Lipid oxidation in relation to fish health and quality

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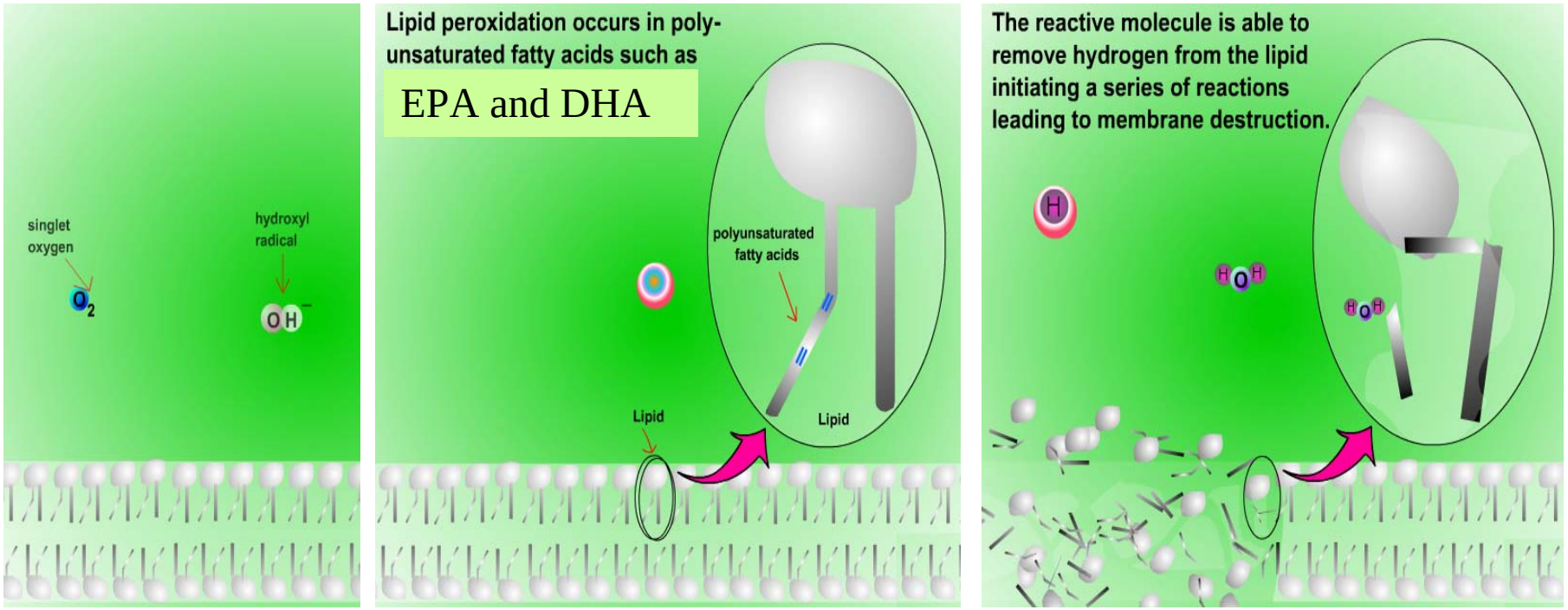

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Background

Positive health effects of n-3 HUFAs may be limited by unfavourably effects due to lipid peroxidation?

- Some examples from mammalian studies
  - Moderate degree of oxidative stress may damage mitochondrial membranes and lead to reduced fatty acid β-oxidation capacity and thereby increased risk of developing obesity (Pomplun et al., 2007).
  - High degree of oxidative stress may on the other hand induce apoptosis (fat cell death) and therefore have a leaning effect (Rutzikova et al., 2004).
  - High dietary levels of fish oil may lead to vitamin E deficiency, oxidation of LDL and increased risk of formation of atherosclerotic plaques.
  - High intake of n-3 HUFAs may inhibit cancer cell growth (*Colquhon, 2001)*
  - Oxidative stress in placenta affects bone formation during foetal development in mice (Prater et al., 2008)

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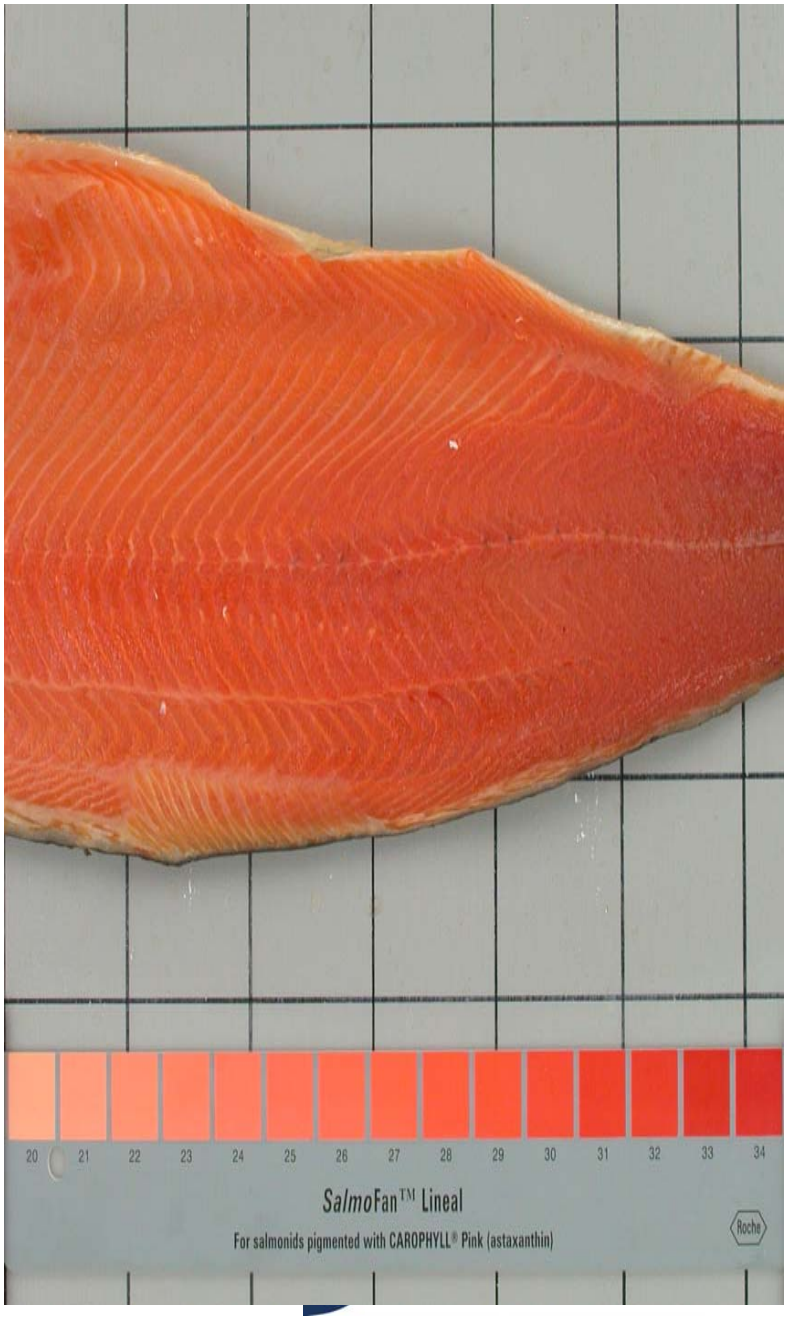
- **Oxidised lipids in fish diets**

  - Fish oils are more prone to peroxidation than plant oils, due to their high content of HUFAs

  - Oxidation products:
    - poor taste of feed, reduced feed intake, poor quality of fish product (decreased storage stability).



