Impact of ω-3 fatty acids and their combination with physical activity on mitochondrial function and antioxidant content in skeletal muscle and liver

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Background

- The aging process is conditioned by the interactions between genetic and environmental factors
- Skeletal muscle is significantly affected by aging
    - skleletal mucle mass starts to ↓ at 40 years of age
    - body fat mass ↑
    - fat is accumulated within muscle
    - muscle fiber composition is shifted

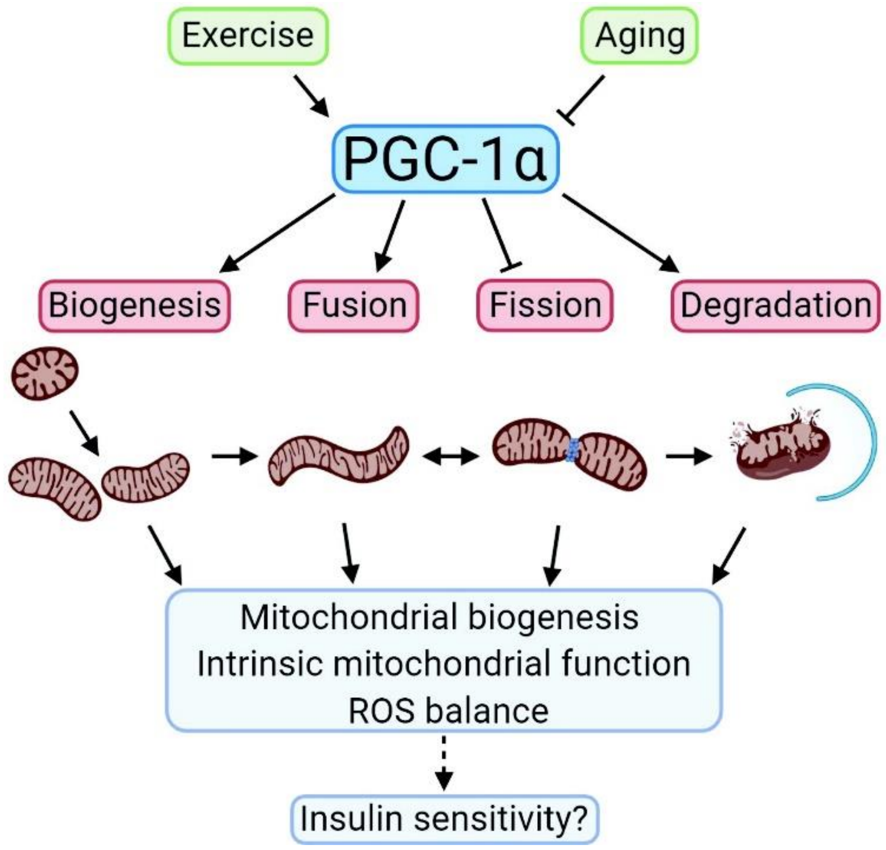
      ↓ in fast-twich glycolytic fibres (Type II)
    - changes in motor neurons

    ⇒ **muscle strenght and functional capacity ↓**
- **In aged skeletal muscle**

  ↑ mitophagy

  ↓ mitochondrial content

Effect of regular exercise on skeletal muscle



Halling JF, Pilegaard H. PGC-1α-mediated regulation of mitochondrial function and physiological implications. Appl Physiol Nutr Metab. 2020 Sep;45(9):927-936. doi: 10.1139/apnm-2020-0005.

Regular exercise

↓ muscle loss
↑ muscle mass and strength
Improves muscle quality and functional capacity

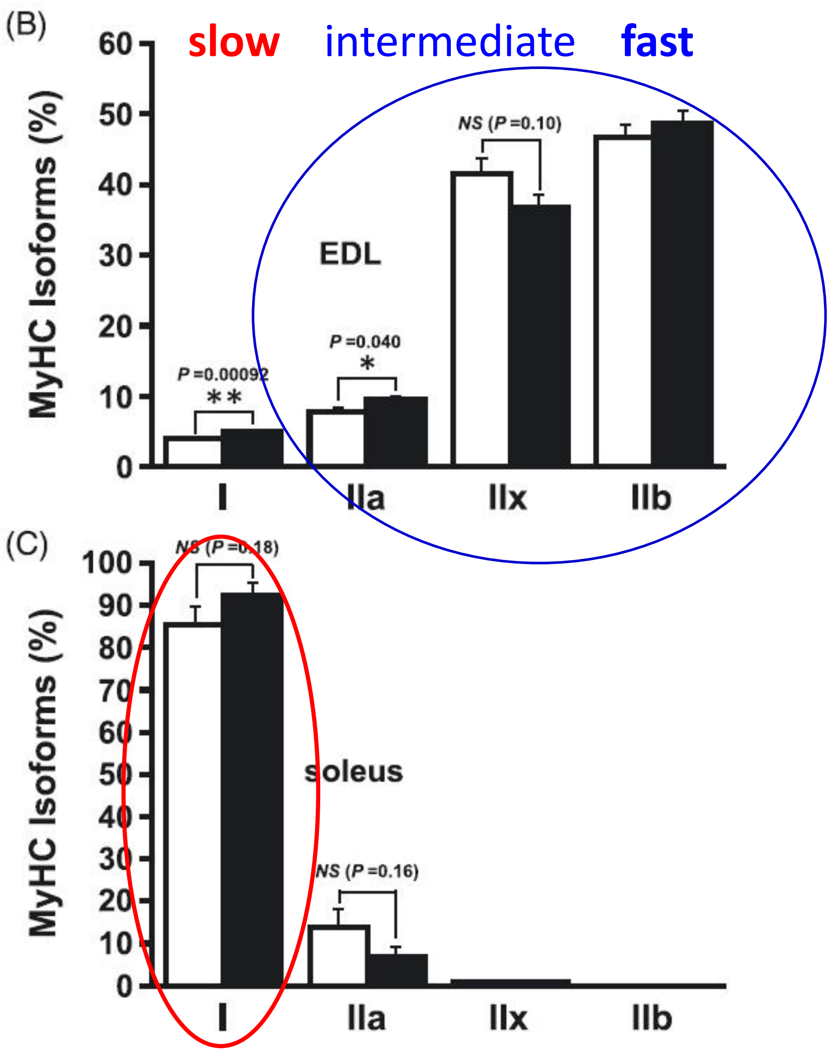
↑ **mitochondrial biogenesis**
↓ mitophagy

The effects of exercise

- smaller in aged animals
- ↑ PGC-1α mRNA **depends on intensity of exercise**

(Peroxisome proliferator-activated receptor γ coactivator 1α) transcriptional coactivator

Muscle fiber type composition



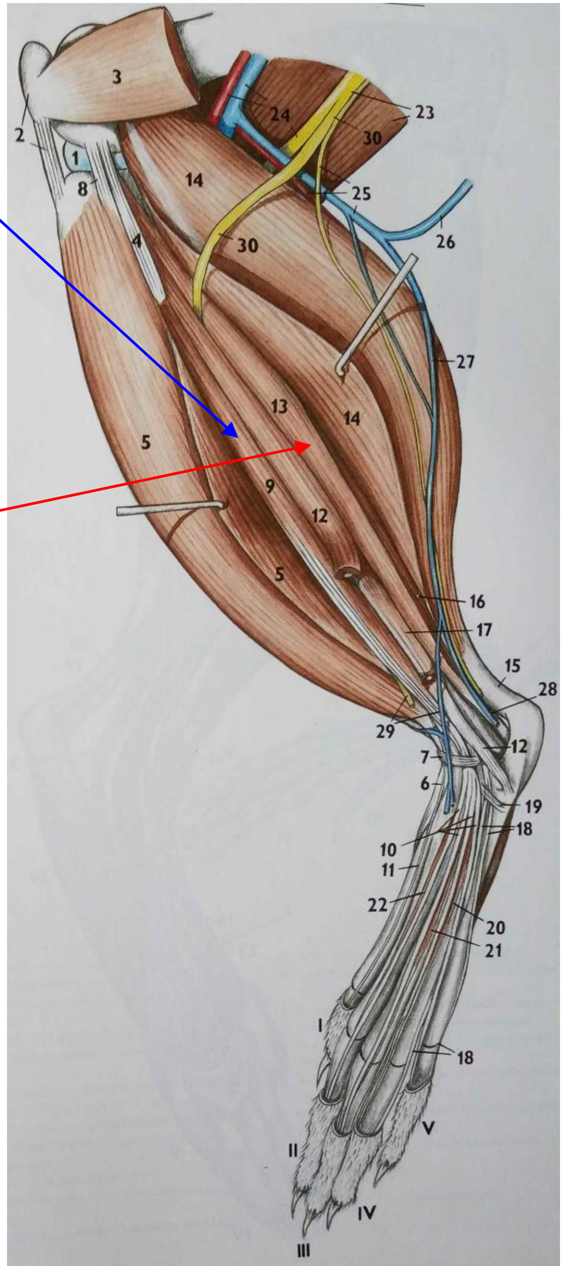
Mizunoya W et al: Fast-to-slow shift of muscle fiber-type composition by dietary apple polyphenols in rats: Impact of the low-dose supplementation. Anim Sci J. 2017 88(3):489-499.

