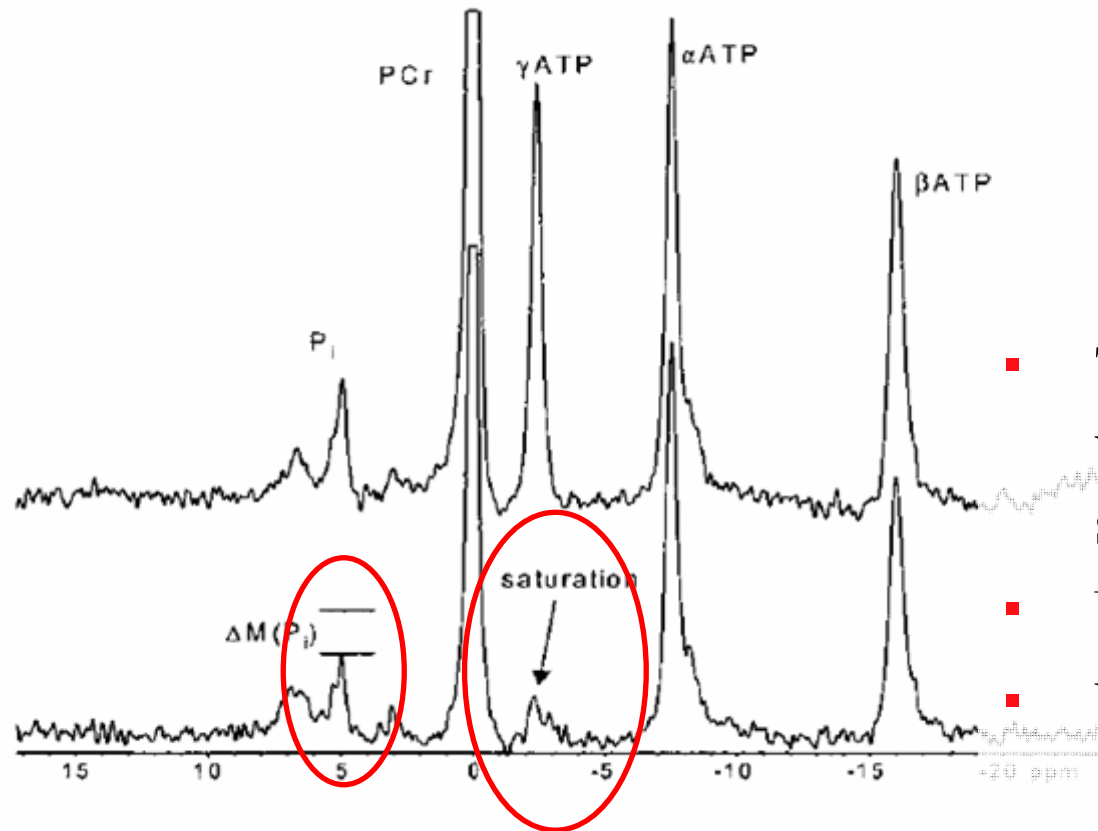
NMR-based methods

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



NMR-based methods

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

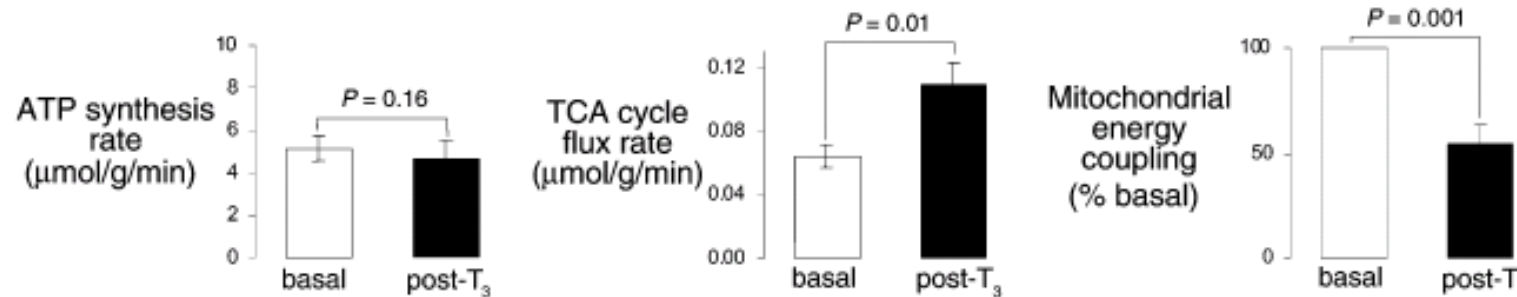


- Transfer of saturated $\gamma\text{-ATP}$, via PCr results in decline in saturated P_i (ΔM)
- Unidirectional
- Very low signal