Some early studies have shown that oxidised lipids in fish diets may lead to;

- Reduced appetite
- Increased mortality
- Myopati of skeletal muscle
- Fatty liver

} Atlantic salmon (Ketola *et al.*, 1989)
Rainbow trout (Cowey *et al.*, 1984)

- Oxidised dietary lipids may lead to increased incidence of deformities in marine fish larvae

↓

Lewis-McCrea,L.M., Lall,S.P., 2007. Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 262, 142-155).

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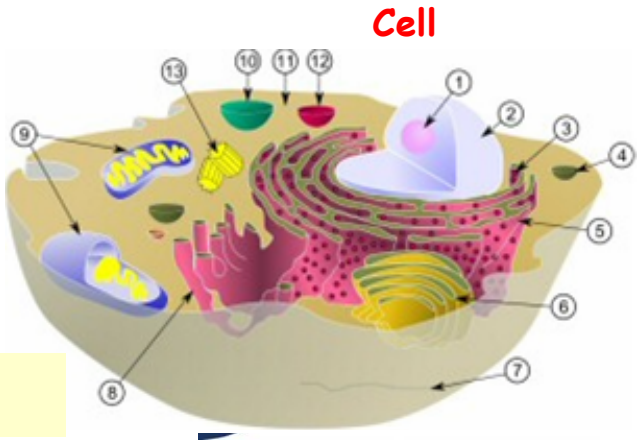
Aims

To study the effects of high dietary n-3 HUFAs on susceptibility to oxidative stress in liver, visceral adipose tissue and skeletal muscle in Atlantic salmon;

Focus on:

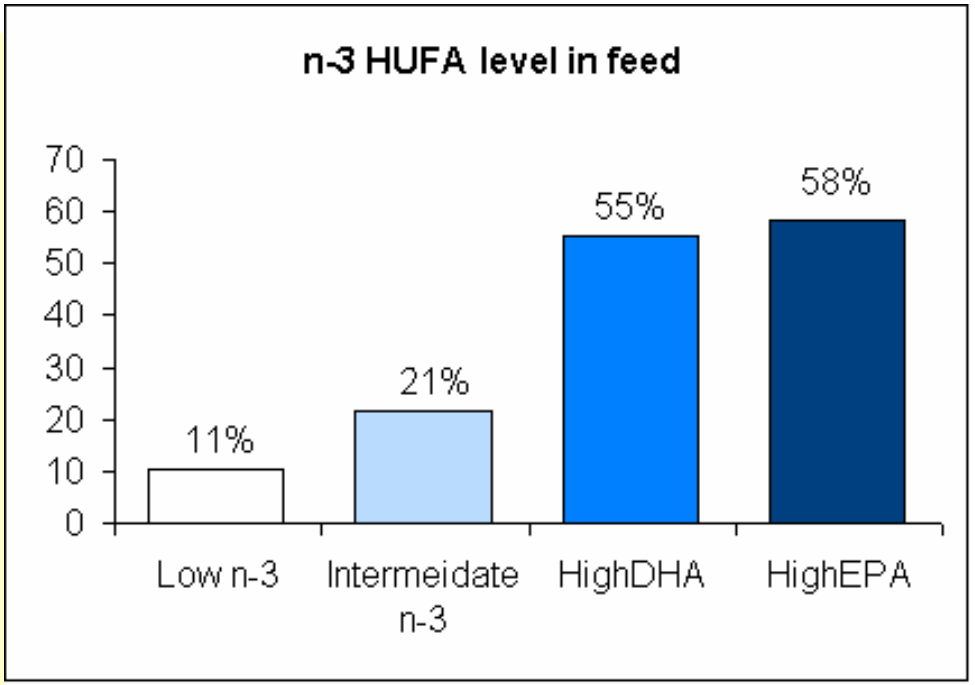
- Membrane lipids in cells and mitochondria
- Mitochondrial function
- Oxidative stress enzyme- and gene markers
- Apoptosis (particularly in white adipose tissue)
- Liver and muscle structure



1: Nucleolus 2: Cell nuclei 3: Ribosome 4: Vesicle 5: ER 6: Golgi 7: Cytoskeleton
8: Smooth ER 9: Mitochondria 10: Vacuole 11: Cytoplasm 12: Lysosome

Fish trial

- Atlantic salmon were fed for 21 weeks in fresh water on one of four diets, with increasing levels of n-3 HUFAs
- The fish grew from an initial average weight of 90 g to a final weight of 344 g ± 68 g