m. extensor digitorum longus **(EDL)**
fast-twich

95% of Type II fibers
5% of Type I fibers

m. soleus
slow-twich

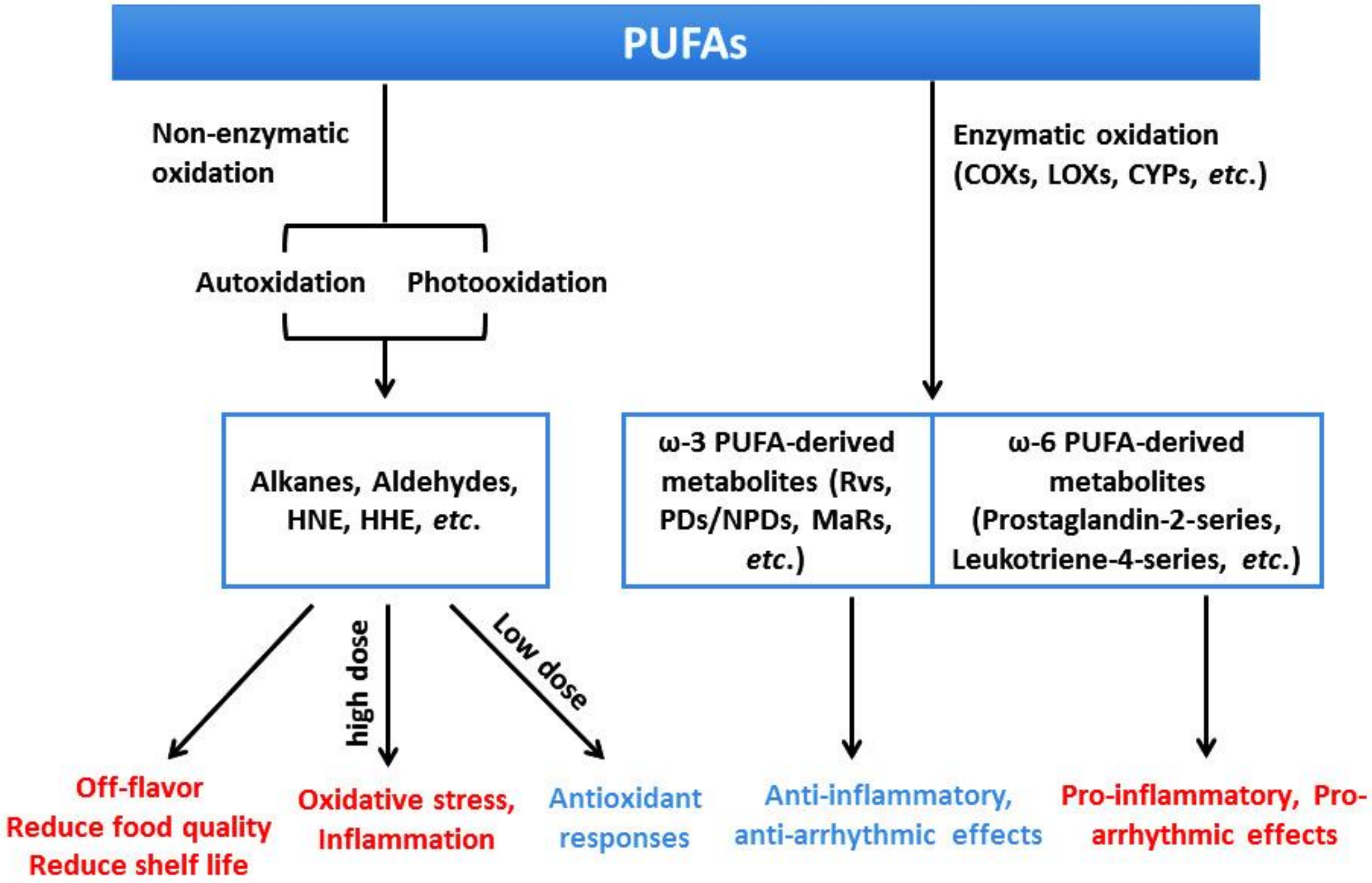
95% of Type I fibers
5% of Type II fibers



Muscle fiber types affect

- Exercise performance
- Fatigue resistance
- Metabolic capacity

ω-3 PUFA



Tao L. Oxidation of polyunsaturated fatty acids and its impact on food quality and human health. Adv Food Technol Nutr Sci Open J. 2015; 1(6): 135-142. doi: 10.17140/AFTNSOJ-1-123.

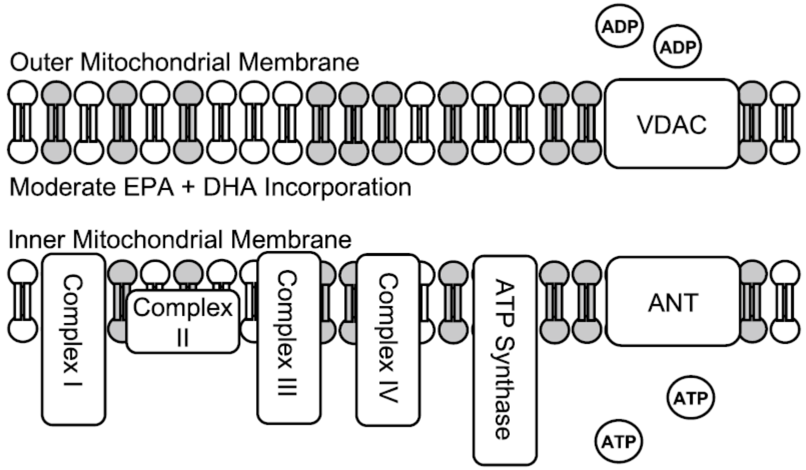
In aging

↓ ω-3/ω-6 PUFAs in membranes

Supplementation with ω-3 PUFA

↑ content of ω-3 PUFA in membranes

- Antioxidant, anti-inflammatory effect
- Makes membranes more fluid
- May affect function of membrane associated proteins, respiratory chain complexes
- Effect of dosage - not well documented



Murphy, C.H., McGlory, C. Fish Oil for Healthy Aging: Potential Application to Master Athletes. Sports Med 51 (Suppl 1), 31–41 (2021).

ω-3 PUFA +/- Exercise
Young/Old rats

14 Experimental groups, 6 male Wistar rats in each group:

Young rats (average weight 0.4 kg), age 9 – 10 months

MOC2 – Placebo daily for 3 weeks
MO1 – ω-3 PUFA in a dose of 160 mg/kg body weight for 3 weeks
MO2 – ω-3 PUFA in a dose of 320 mg/kg body weight for 3 weeks
MOC6 – Placebo daily for 8 weeks
MOCEx – Placebo daily for 3 weeks + 5 weeks placebo + exercise 5x/week 10 min
MO1Ex – ω-3 PUFA in a dose of 160 mg/kg b. w. for 3 weeks + 5 weeks daily ω-3 PUFA + exercise 5x/week 10 min
MO2Ex – ω-3 PUFA in a dose of 320 mg/kg b. w. for 3 weeks + 5 weeks daily ω-3 PUFA + exercise 5x/week 10 min

Old rats (average weight 0.5 kg), age 24 – 25 months

SOC2 – Placebo daily for 3 weeks
SO1 – ω-3 PUFA in a dose of 160 mg/kg body weight for 3 weeks
SO2 – ω-3 PUFA in a dose of 320 mg/kg body weight for 3 weeks
SOC6 – Placebo daily for 8 weeks
SOCEx – Placebo daily for 3 weeks + 5 weeks placebo + exercise 5x/week 10 min
SO1Ex – ω-3 PUFA in a dose of 160 mg/kg b. w. for 3 weeks + 5 weeks daily ω-3 PUFA + exercise 5x/week 10 min
SO2Ex – ω-3 PUFA in a dose of 320 mg/kg b. w. for 3 weeks + 5 weeks daily ω-3 PUFA + exercise 5x/week 10 min