NMR-based methods

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



Lebon et al., JCI 2001

- T₃ treatment reduces mitochondrial coupling

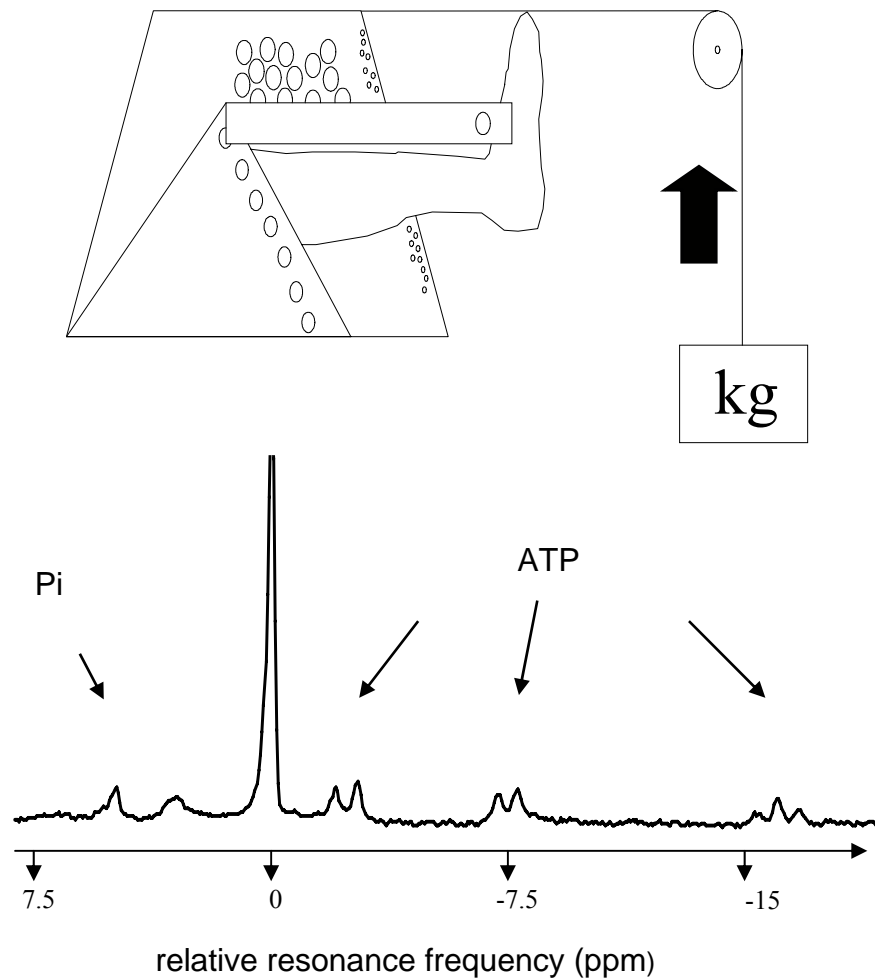
	Basal rates of glucose production (mg/kg of LBM/min)	Clamp peripheral glucose metabolism rate (mg/kg of LBM/min)	Intramyocellular lipid content (%)	Intrahepatic lipid content (%)	Mitochondrial TCA flux rate (nmol/g of muscle/min)	Mitochondrial ATP synthesis rate (μmol/g of muscle/min)
Young	2.3 ± 0.1	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