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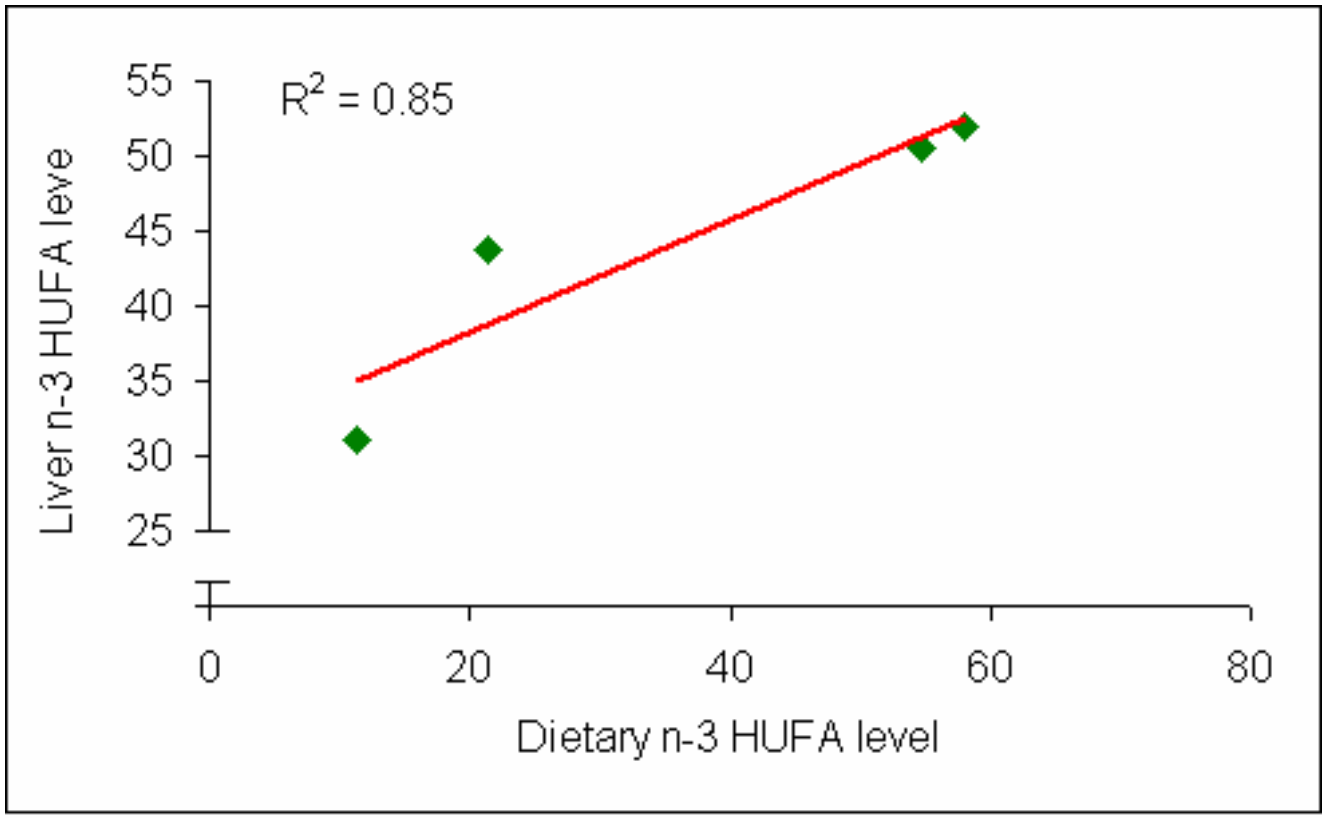
Increasing dietary levels of n-3 HUFAs led to increasing percentages of these FAs in fish tissues (here example from liver)



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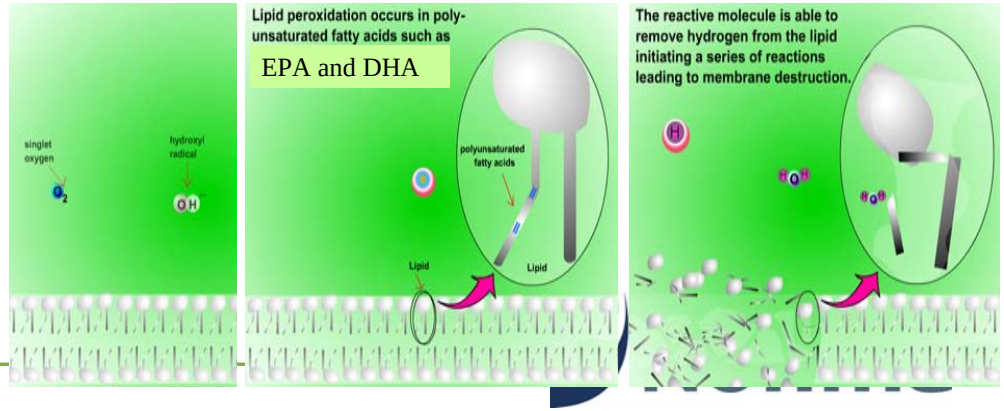
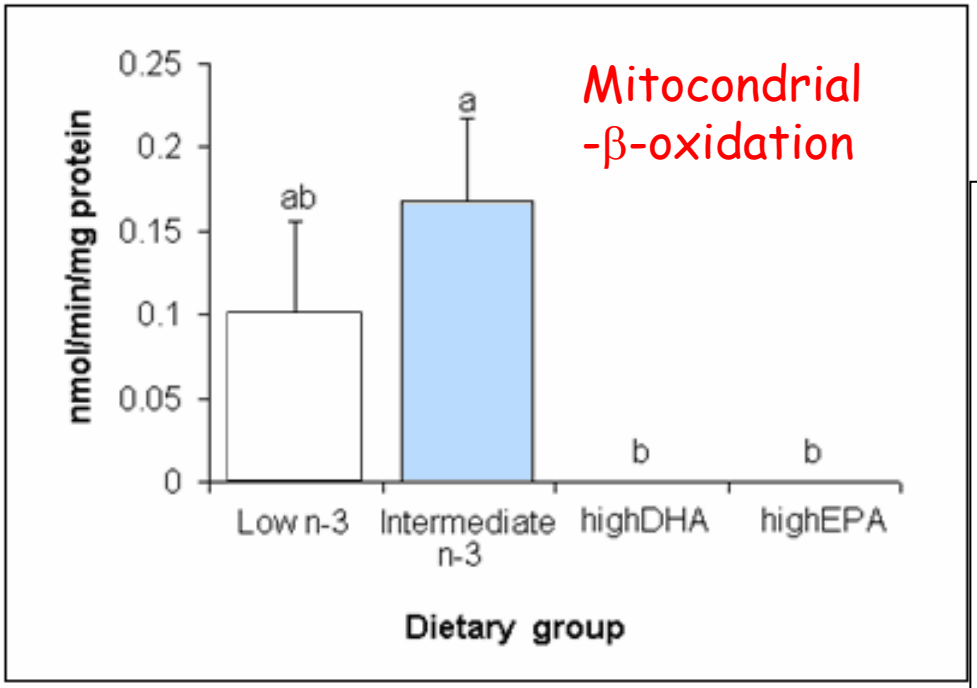
Lipid class composition of mitochondria isolated from liver

