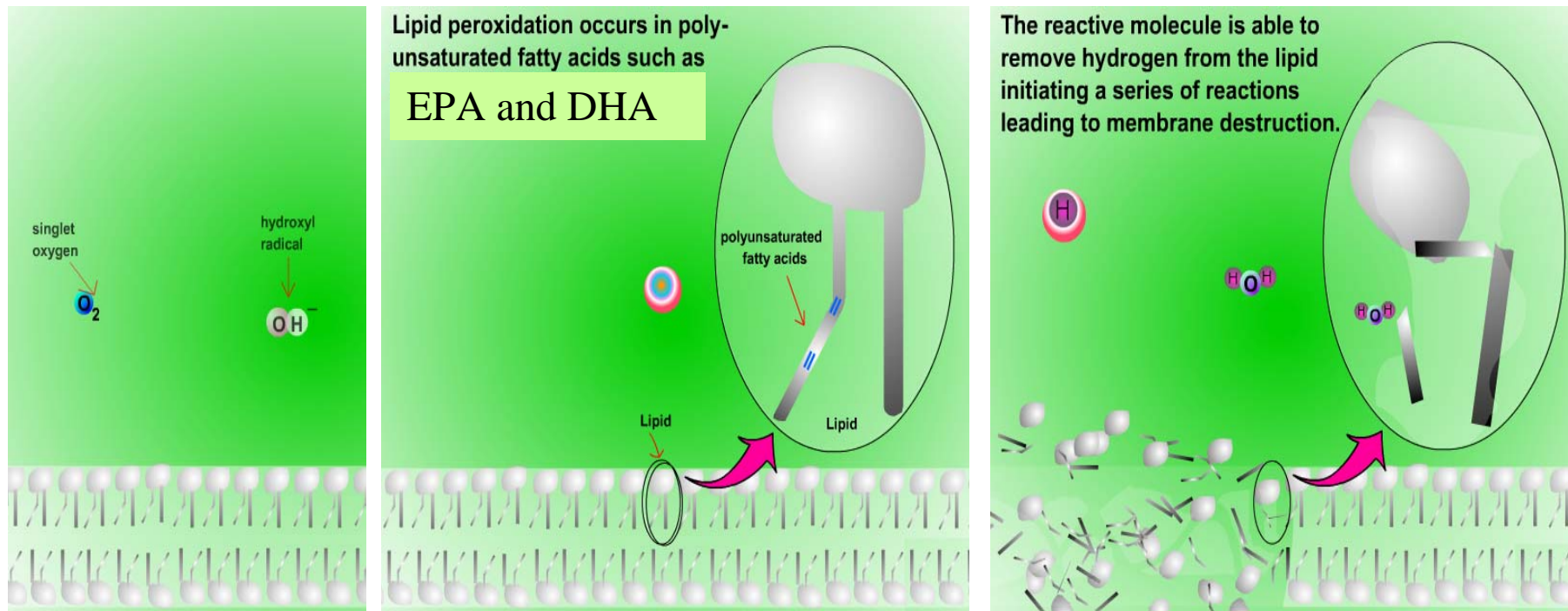


Lipid oxidation in relation to fish health and quality

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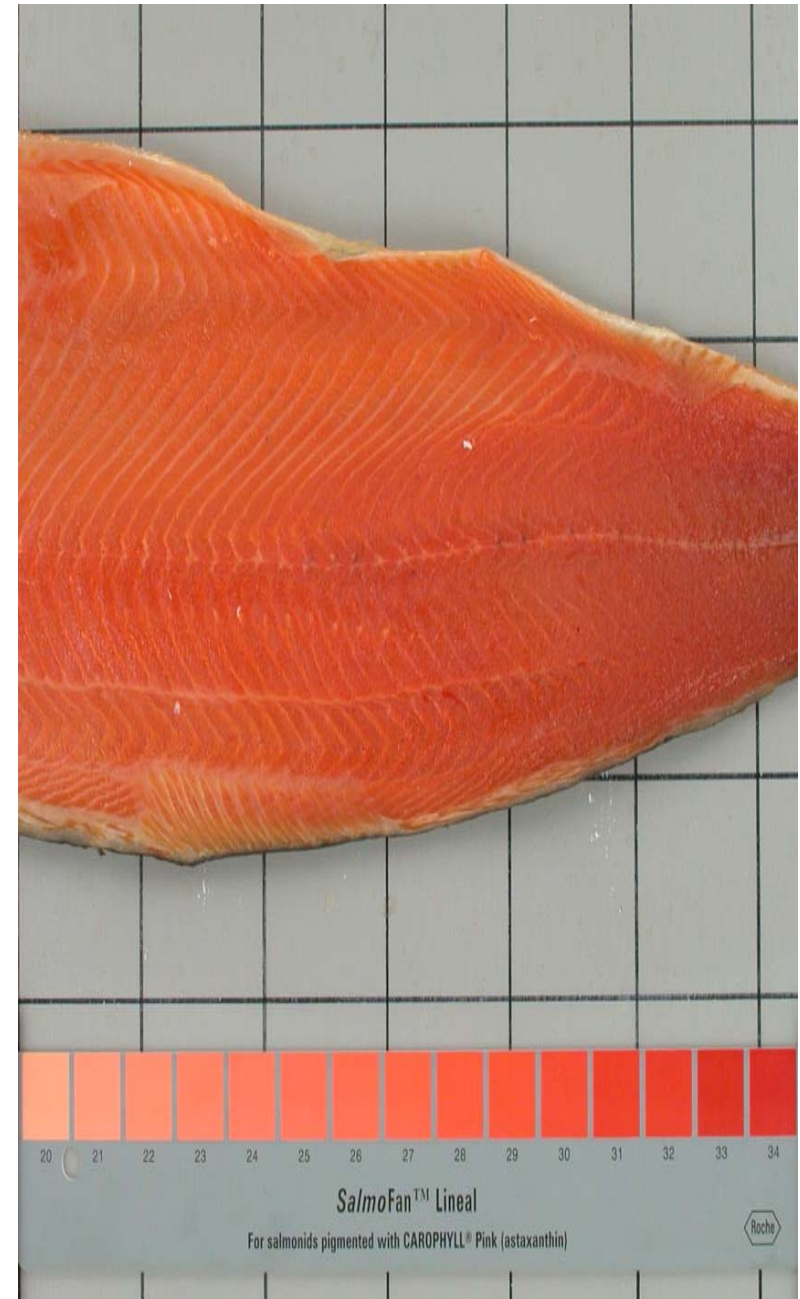
Background