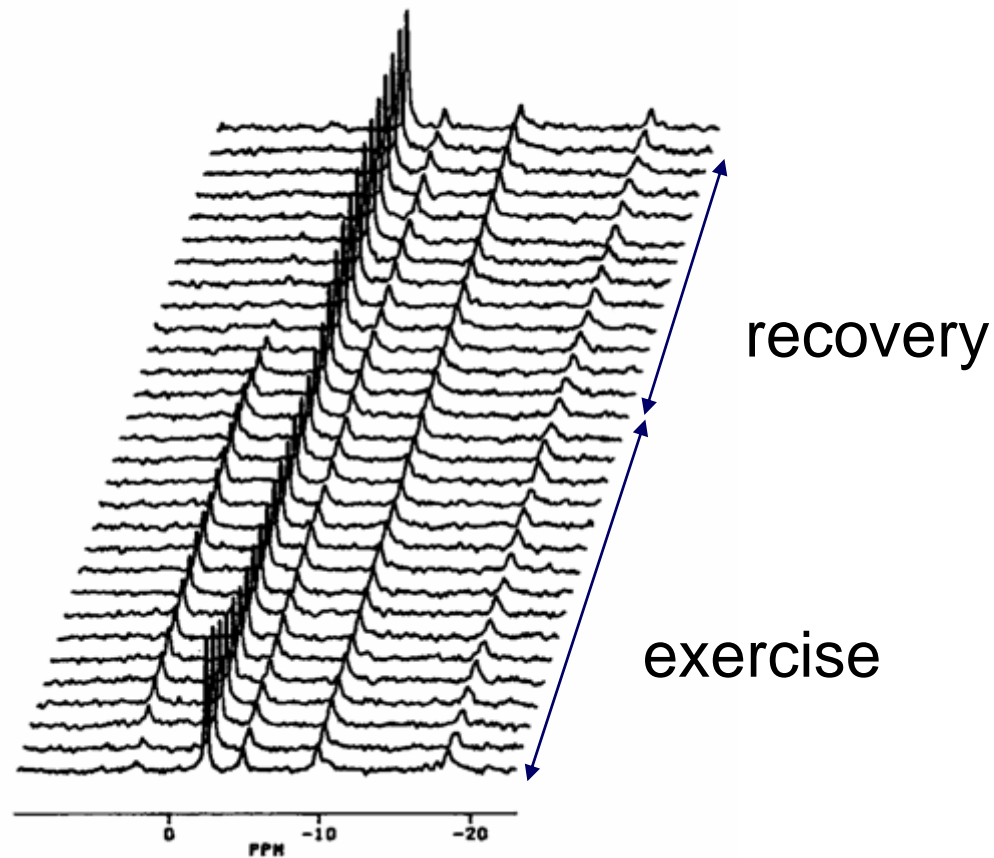
NMR-based methods

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



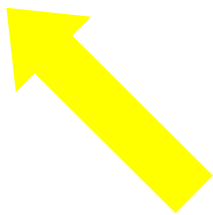
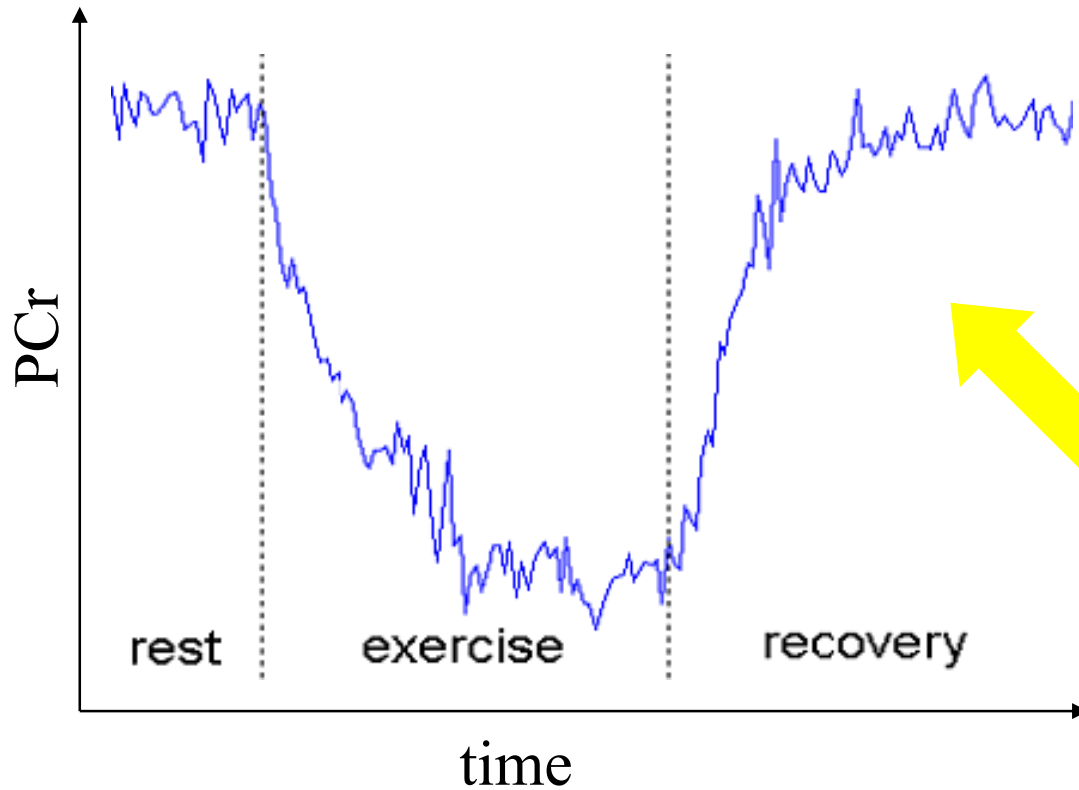
NMR-based methods