Exercise: running on treadmill inclined 10°, 12 m/min for 10 min

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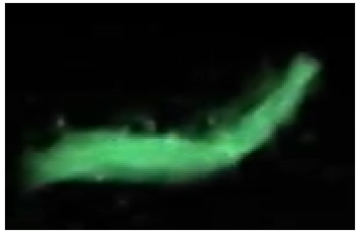
Methods

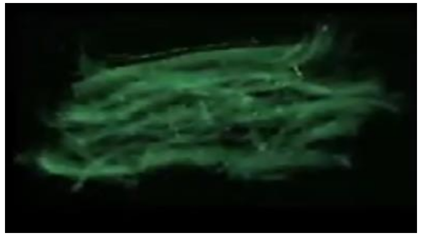
- **High-resolution respirometry** for determination of **mitochondrial function** in permeabilized muscle fibres from *m. extensor digitorum longus* (EDL)
- **Classical respirometry** for determination of mitochondrial function in isolated **liver mitochondria**
- **HPLC** with spectrophotometric detection for determination of **coenzyme Q** in EDL tissue and **liver mitochondria**
- Colorimetric methods for determination of **total cholesterol** and **triacylglycerols (TAG)** concentration in **liver tissue**

Mitochondrial respiration in permeabilized fibres

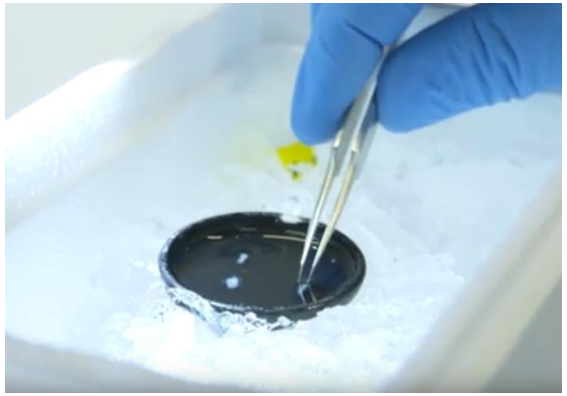
Preparation of permeabilized muscle fibres:



Muscle fibres



mechanically separated then permeablized with saponin (50 μg/mL) in BIOPS 30 min, washed 10 min in MiR05+Cr



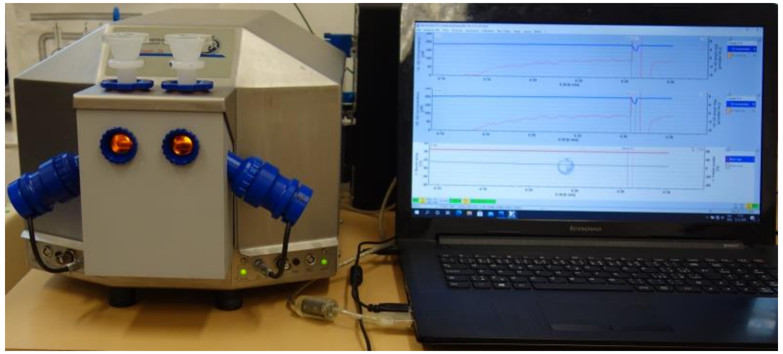
2 mg of permeabilized muscle fibres (pfi)

https://www.youtube.com/watch?v=iN38rOkiBUQ

→

O2k-Respirometer
(Oroboros Instruments, Austria)

High-resolution respirometry

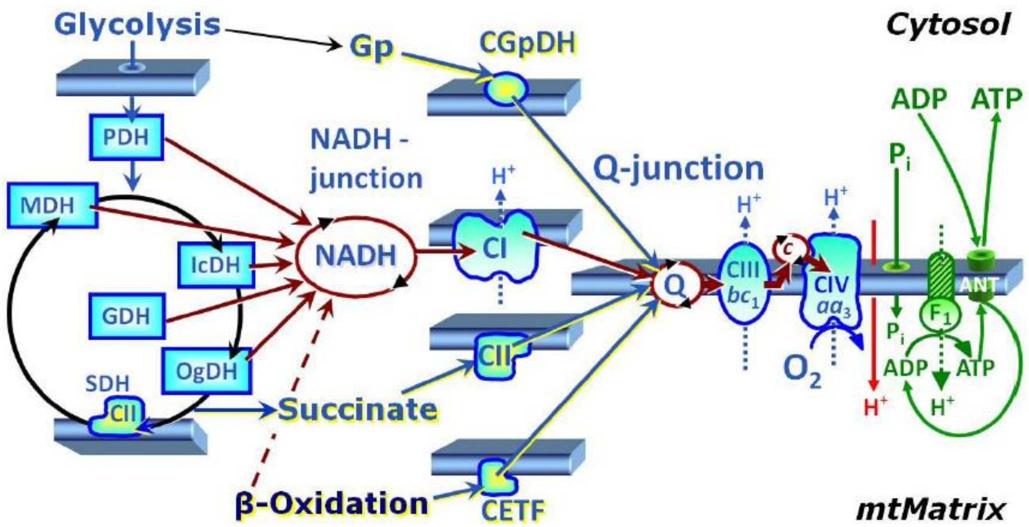


- measurement in MiR05+20 mM Creatine, at 37°C and high O_2 concentration
- **O_2 concentration** and **O_2 consumption** is recorded by DatLab software
- **SUIT protocol** (substrate – uncoupler – inhibitors titration) protocol for assessing several pathways of mitochondrial respiratory system

SUIT protocol (SUIT-005)

substrate – uncoupler – inhibitors titration protocol

https://wiki.oroboros.at/index.php/SUIT-005_O2_pfi_D011

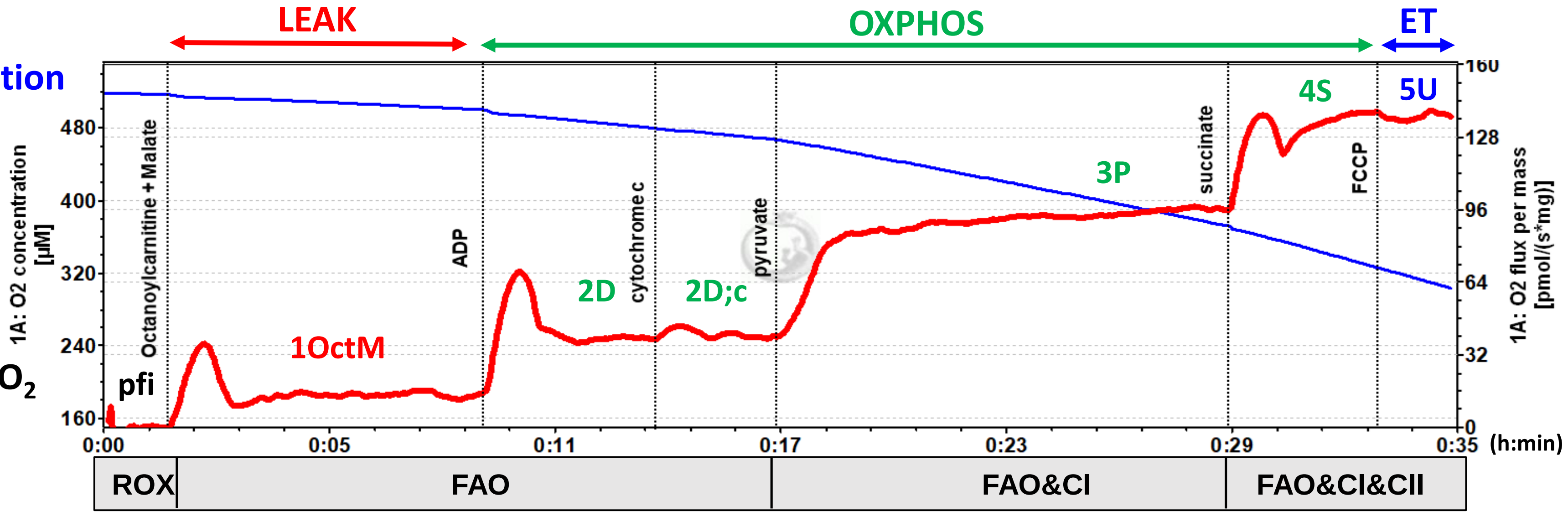


2 mg of permeabilized muscle fibres (**pfi**)