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)

- 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



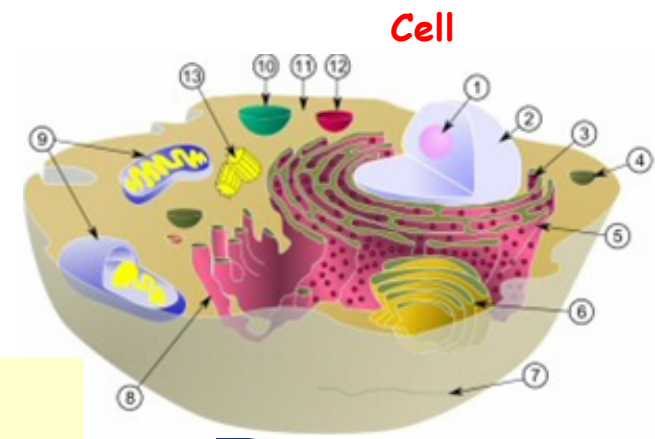
Lewis-McCrea, L.M., Lall, S.P., 2007. Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 262, 142-155).

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:

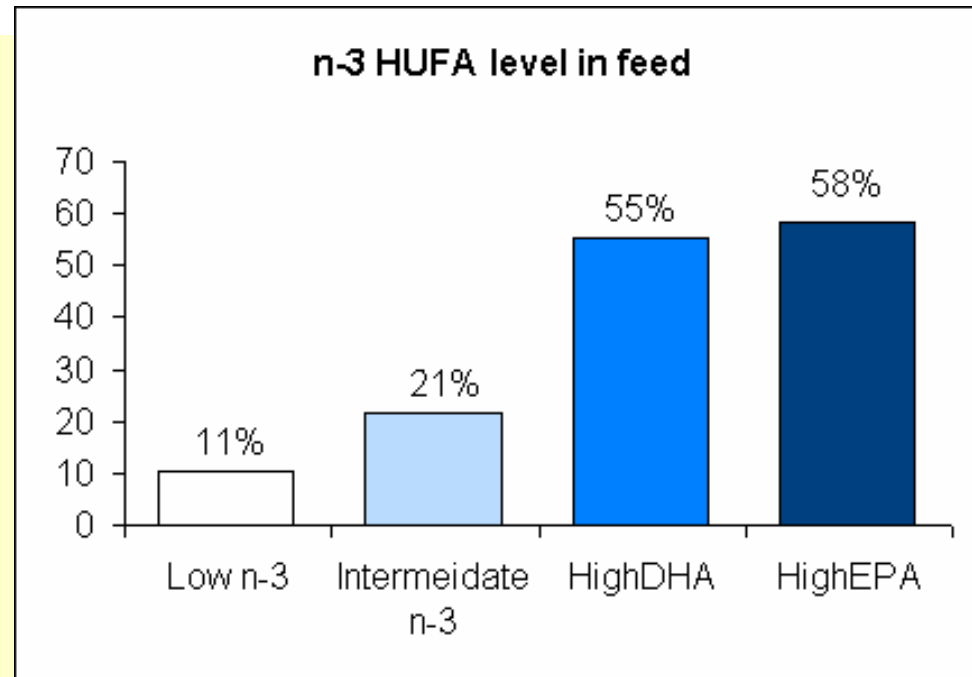
- Membrane lipids in cells and mitochondria
- Mitochondrial function
- Oxidative stress enzyme- and gene markers
- Apoptosis (particularly in white adipose tissue)
- Liver and muscle structure