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



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

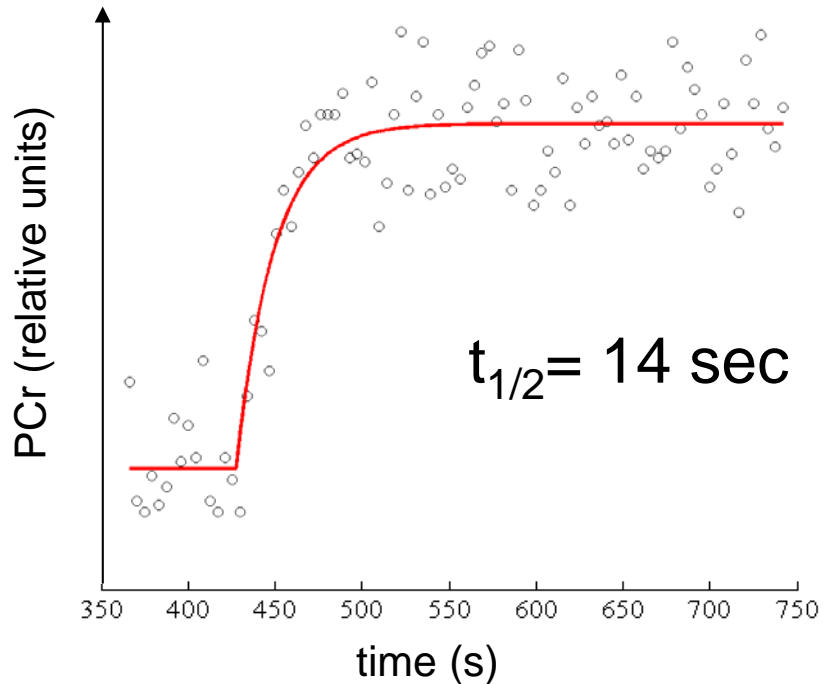
^{31}P -NMR post-exercise PCr resynthesis rate