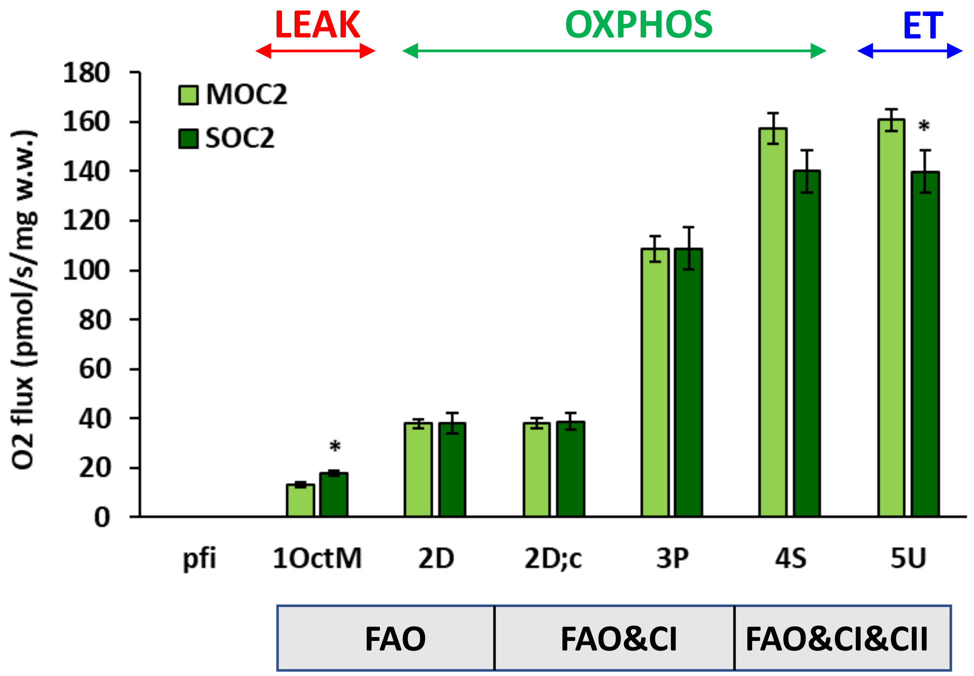
Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. Bioenerg Commun 2020.2: 112 pp. doi:10.26124/bec:2020-0002.

High O_2 concentration ~ 500 µM

normal ~ 200 µM O_2



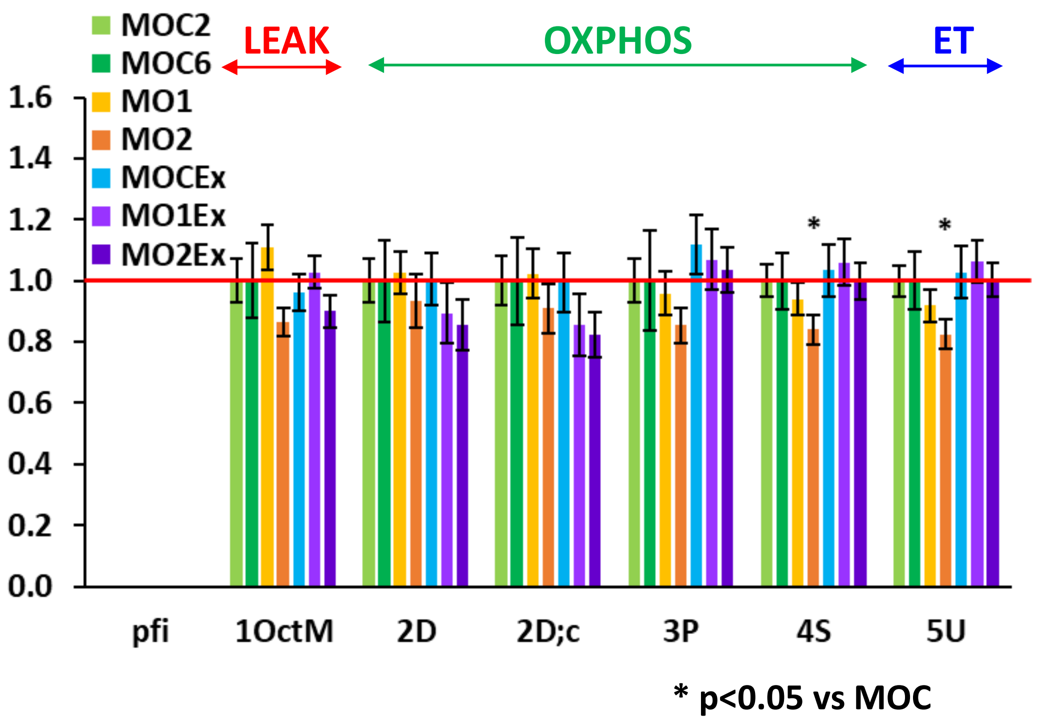
Mitochondrial respiration in permeabilized fibers of EDL muscle

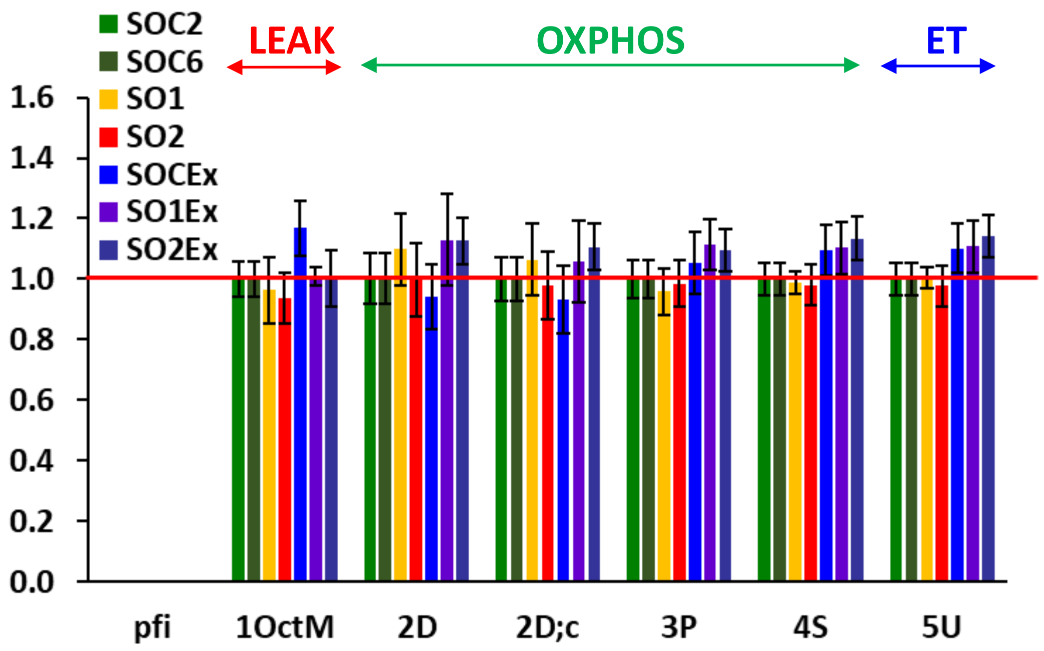


In old rats compared to young rats:

- **LEAK respiration** with Octanoylcarnitine + malate (1OctM) was higher
- The **capacity of electron transfer** with fatty acids and substrates of CI and CII (5U) **was ↓**

Mitochondrial respiration in EDL
(a ratio to control)

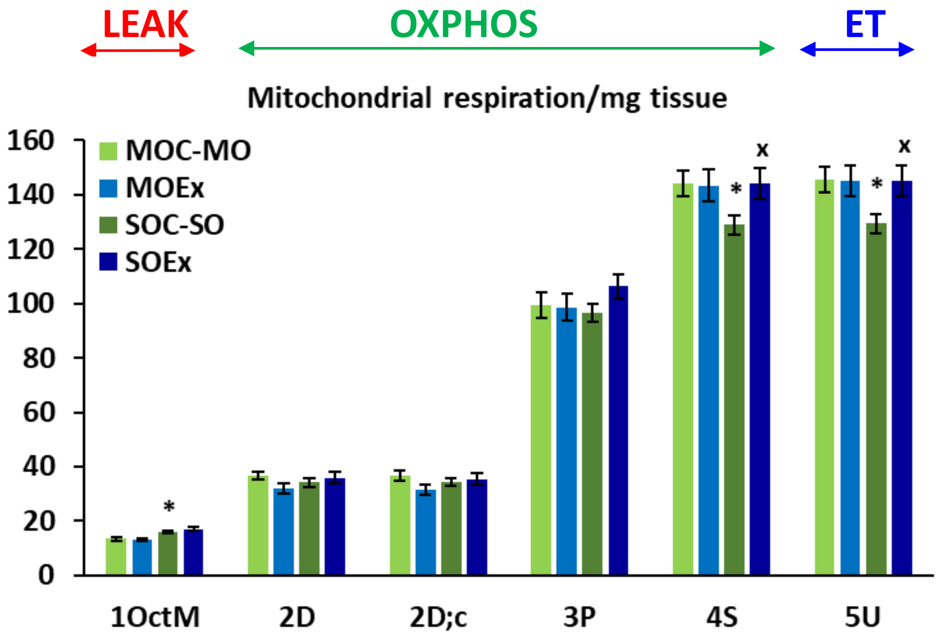




In young rats with **high dose of fish oil** the **capacity of OXPHOS** and **ET** with substrates linked to **FAO&CI&CII was ↓**