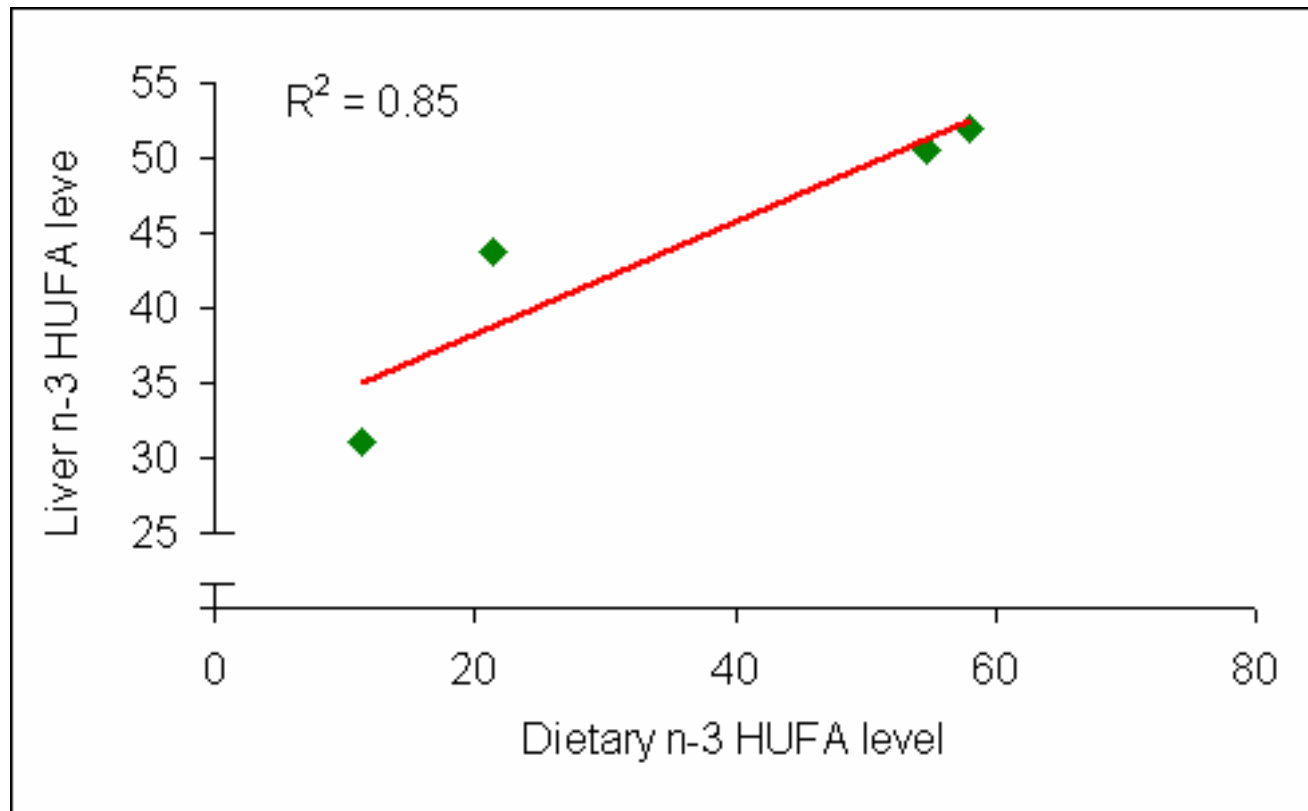
1: Nucleolus 2: Cell nuclei 3: Ribosome 4: Vesicle 5: ER 6: Golgi 7: Cytoskeleton
8: Smooth ER 9: Mitochondria 10: Vacuole 11: Cytoplasm 12: Lysosome

Fish trial

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



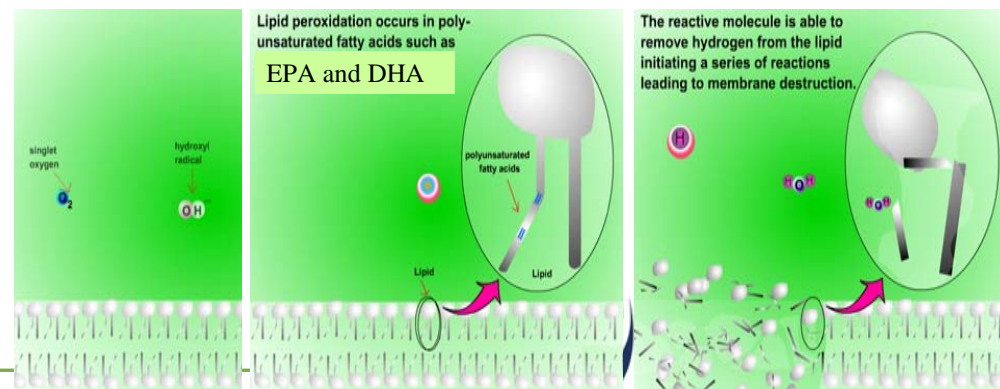
Increasing dietary levels of n-3 HUFAs led to increasing percentages of these FAs in fish tissues (here example from liver)