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

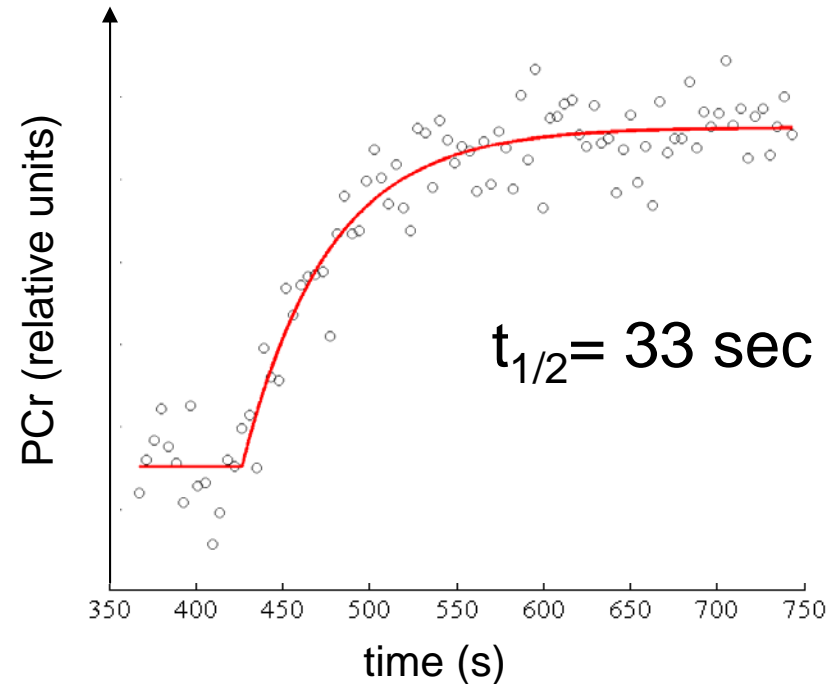
NMR-based methods

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

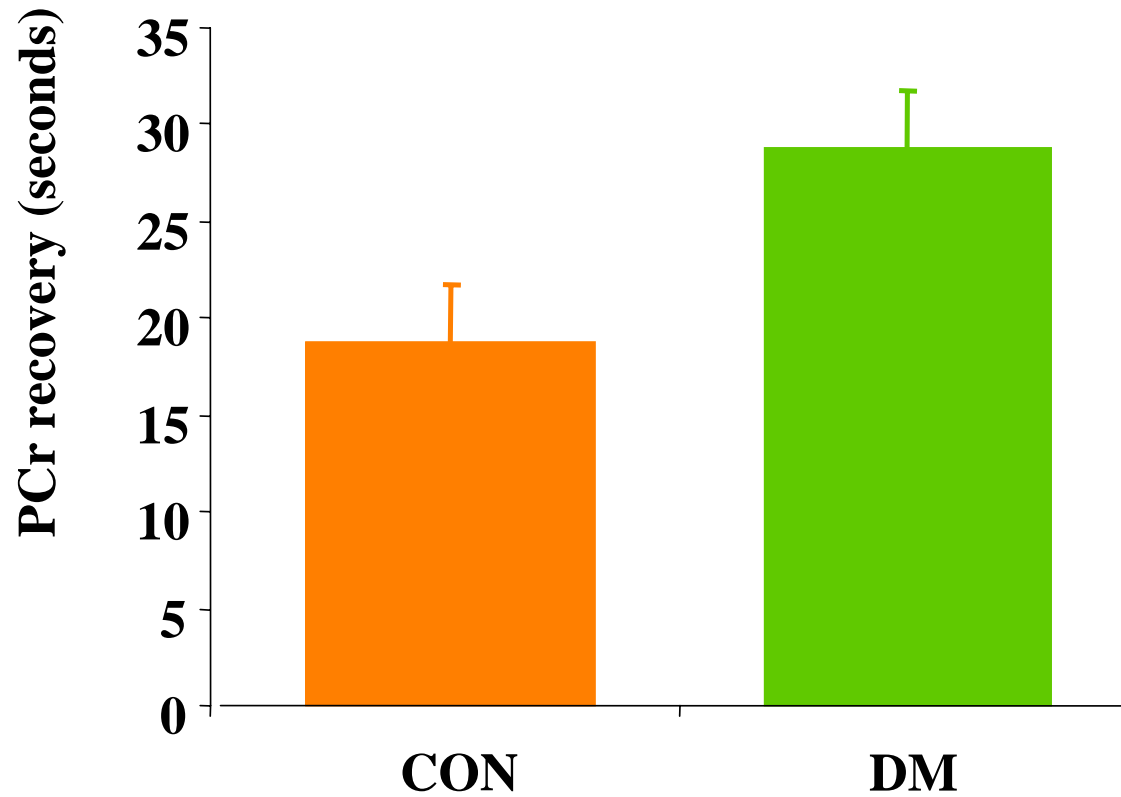
fast recovery
'good' mito function