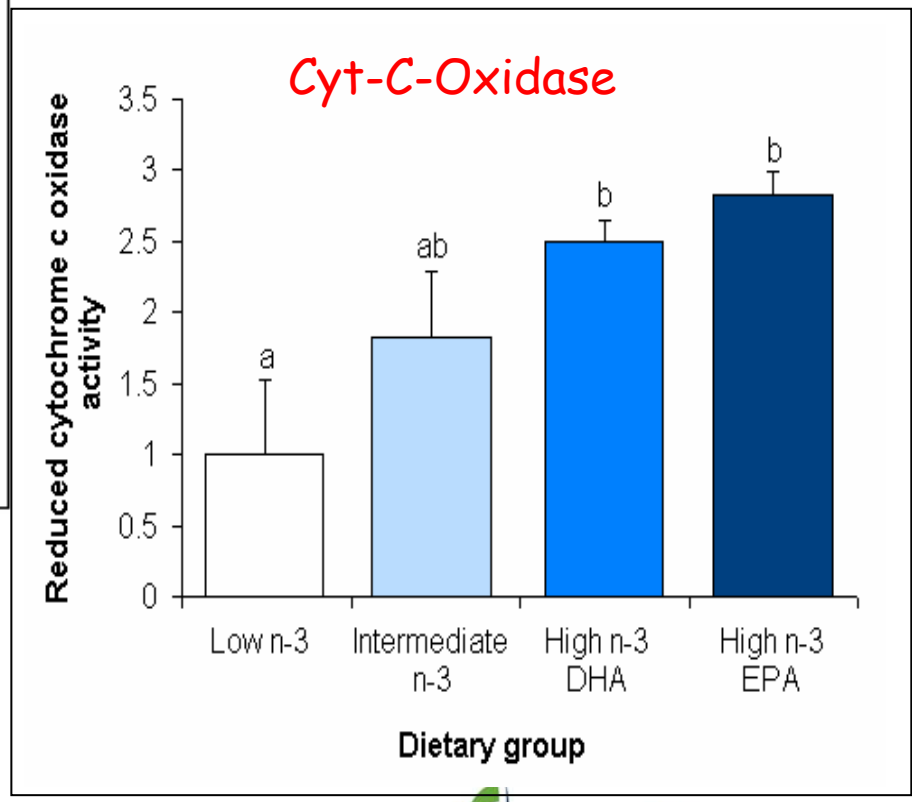
- Increasing dietary levels of n-3 HUFAs clearly decreased the percentage of major phospholipids (PLs) in mitochondrial membranes.
  - The percentage of cardiolipin (CL) in the mitochondrial membrane of the high EPA group was less than half of the percentage in the intermediate n-3 group.

	Mitochondria			
	Low n-3	Intermediate n-3	highDHA	highEPA
% distribution				
PC	22.5±1.02^{ab}	28.1±2.51^{a}	23.5±2.89^{ab}	16.1±2.82^{b}
PI	4.1±0.32^{ab}	4.3±0.77^{ab}	5.2±0.57^{b}	2.3±0.59^{a}
CL	5.1±0.31^{ab}	6.6±0.96^{a}	5.1±0.63^{ab}	3.1±0.61^{b}
PE	11.7±0.12^{ab}	14.6±0.99^{a}	11.2±1.23^{ab}	8.9±1.46^{b}
DAG	1.5±0.10^{a}	1.3±0.23^{a}	1.8±0.18^{ab}	2.5±0.39^{b}
CHOL	7.5±0.27	8.9±1.03	9.0±0.97	5.7±1.35
FFA	16.4±0.34^{a}	13.6±1.65^{ab}	11.0±0.82^{b}	13.4±0.33^{ab}
TAG	20.5±1.52^{a}	13.3±2.87^{a}	24.6±7.26^{ab}	41.3±7.20^{b}
∑ PL	54.1±1.10^{ab}	62.9±5.74^{a}	53.6±5.98^{ab}	37.1±5.96^{b}
∑ NL	45.9±1.10^{ab}	37.1±5.74^{a}	46.4±5.98^{ab}	62.9±5.96^{b}



Oxidative stress and effects on mitochondrial function in salmon liver





Avranden Kjær et al., 2008, Lipids

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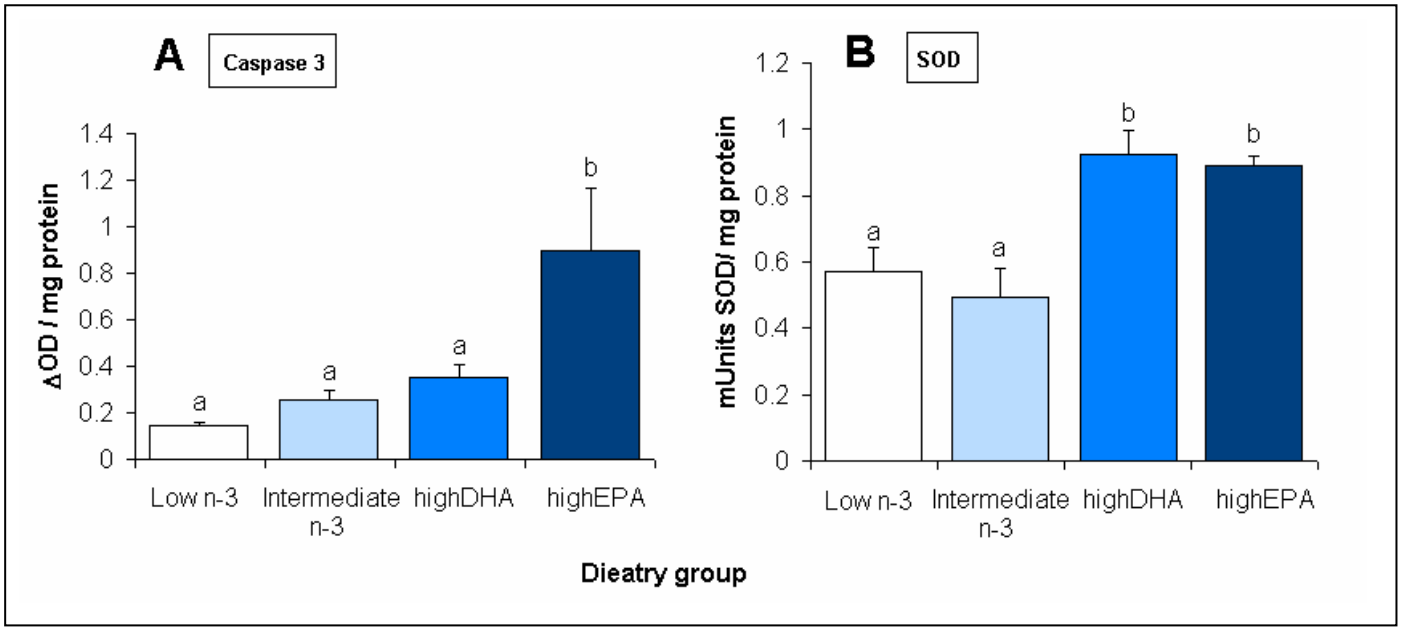
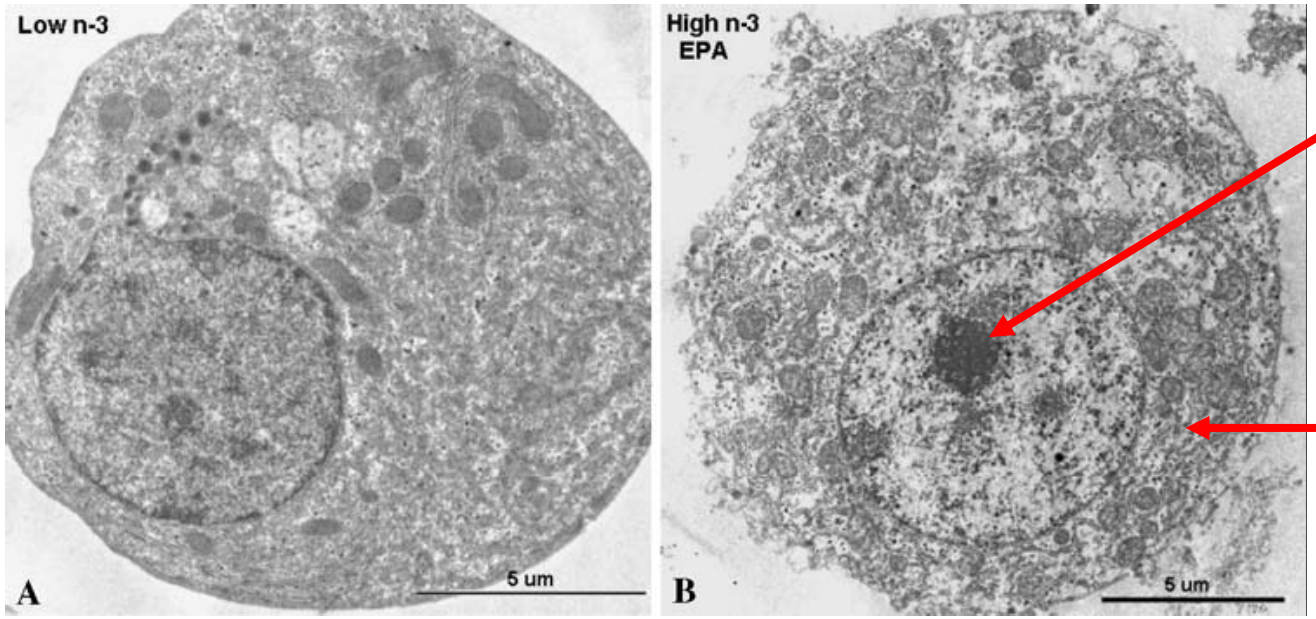