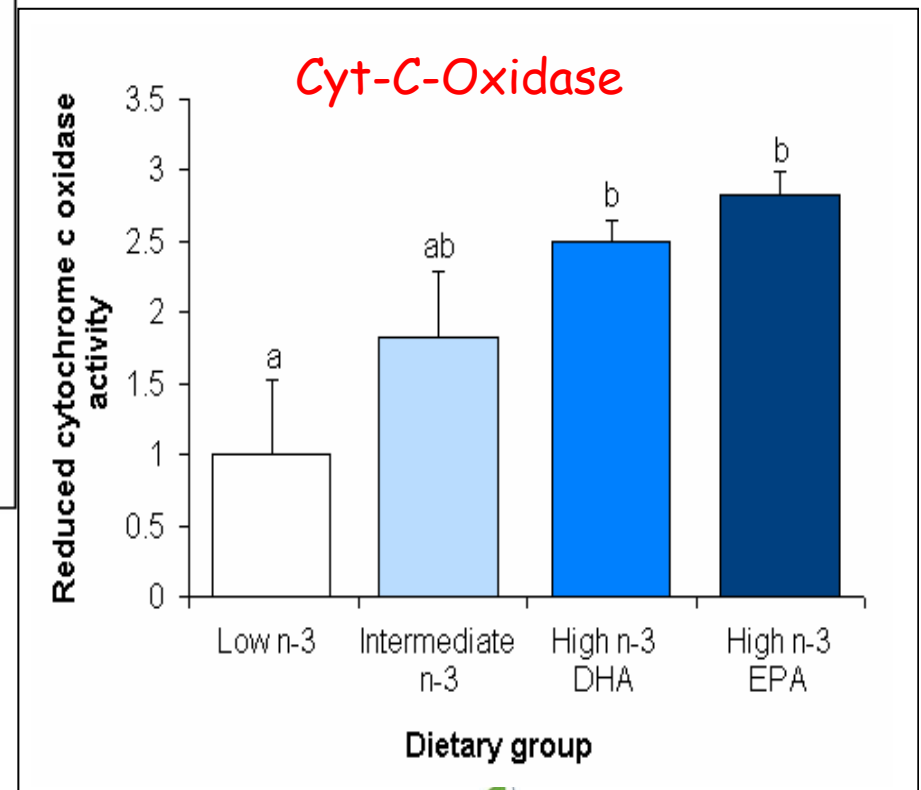
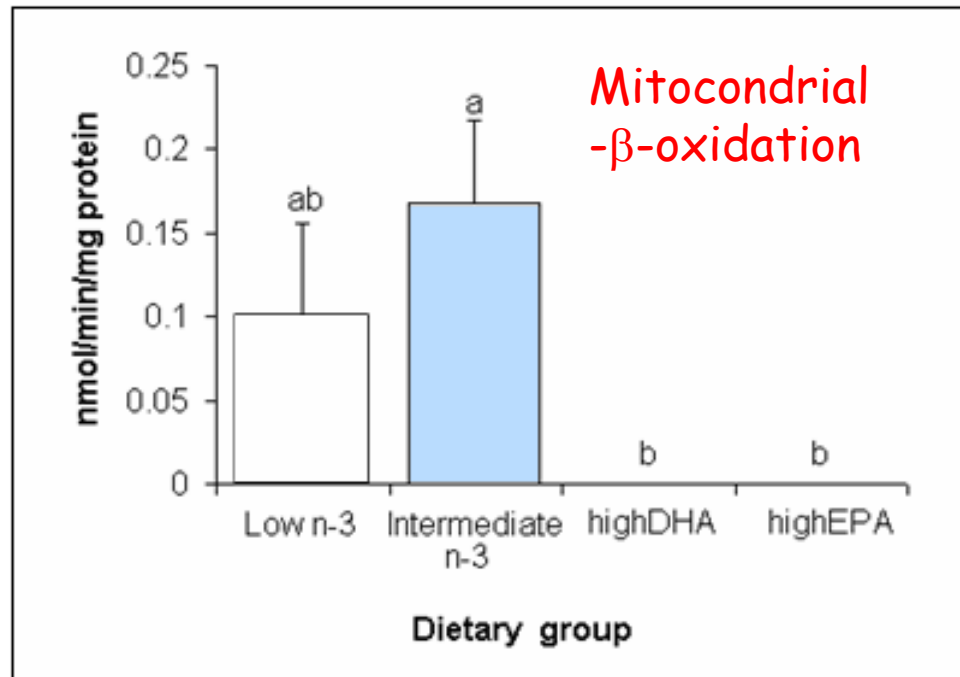
Lipid class composition of mitochondria isolated from liver