In old rats exercise may ↑the capacity of **OXPHOS** with substrates linked to FAO, FAO&CI and FAO&CI&CII and **↑ ET** capacity with mixture of substrates **vs control independently of ω-3 PUFA dose**

Mitochondrial respiration in EDL



*p<0.05 vs MOC-MO

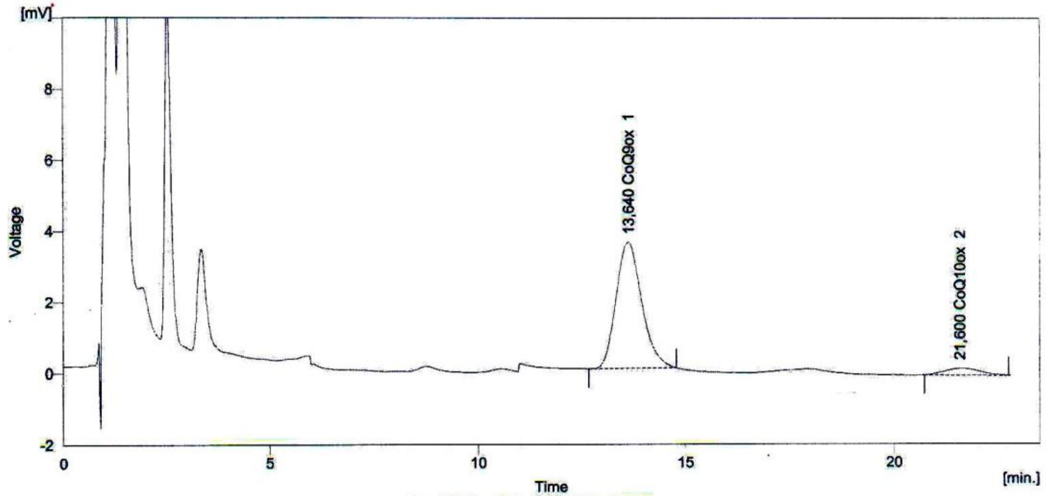
ˣp<0.05 vs SOC-SO

In old rats with exercise the capacity of OXPHOS and ET with mixture of substrates linked to FAO&CI&CII were ↑ **vs control**

Coenzyme Q

- component of the mitochondrial respiratory system essential for energy production
- powerful lipid-soluble antioxidant
- CoQ_9 is the main form of coenzyme Q in rat
- In EDL – CoQ_{TOTAL}
- In liver mitochondria – oxidized and reduced forms

HPLC determination of coenzyme Q_9 a Q_{10} in rat extensor digitorum longus (EDL)



Result Table - Calculation Method ESTD

Reten. Time	Area [mV.s]	Area [%]	Amount [µl]	Amount [%]	Peak Type	Compound Name
13,640	152,023	93,1	17,900	94,7	Free	CoQ9ox
21,600	11,319	6,9	1,007	5,3	Ordnr	CoQ10ox
Total	163,342	100,0	18,907	100,0		

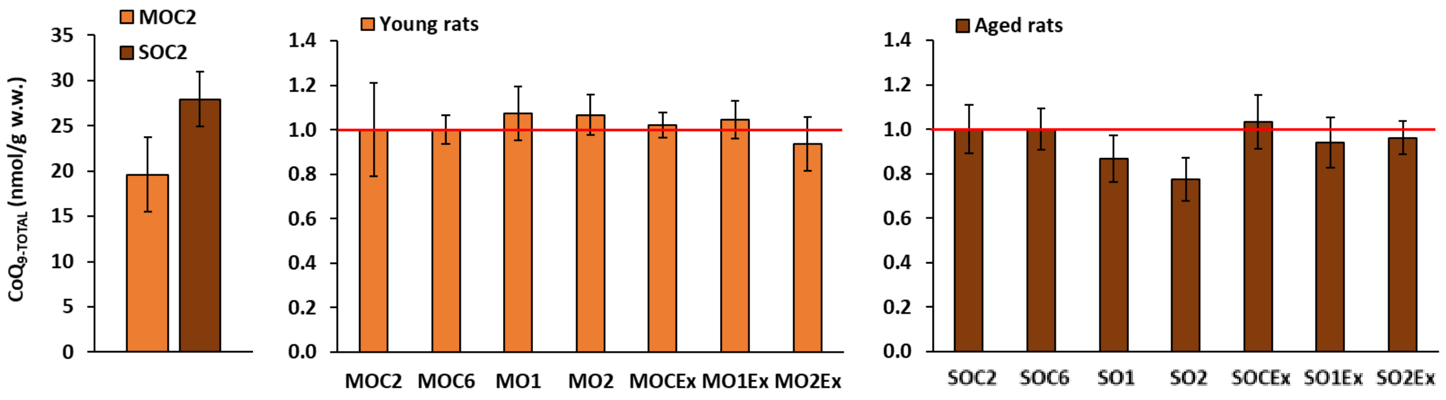
Sample preparation: The **EDL tissue** (aprox. 50 mg) was **homogenized** using an Ultra-Turrax in 1 ml of redestilled **water**, extraction mixture **hexane/ethanol** (5/2, vv) was added. For the oxidation of ubiquinol to ubiquinone **1,4-benzoquinone** was added, mixed and incubated for 10 minutes at room temperature (Mosca et al 2002). After adding **SDS** (sodium dodecyl sulphate), **shaking for 5 minutes** and centrifuging, the **organic layer** was separated and **evaporated** under nitrogen.

Detection: The residues were taken up in 99,9% ethanol and injected into a reverse phase **HPLC column**. Elution was performed with **methanol/acetonitrile/ethanol** (6/2/2, v/v). Concentrations of compounds were detected spectrophotometrically at **275 nm**, using external standards. Data were collected and processed using CSW 32 chromatographic station.

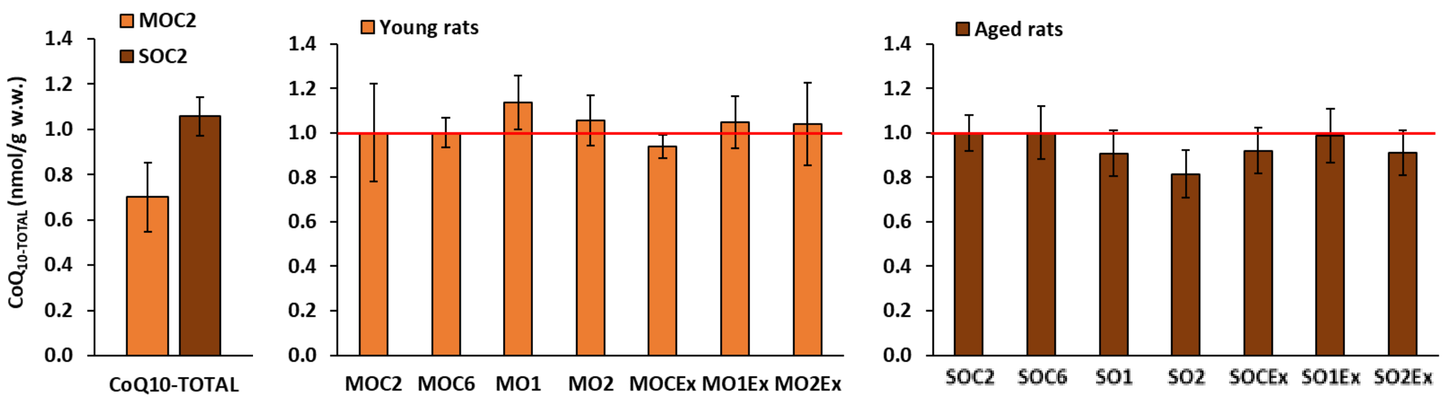
CoQ_9 and CoQ_{10} concentration in EDL (extensor digitorum longus)

$CoQ_{9\text{-TOTAL}}$

a ratio to control



$CoQ_{10\text{-TOTAL}}$



CoQ_9 and CoQ_{10} content in EDL

no significant difference between aged vs young control rats in CoQ_9 or CoQ_{10} content in EDL