Both high EPA and high DHA lead to Oxidative stress



Oxidative stress leads to condensation of chromatin

Cellular content seemed to be released

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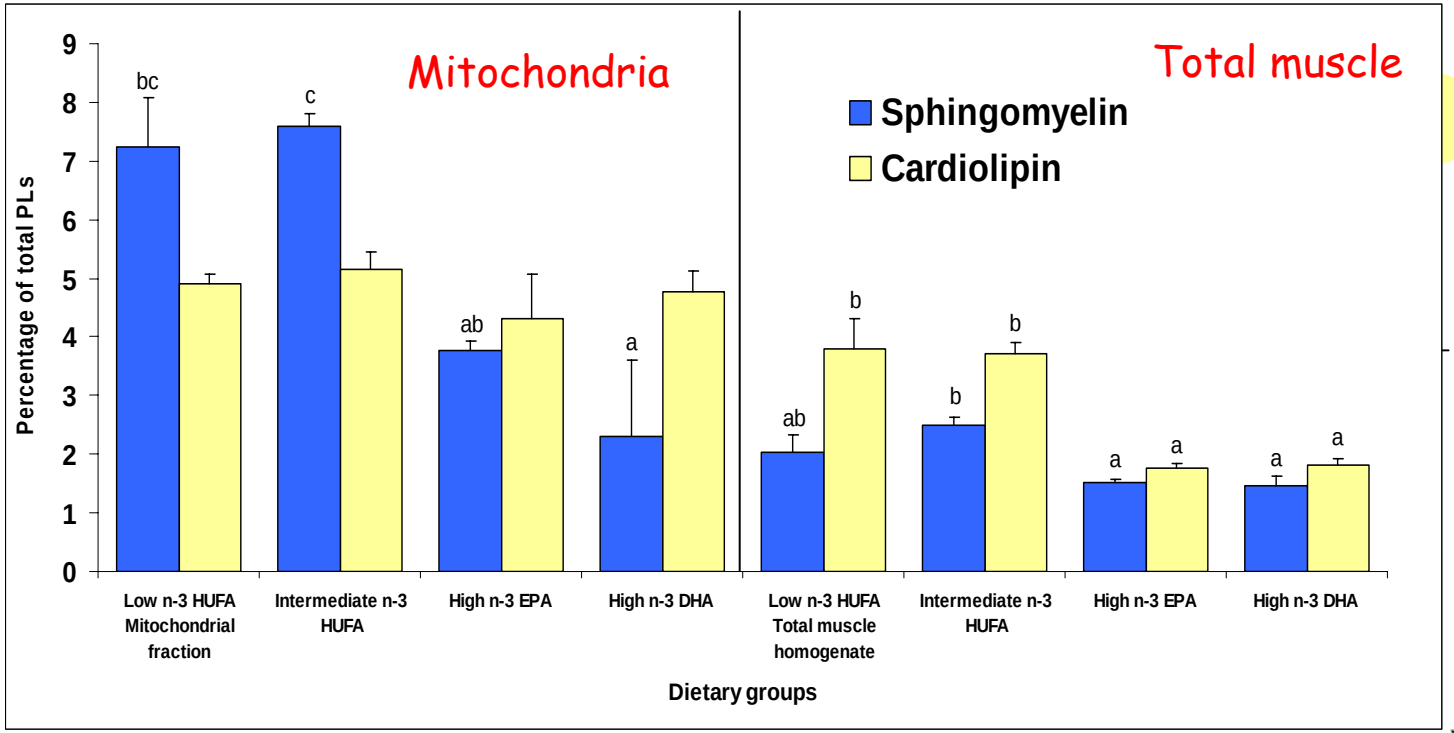
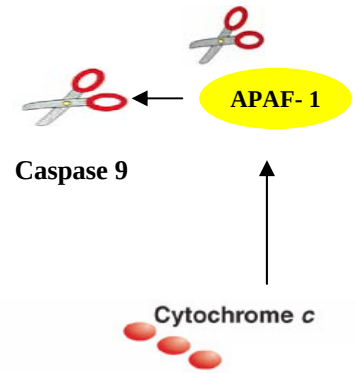
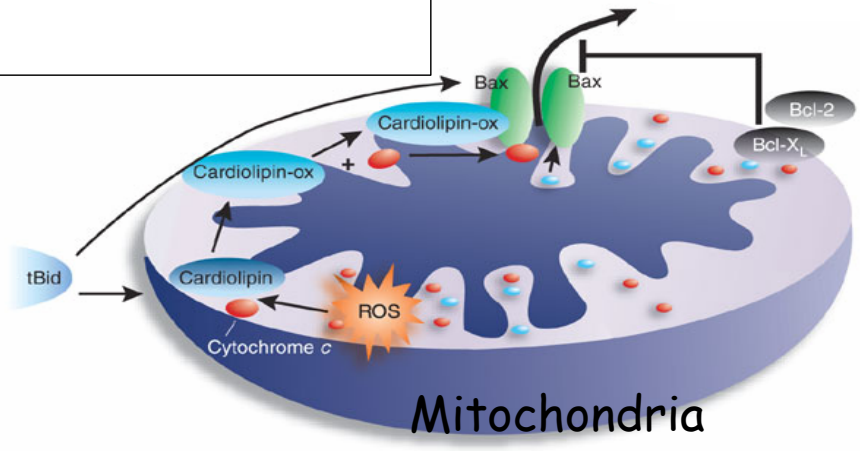
High HUFA diets result in damage of mitochondrial membrane PL in muscle