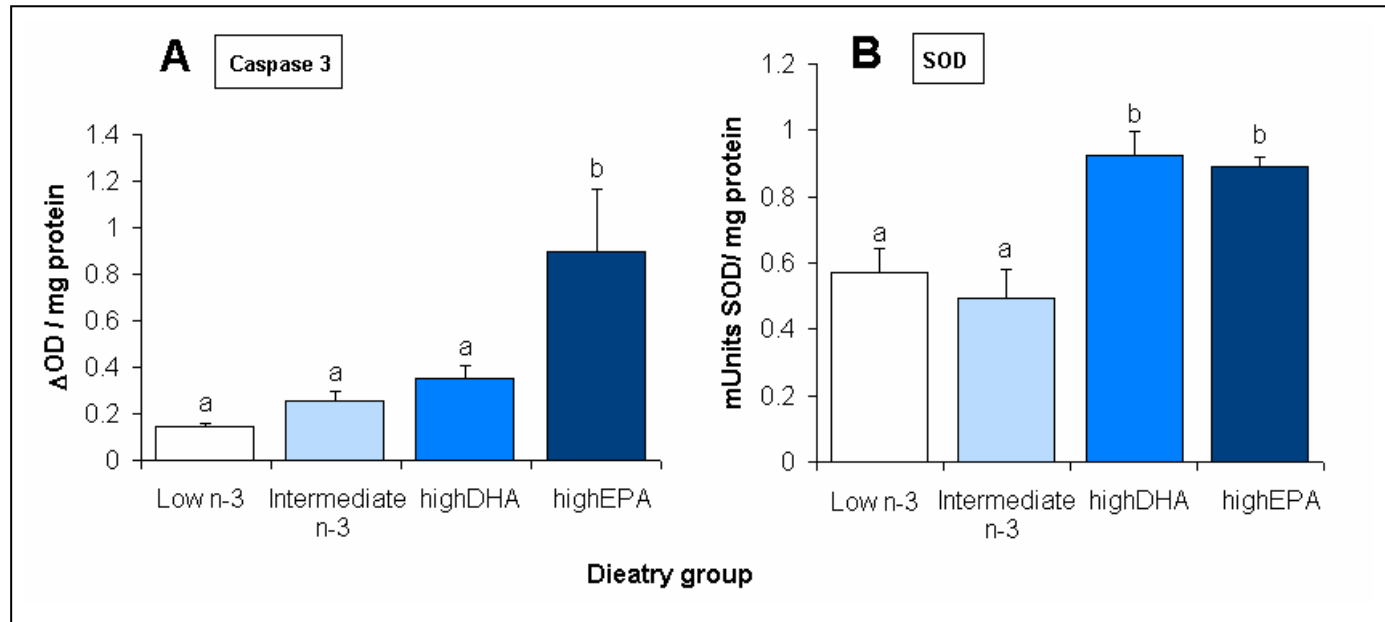
- Increasing dietary levels of n-3 HUFAs clearly decreased the percentage of major phospholipids (PLs) in mitochondrial membranes.
 - The percentage of cardiolipin (CL) in the mitochondrial membrane of the high EPA group was less than half of the percentage in the intermediate n-3 group.

	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
Σ PL	54.1±1.10 ^{ab}	62.9±5.74 ^a	53.6±5.98 ^{ab}	37.1±5.96 ^b
Σ NL	45.9±1.10 ^{ab}	37.1±5.74 ^a	46.4±5.98 ^{ab}	62.9±5.96 ^b