Young
no effect of diet or exercise

Old
no effect of diet or exercise

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Liver

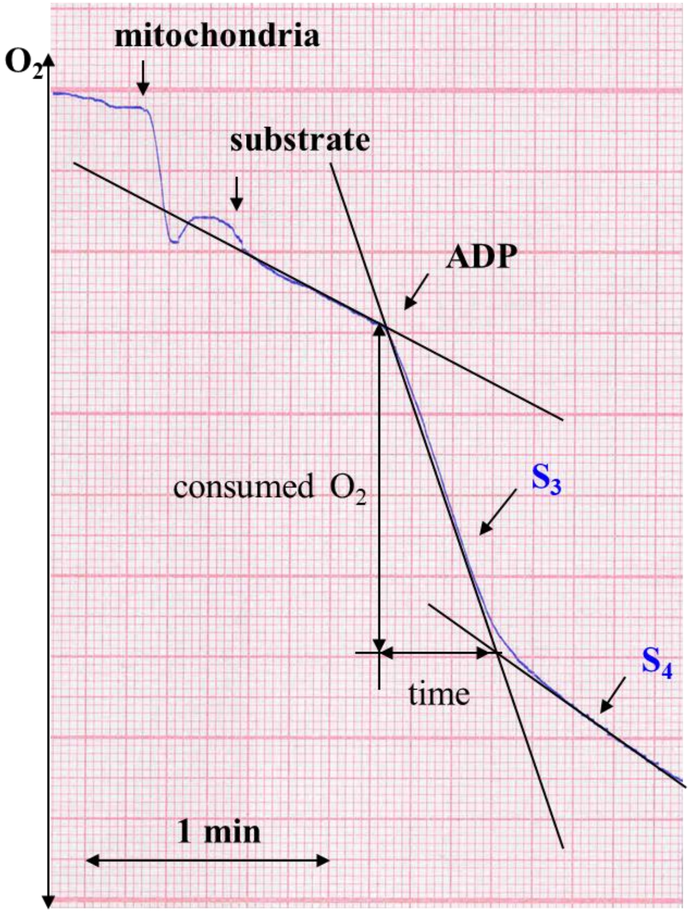
Liver is the main organ **involved in metabolism of nutrients** – various diets can affect liver function, excessive nutrient intake can lead to liver steatosis

We analyzed

- mitochondrial function
- concentration of CoQ_9 and CoQ_{10} in liver mitochondria
- concentration of tChol and TAG in liver tissue

Evaluation of mitochondrial function in liver mitochondria

O_2 consumption measured by Oxygraf Gilson



Evaluated parameters:

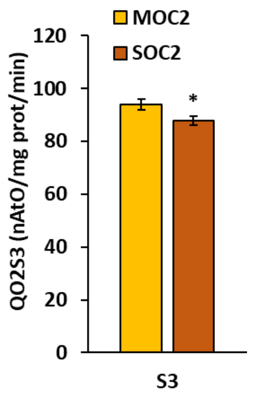
S3 (ADP stimulated respiration)
S4 (respiration after ADP depletion)
RCR = S3/S4 (respiratory control ratio)
ADP:O (efficiency of OXPHOS) = amount of added ADP/consumed O_2
OPR (rate of ATP production) = amount of added ADP/time

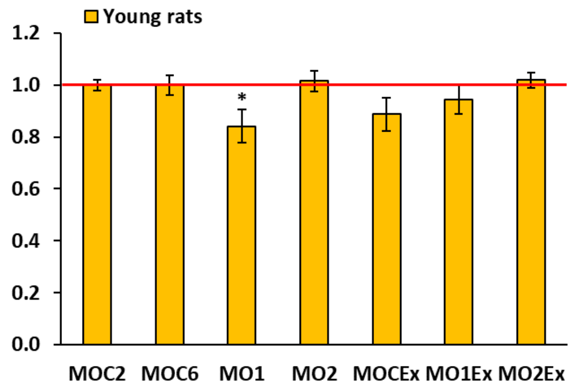
CI-linked substrates: glutamate + malate

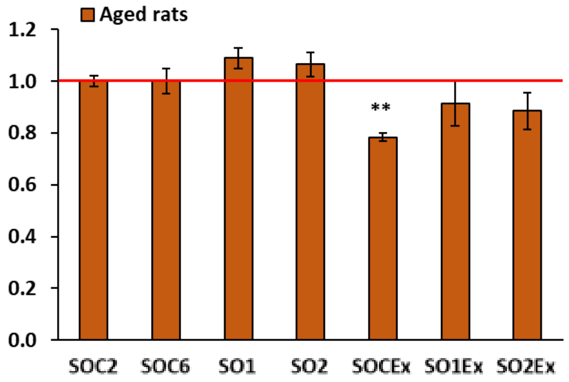
Respiration of liver mitochondria
with CI-linked substrates

State 3 (ADP-stimulated)

a ratio to control







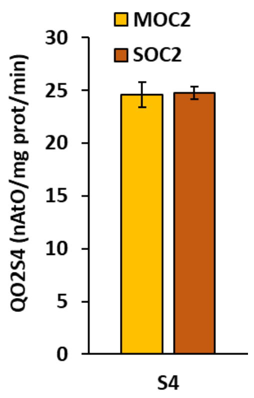
*p<0.05 vs young control

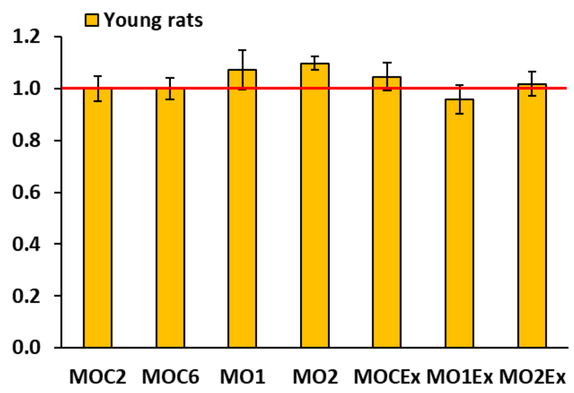
S3 respiration
↓ in aged vs young control

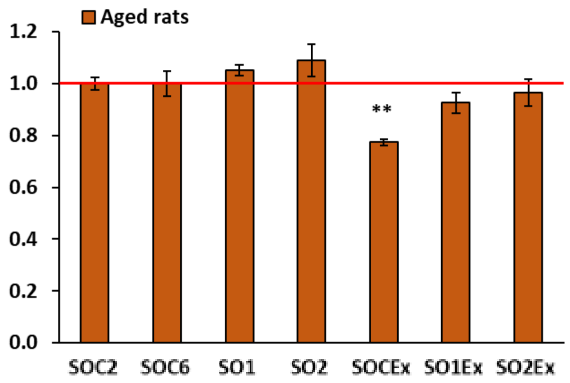
Young
↓ in low dose of ω-3 PUFA

Old
↓ after exercise

State 4 (basal)







S4 respiration
no diff. aged vs young control

Young
no difference between groups

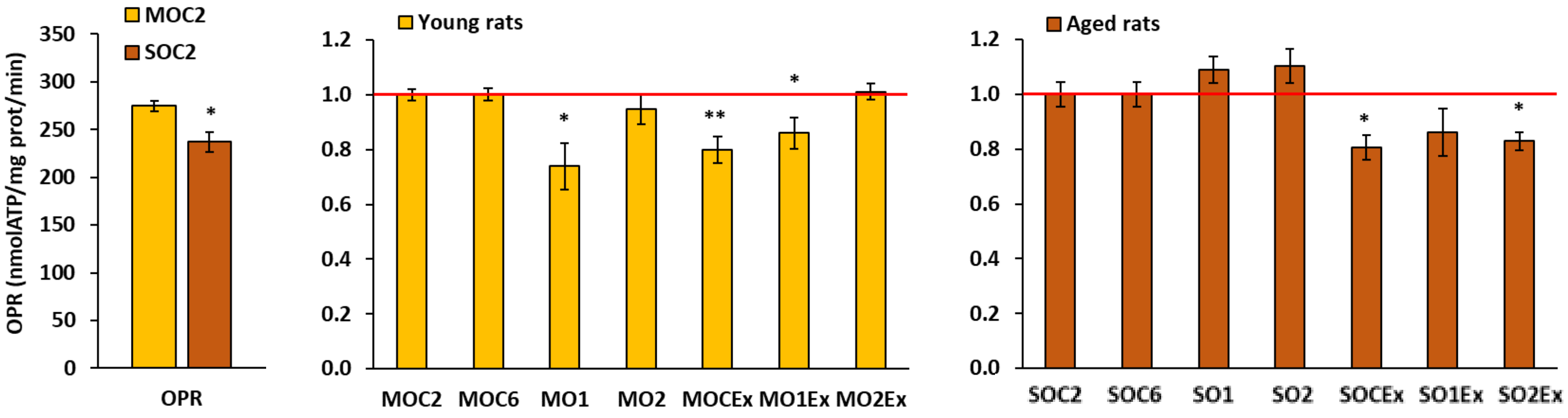
Old
↓ after exercise

*p<0.05, **p<0.01 vs control

The rate of ATP production in liver mitochondria
with CI-linked substrates

OPR (the rate of OXPHOS)