Cardiolipin
Sphingomyeline



Østbye *et al.*, 2008, Manuscript

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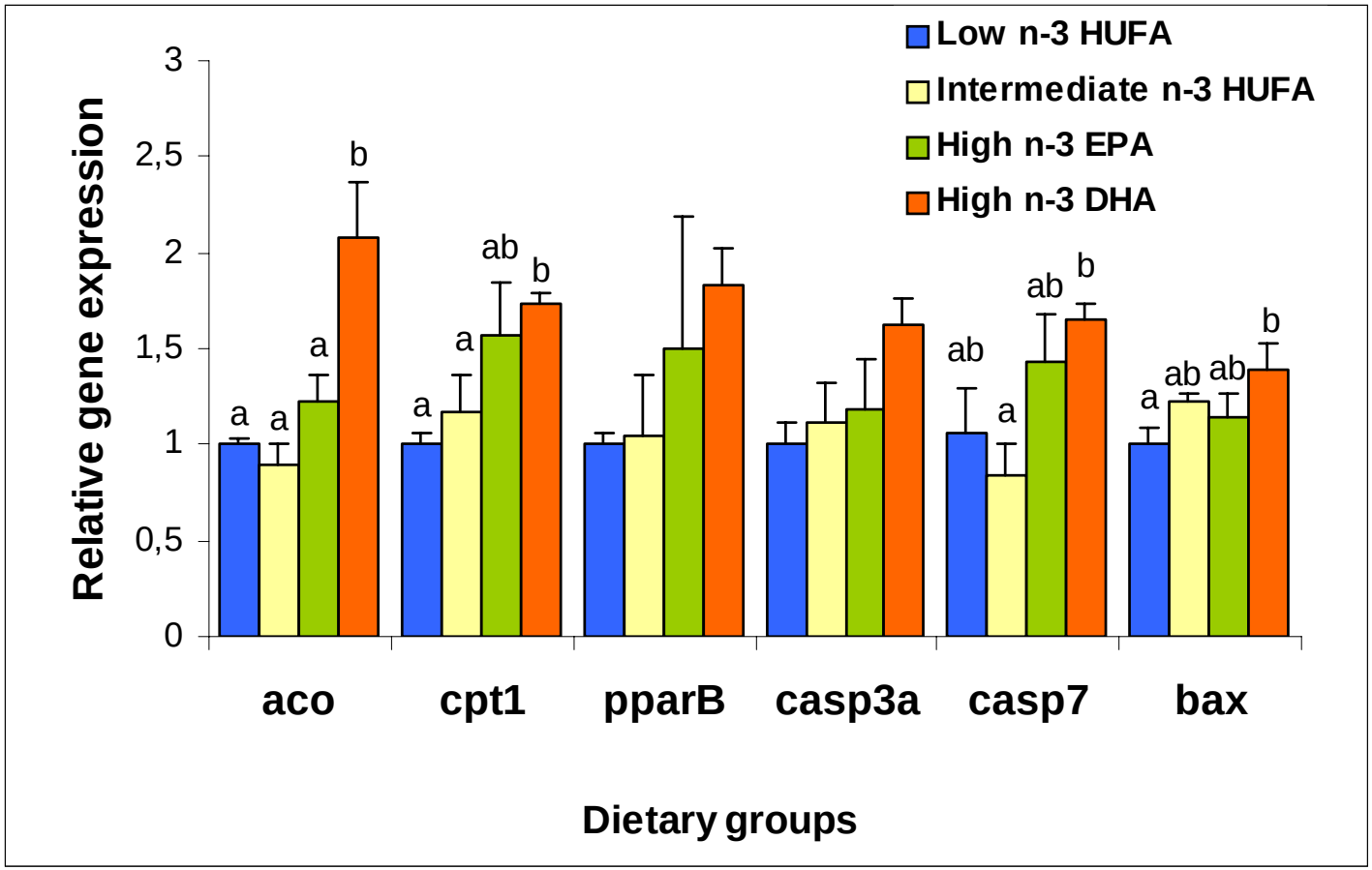
Effects of diets on gene expression in muscle

n-3 HUFAs lead to induction of Casp-7 and Bax genes



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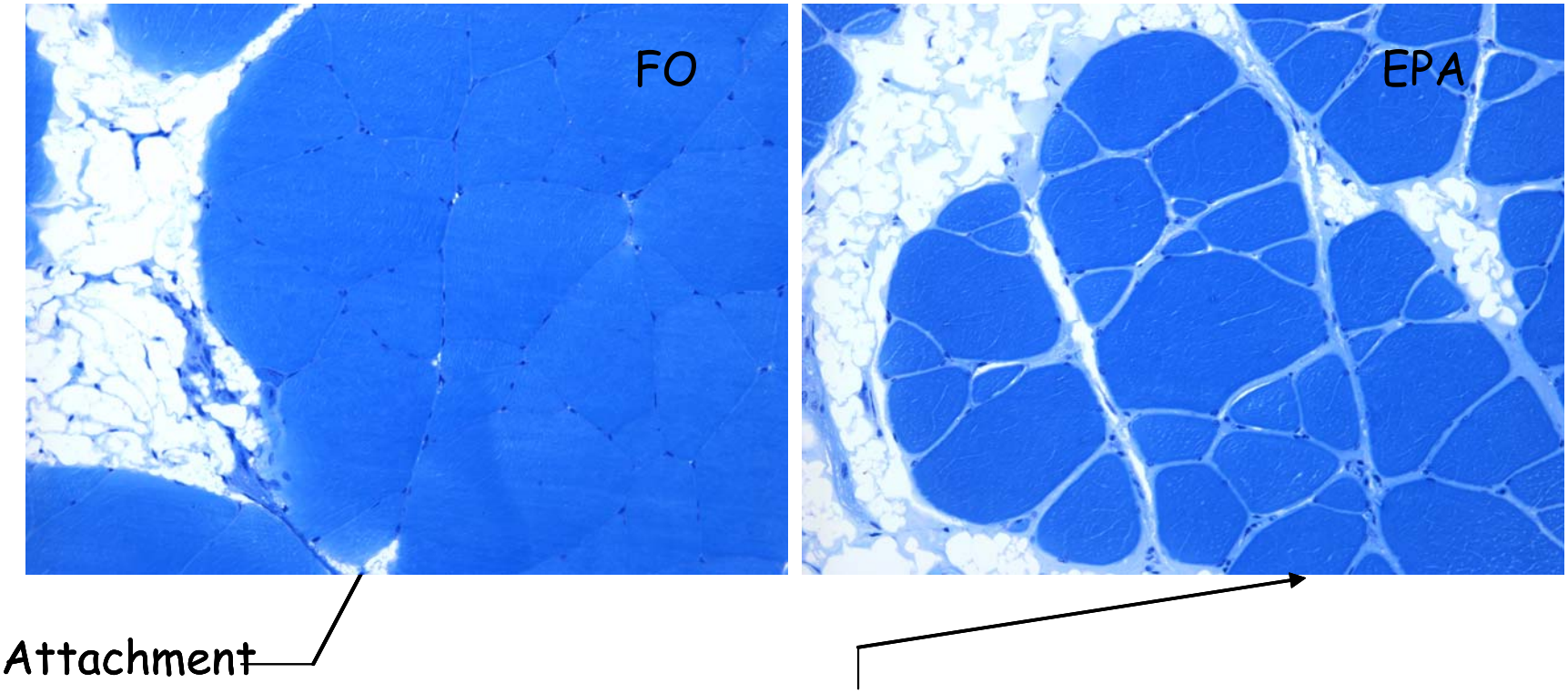
Oxidative stress led to changes in muscle structure (light microscopy)