slow recovery
'bad' mito function

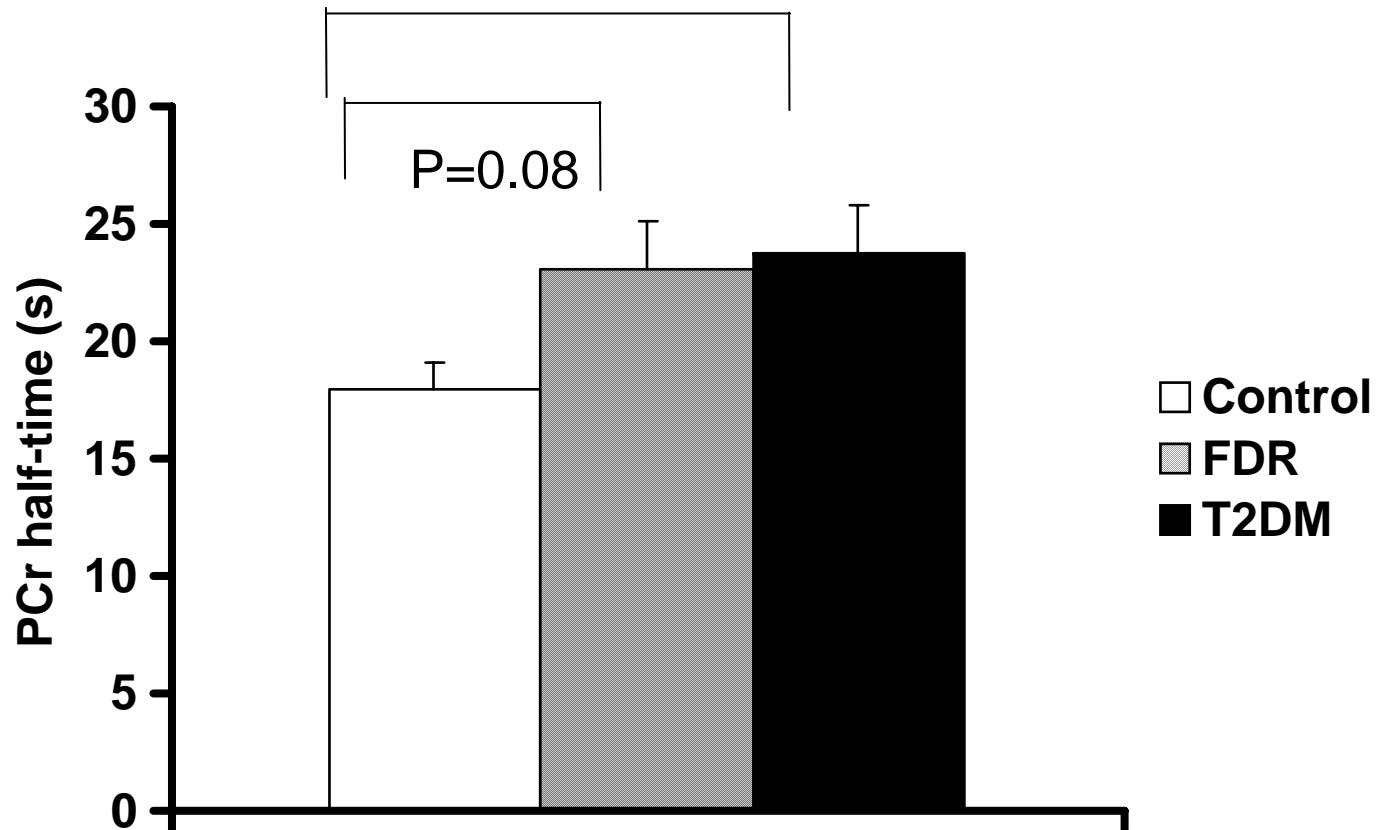


In vivo mitochondrial function reduced in T2DM



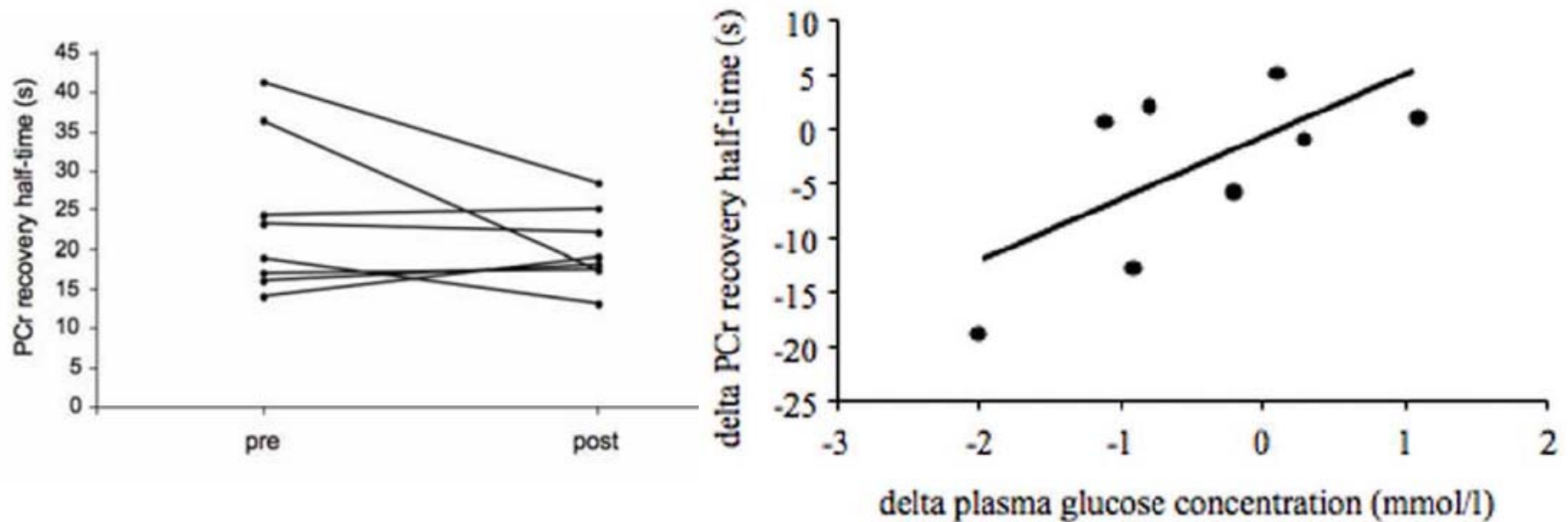
Lower *in vivo* mitochondrial function in T2DM and first-degree relatives