a ratio to control



OPR

↓ in aged vs young control

Young

↓ in low dose of ω-3 PUFA

↓ after exercise

↓ after exercice+low ω-3 PUFA

Old

↓ after exercise

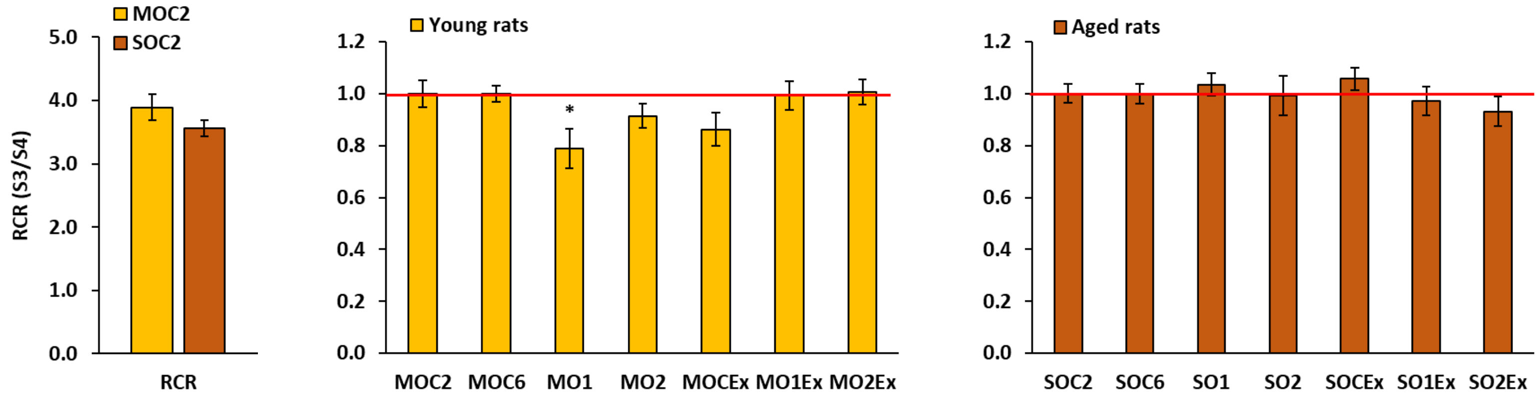
↓ after exercise+high ω-3 PUFA

Respiration indexes in liver mitochondria

with CI-linked substrates

RCR (State3/State4)

a ratio to control

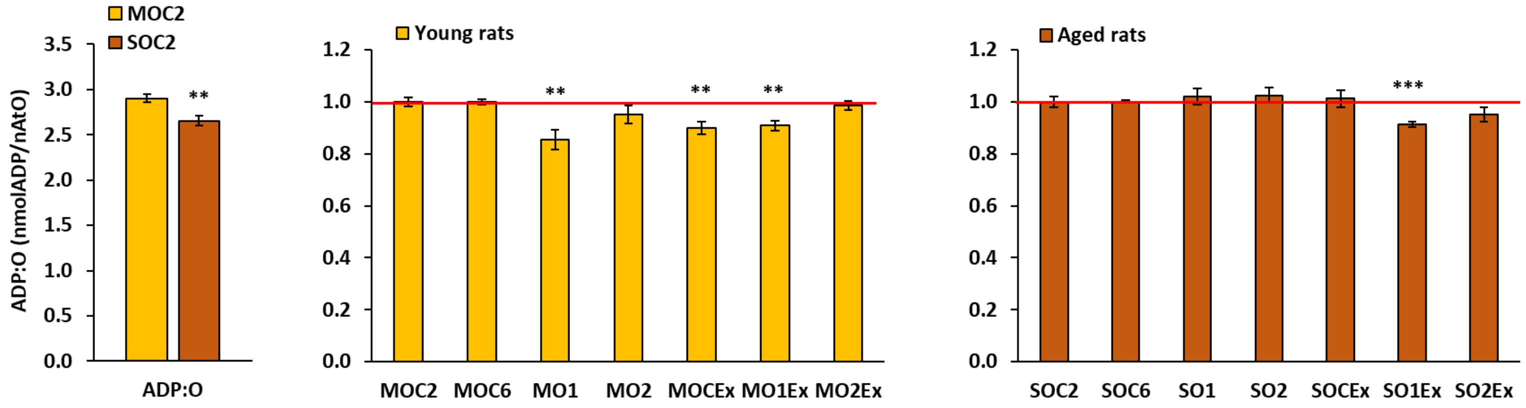


RCR
no diff. aged vs young control

Young
↓ in low dose of ω-3 PUFA

Old
no difference between groups

ADP:O (efficiency of OXPHOS)



ADP:O
no diff. aged vs young control

Young
↓ in low dose of ω-3 PUFA
↓ after exercise
↓ after exercice+low ω-3 PUFA

Old
↓ after exercise with low ω-3 PUFA

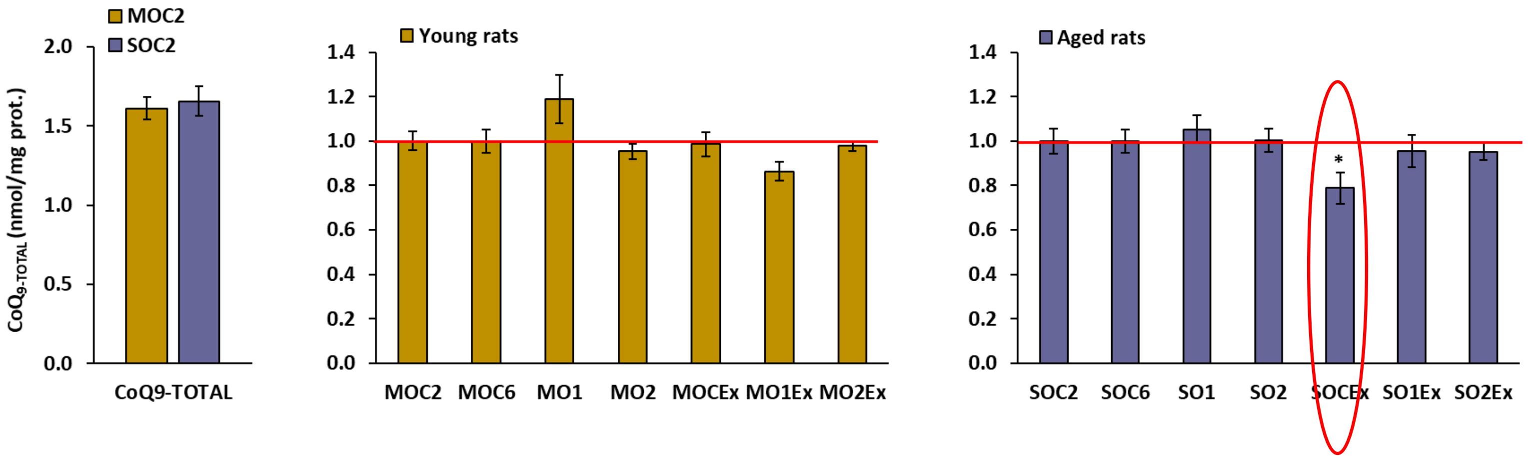
**$p<0.01$ vs young control

*$p<0.05$, **$p<0.01$, ***$p<0.01$ vs corresponding control

CoQ_9 in liver mitochondria

$CoQ_{9\text{-TOTAL}}$ (oxidized + reduced)