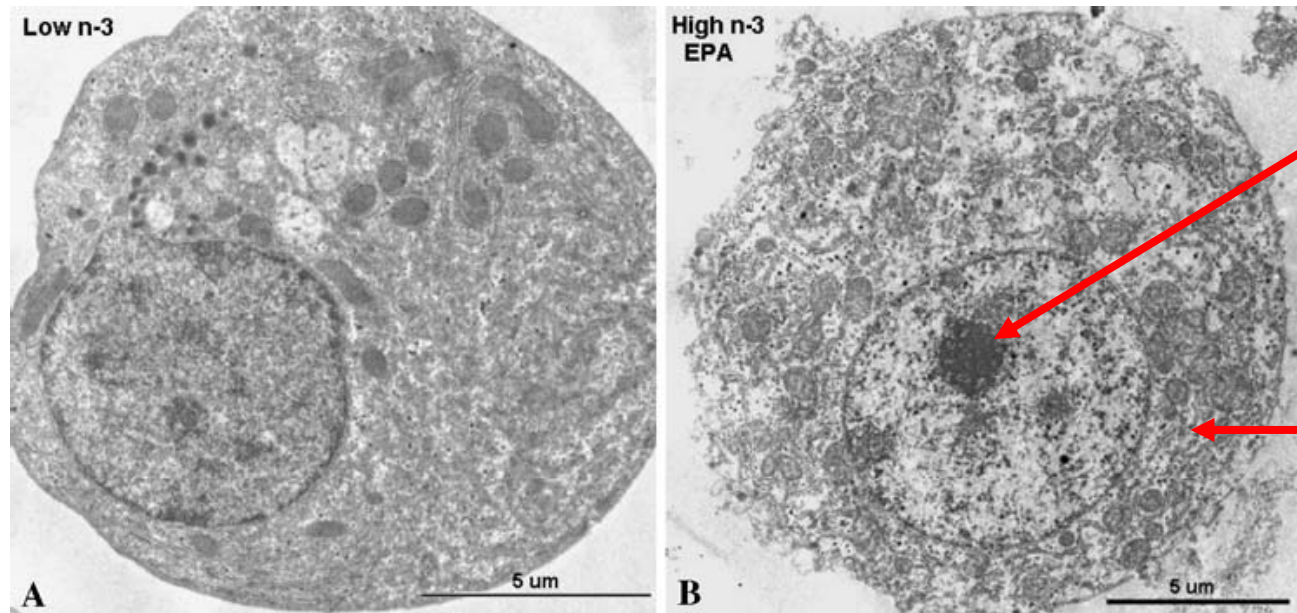


Oxidative stress and effects on mitochondrial function in salmon liver





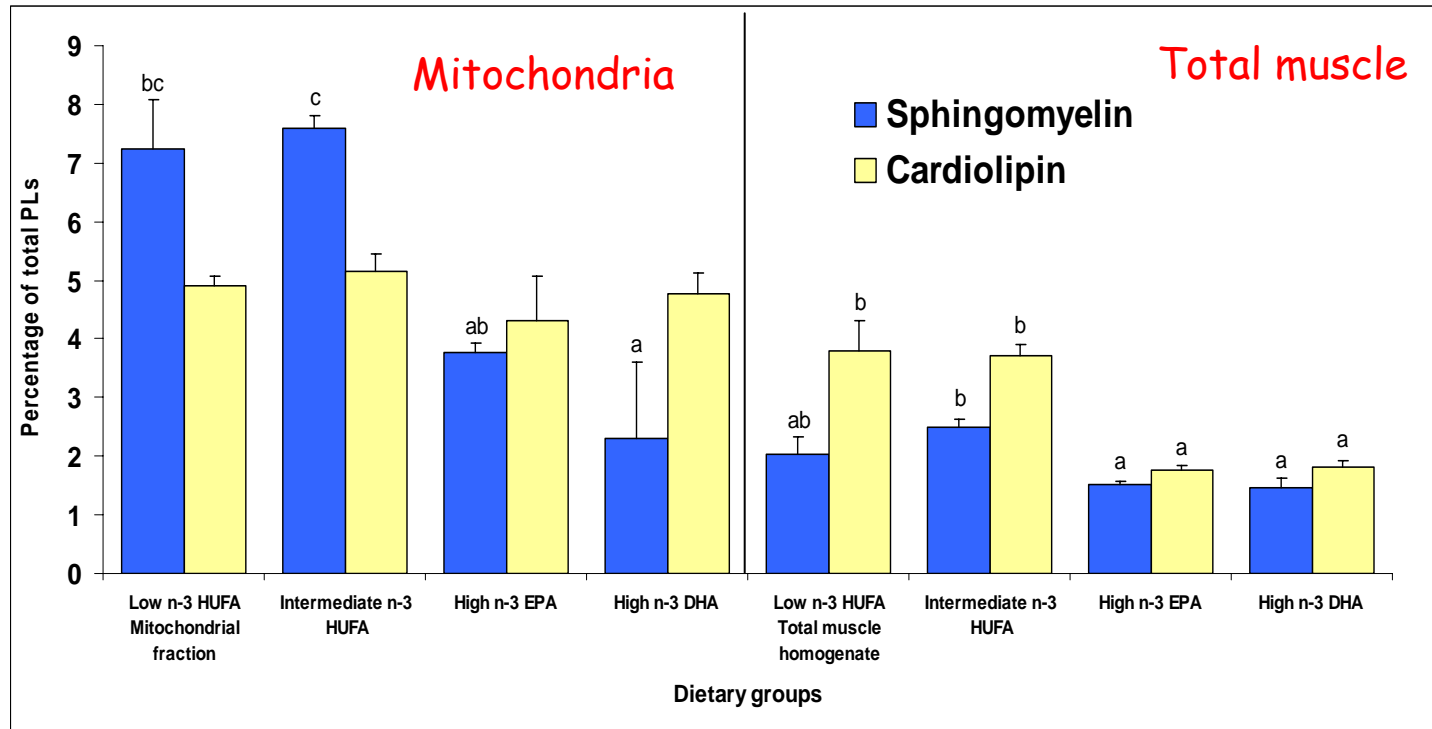
Both high EPA and high DHA lead to Oxidative stress