Bahuaud et al., 2008

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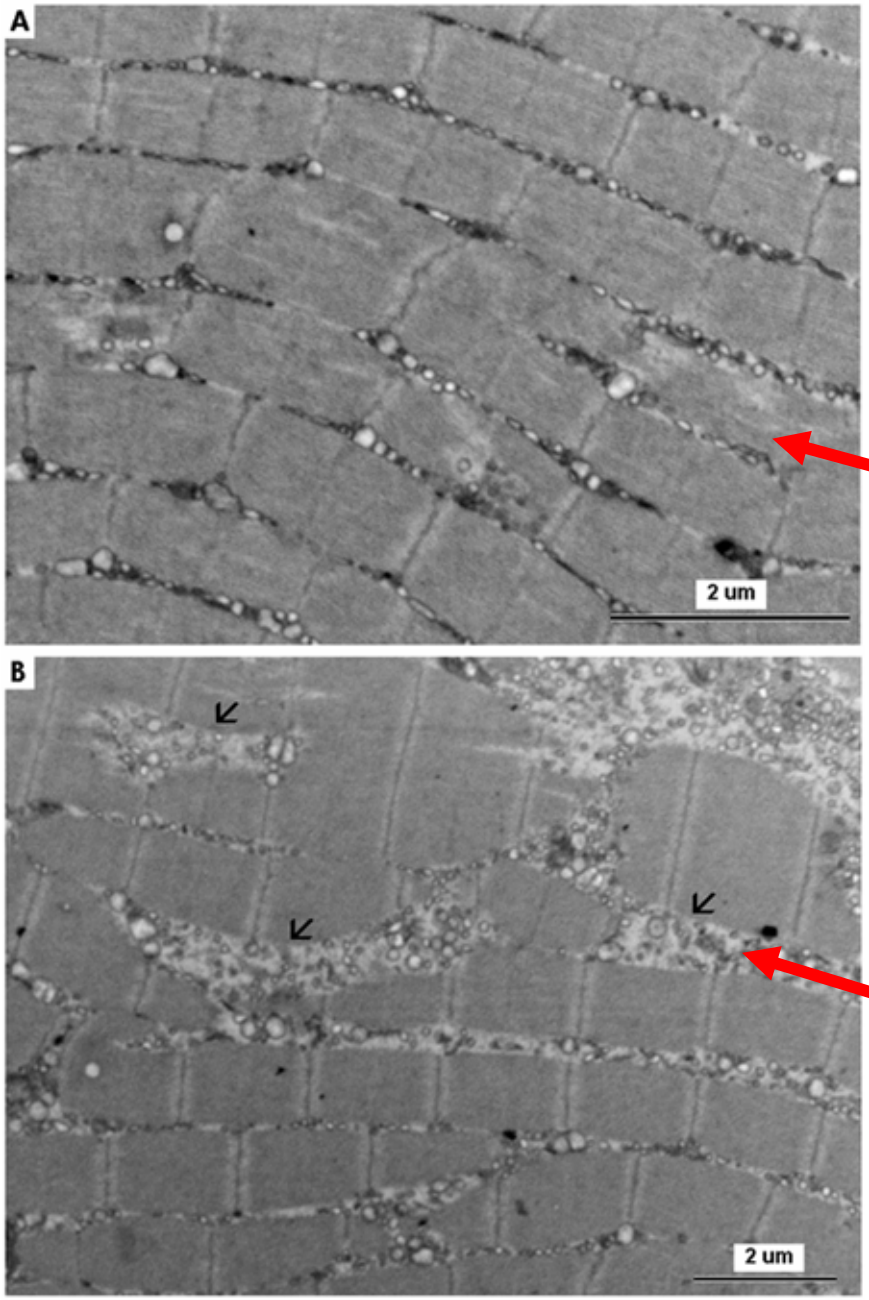