a ratio to control

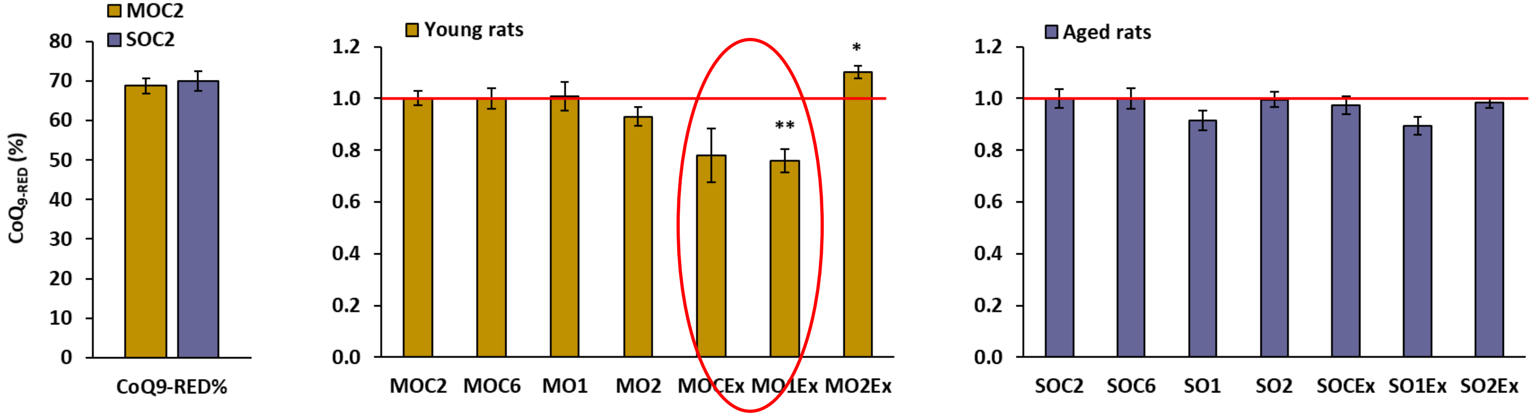


$CoQ_{9\text{-TOTAL}}$
no diff. aged vs young control

Young
no differences between groups

Old
↓ after exercise

Proportion (%) of $CoQ_{9\text{-RED}}$ to $CoQ_{9\text{-TOTAL}}$



% of $CoQ_{9\text{-RED}}$
no diff. aged vs young control

Young
↓ after exercise with low ω-3 PUFA
↑ after exercise with high ω-3 PUFA

Old
no differences between groups

Cholesterol and triacylglycerols concentration in the liver tissue

- **to find out if the intervention does not cause unwanted storage of neutral fats in the liver**

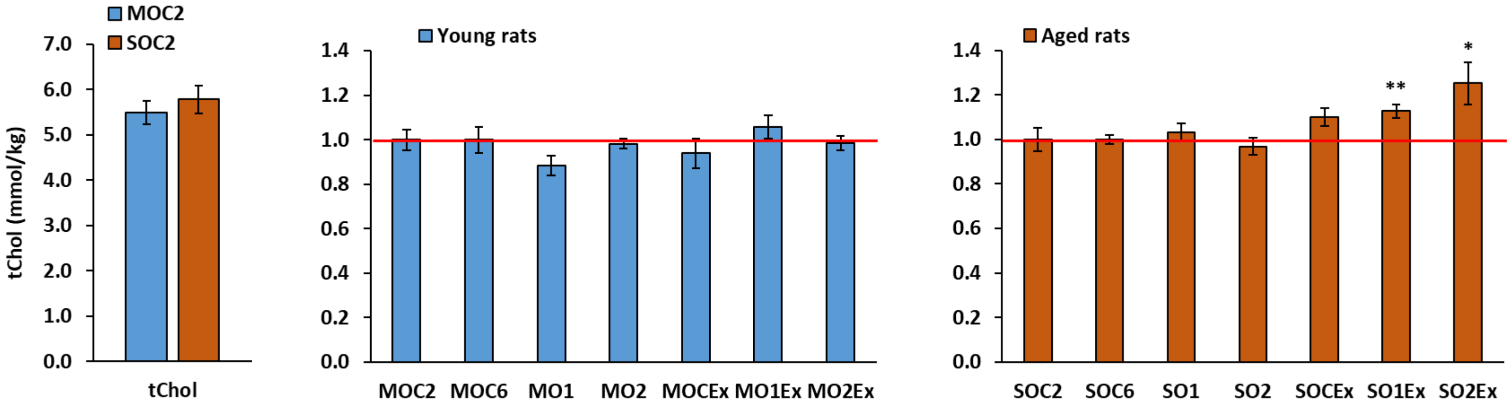
- **Cholesterol** was determined by the modified method of Abel et al. 1952. Liver tissue (100 mg) was **homogenized in chloroform/methanol** (1:1). After lipid extraction, the Lieberman-Burchard colorimetric assay was used for the detection of cholesterol. Cholesterol concentrations were determined spectrophotometrically at 650 nm.

- **Triacylglycerols** concentrations in the liver were determined by the modified method of Jover (1963). Liver tissue (100 mg) was **homogenized** and **extracted in chloroform/methanol** (2:1). The interfering phospholipids were removed by absorption from the liver extract on silica gel. Purified extracts were evaporated and triglycerides hydrolyzed with potassium hydroxide. Released fatty acids were removed by extraction into heptane. Finally, the released glycerol was oxidized by periodic acid, and after the reaction with phenylhydrazine, a colored complex was measured spectrophotometrically at 530 nm.

Total cholesterol and triacylglycerols in liver tissue

Total cholesterol

a ratio to control

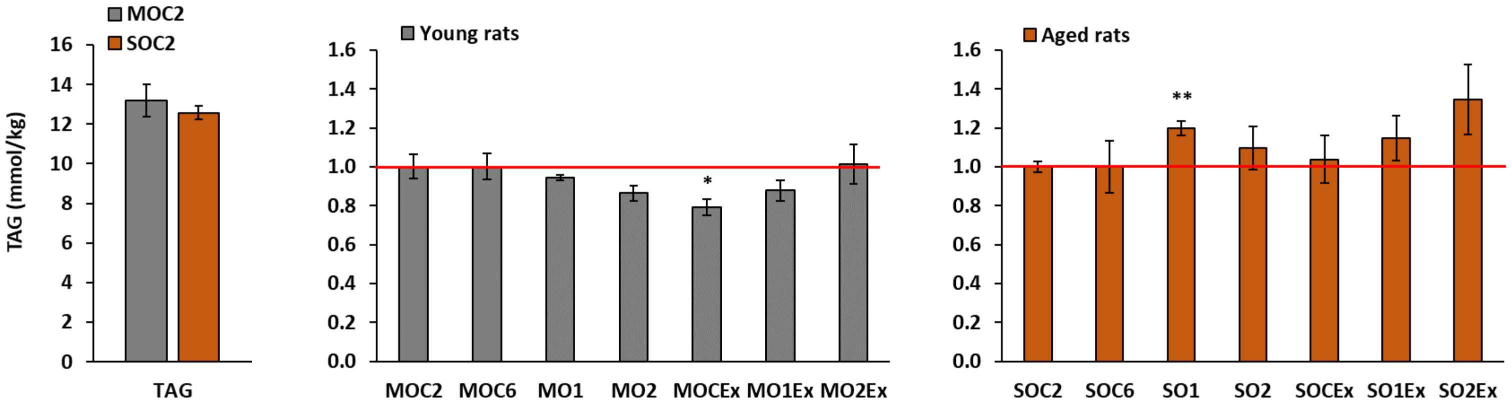


tChol
no diff. in aged vs young control

Young
no difference between groups

Old
↑ after exercise with ω-3 PUFA

Triacylglycerols (TAG)



TAG
no diff. aged vs young control

Young
↓ after exercise

Old
↑ in group with low dose of ω-3 PUFA
In 3 rats of SO2Ex steatosis was found

Summary

	Young rats	Old rats
EDL muscle	• ↓ of **OXPHOS and ET capacity** of mass-specific mitochondrial respiration with **high ω-3 PUFA** • no effect of exercise	• no effect of ω-3 PUFA alone • ↑ of **OXPHOS and ET capacity** of mass-specific mitochondrial respiration with **exercise** and **exercise + ω-3 PUFA (effect of exercise)**
Liver	↓ **%$CoQ_{9\text{-}RED}$** with **exercise** and **exercise + low ω-3 PUFA** ↓ **rate and efficiency of ATP production** after **low ω-3 PUFA, exercise** and **exercise + low ω-3 PUFA**	↓ **CoQ_9 content** after **exercise** ↓ **rate and efficiency of ATP production** after **exercise** and **exercise + ω-3 PUFA** ↑ **tChol and TAG concentration in aged rats with exercise + high ω-3 PUFA**

Conclusion

In young rats

the intensity of exercise was insufficient to improve quality of the muscle

In old rats

the intensity of **exercise** was sufficient to **improve quality of the muscle**

the dose of **ω-3 PUFA 320 mg/kg b.w.** may be **detrimental for liver** health

Thank you for your attention