*



Insulin sensitizing by rosiglitazone

Effect on in vivo mitochondrial function in T2D

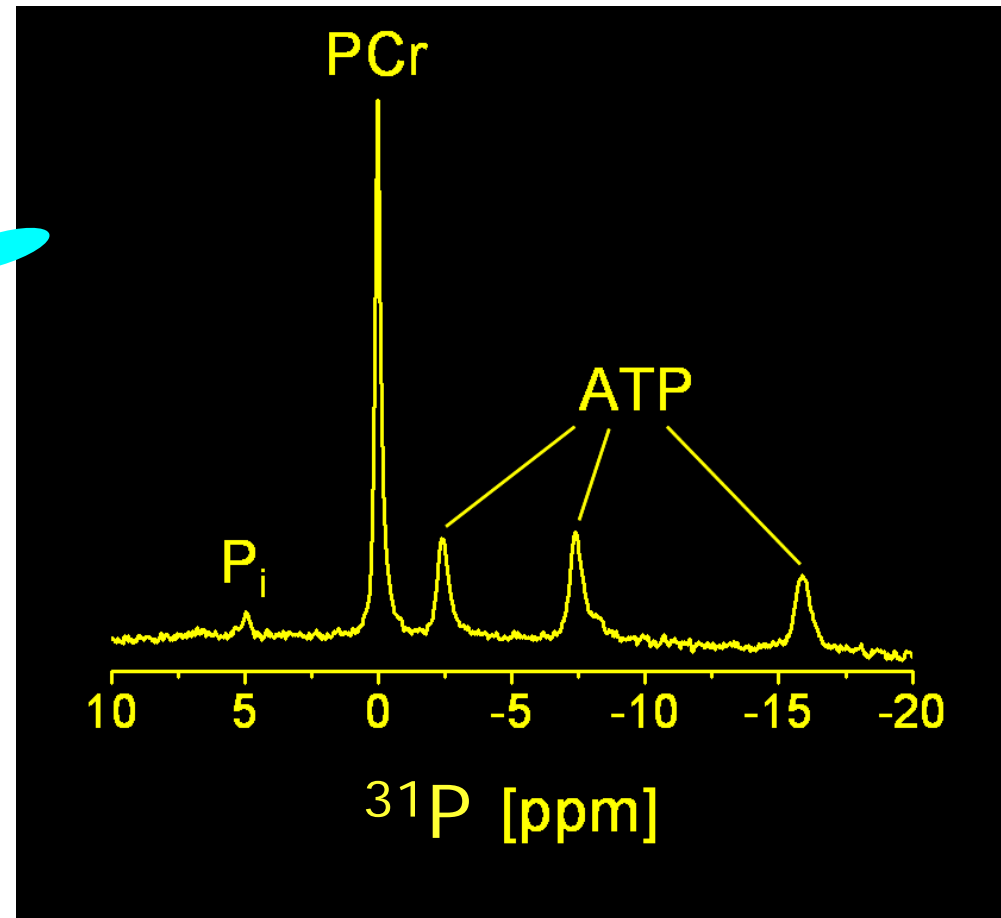
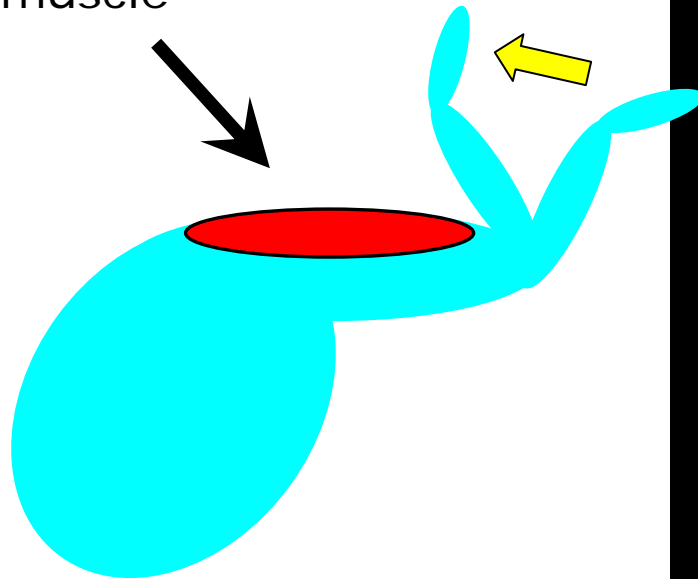


- Rosiglitazone treatment does not improve mitochondrial function
- Delta blood glucose correlates with delta mitochondrial function

NMR-based methods

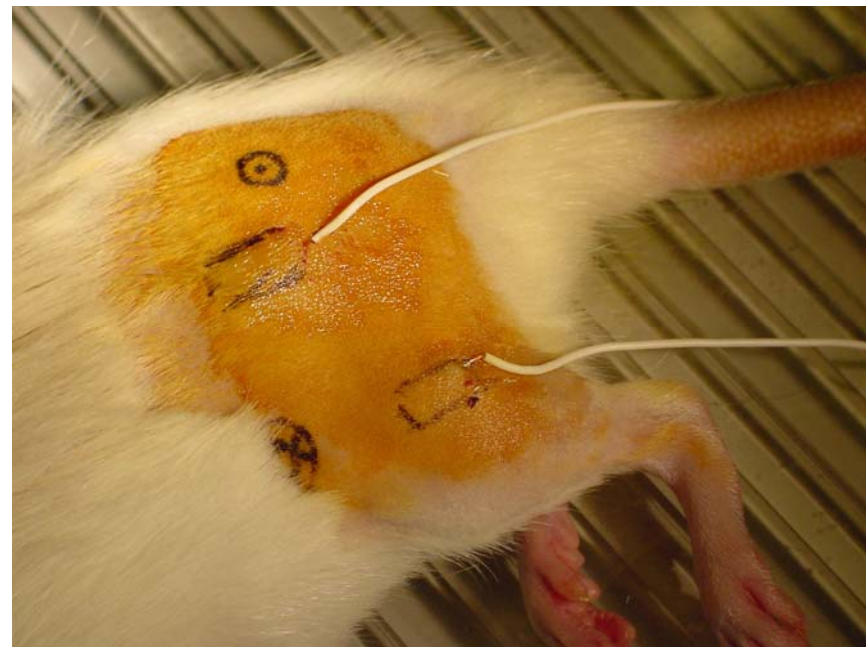
^{31}P -NMR post-exercise PCr resynthesis rate (rats)