Electron microscopy pictures of muscle

normal

detachment, particularly observed in the high HUFA groups

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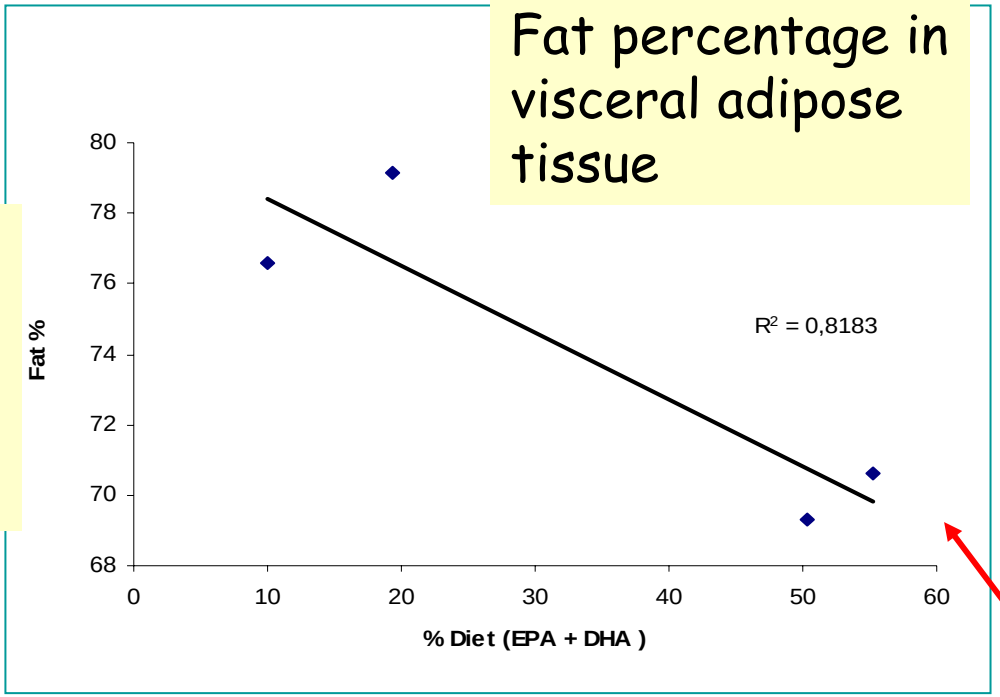
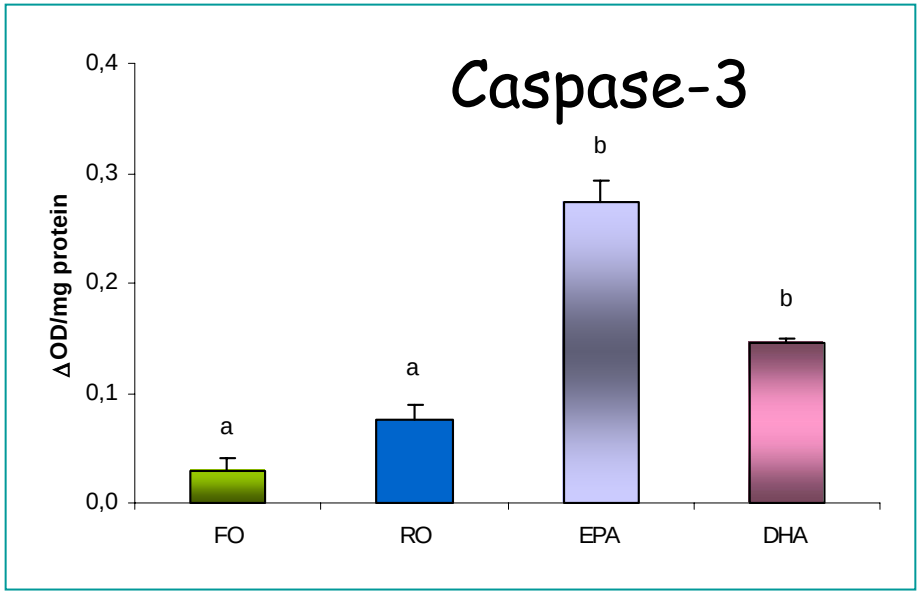
Visceral adipose tissue



Lower Fat%



EPA and DHA induce Caspase-3 activity which indicate that there is higher incidence of apoptosis (cell death) in these groups, which may explain the lower fat%

Todorcevic et al., 2008, Accepted CBP

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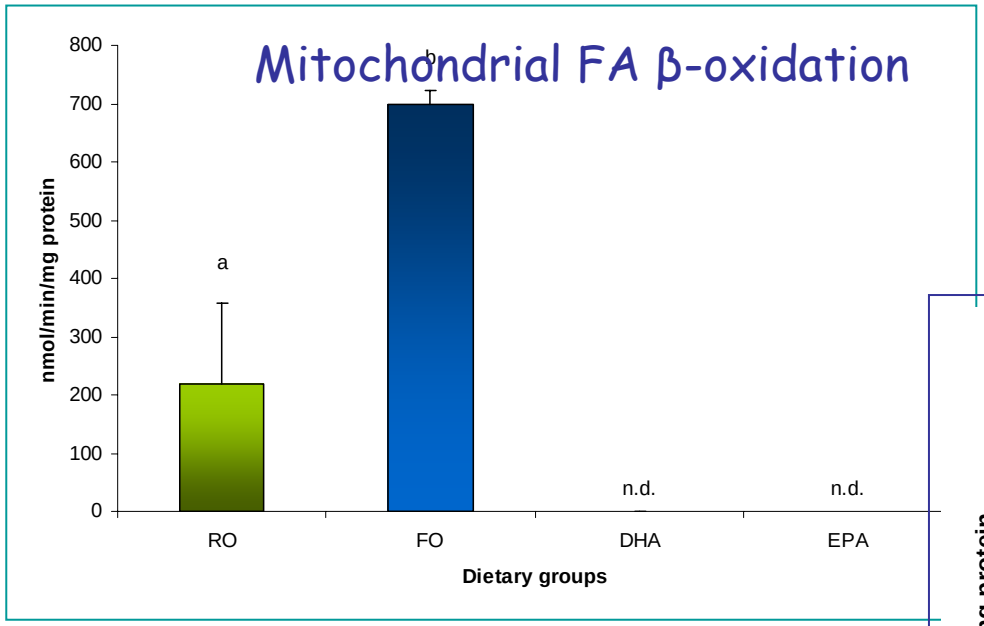
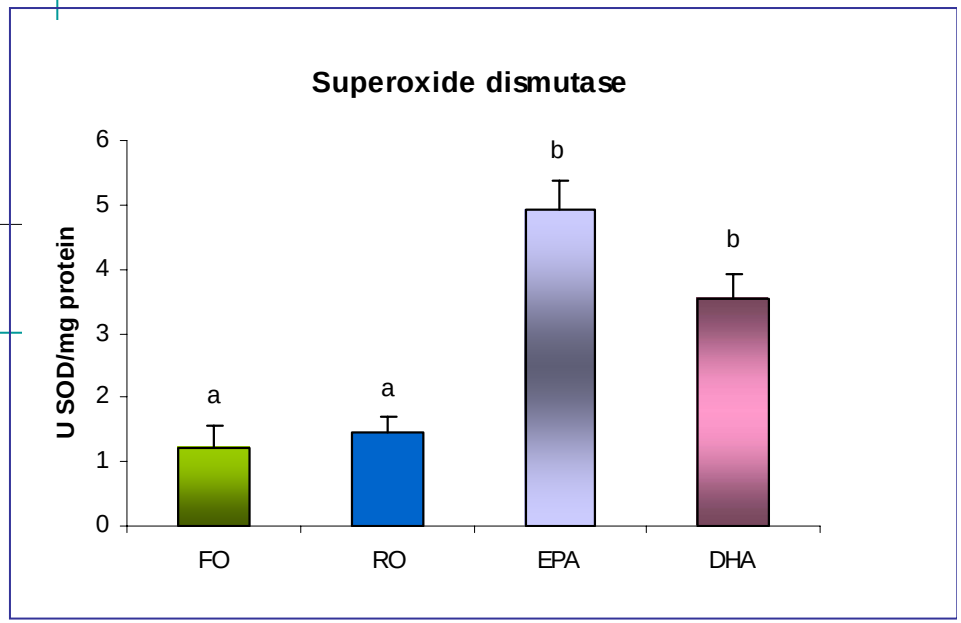
Also in adipose tissue does oxidative stress lead to induced SOD and loss of mitochondrial function





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Conclusions

- Feeding Atlantic salmon diets with very high EPA and DHA levels leads to;

- Oxidative stress symptoms in liver, muscle and adipose tissue

- Up-regulation of oxidative stress- and apoptosis markers

  - Reduced level of membrane phospholipids
  - Reduced mitochondrial function
  - Affected liver and muscle structure
  - Led to condensation of chromatin
  - Affected adipose tissue development

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