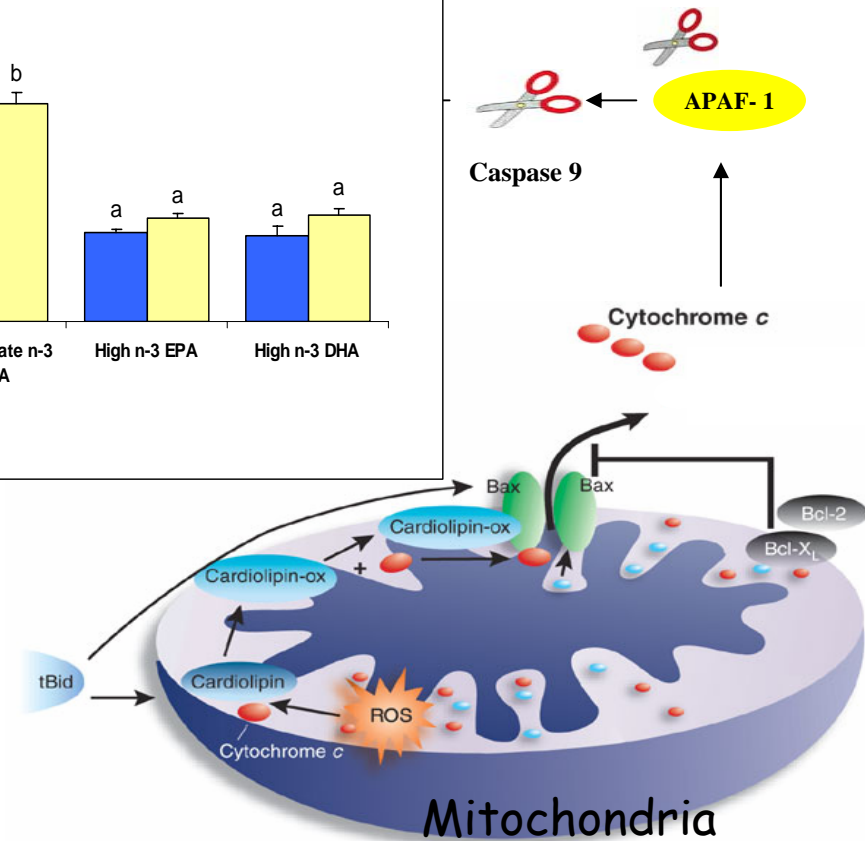
Oxidative stress leads to condensation of chromatin