Tibialis anterior
muscle



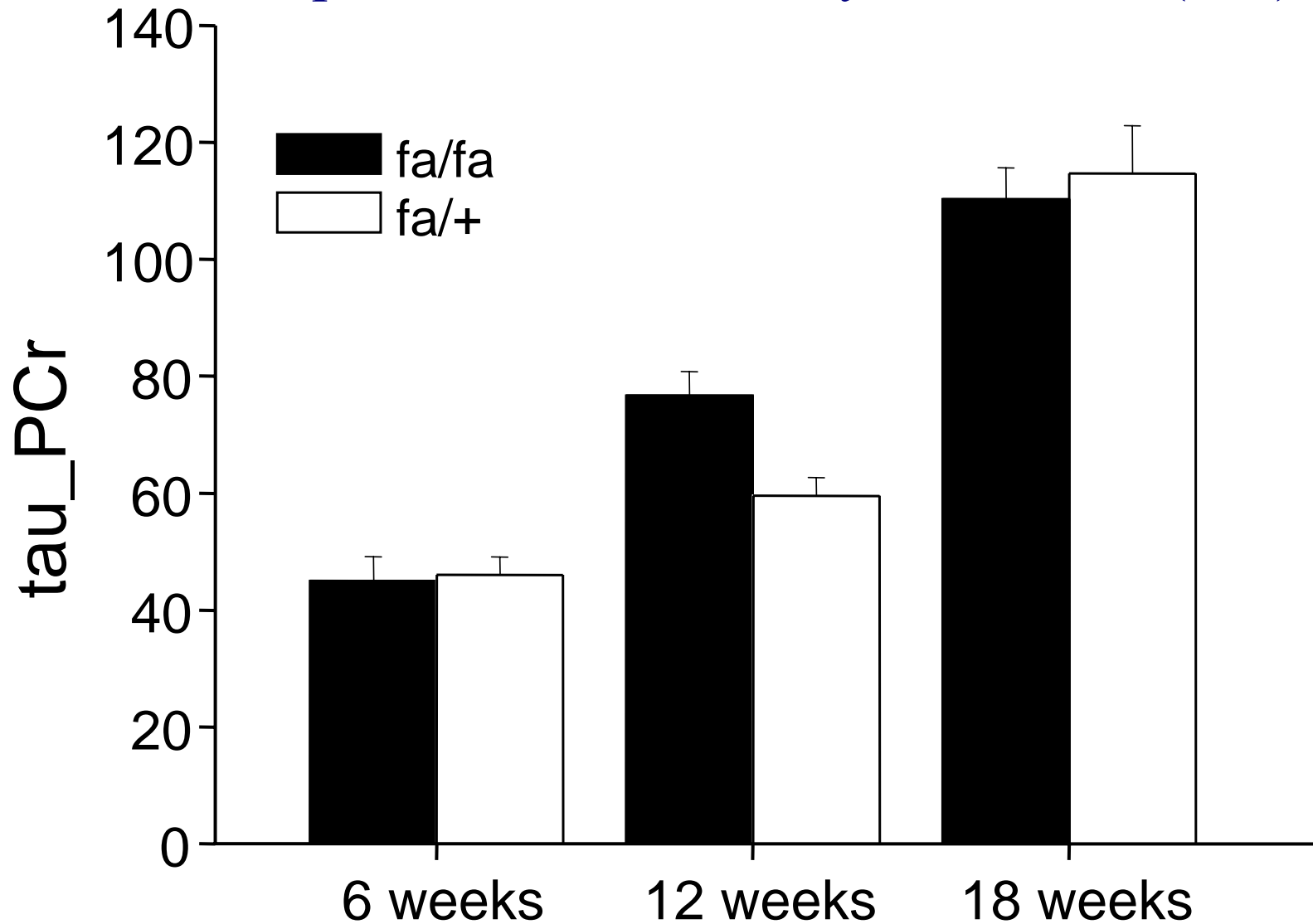
NMR-based methods

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



NMR-based methods

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



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

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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