Cellular content seemed to be released

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



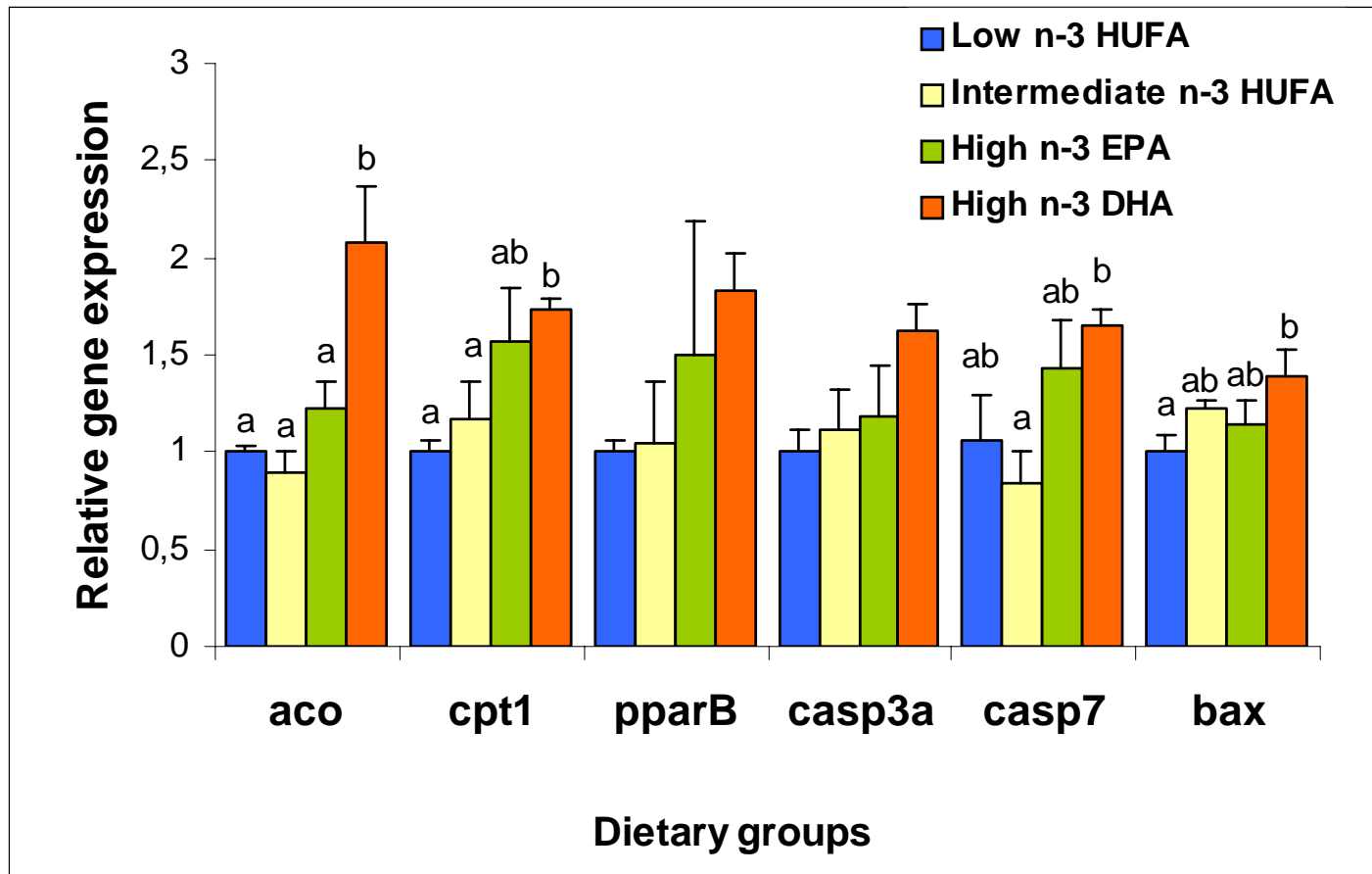
Cardiolipin
Sphingomyeline



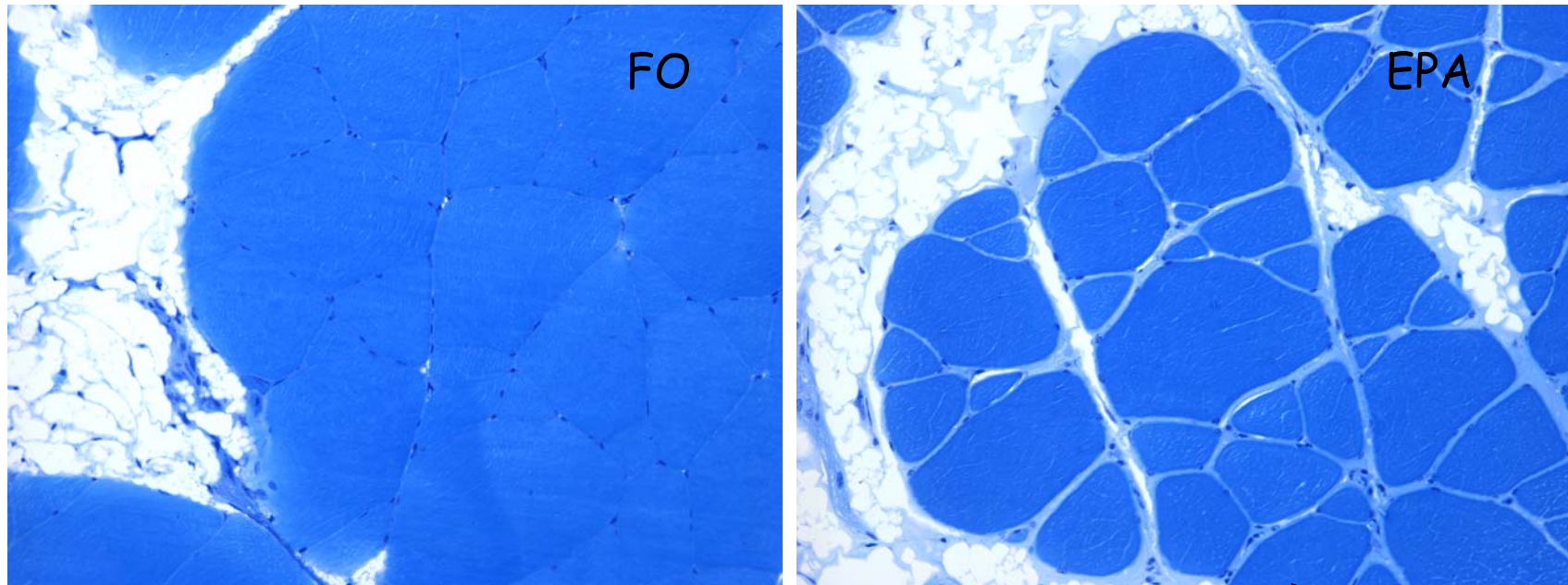
Østbye et al., 2008, Manuscript

Effects of diets on gene expression in muscle

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



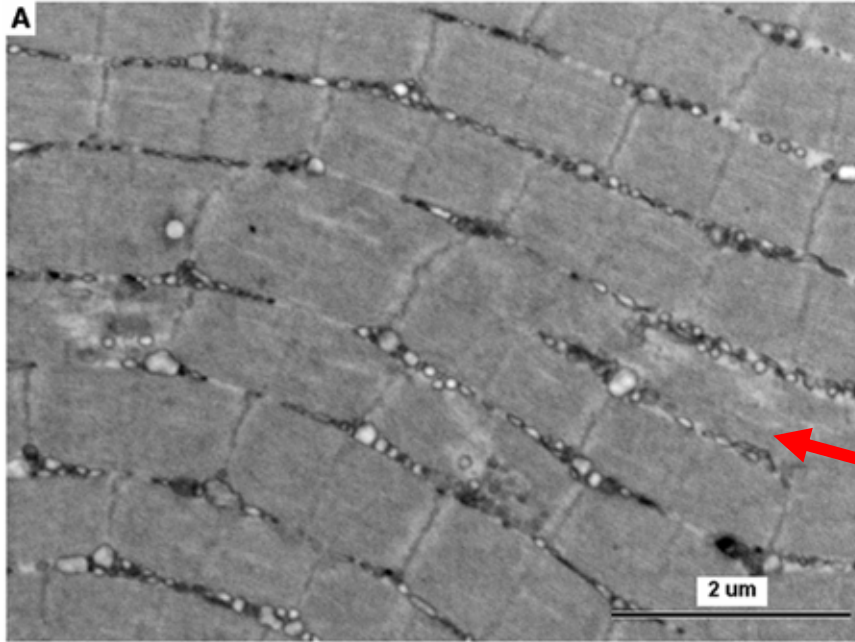
Oxidative stress led to changes in muscle structure (light microscopy)



Attachment

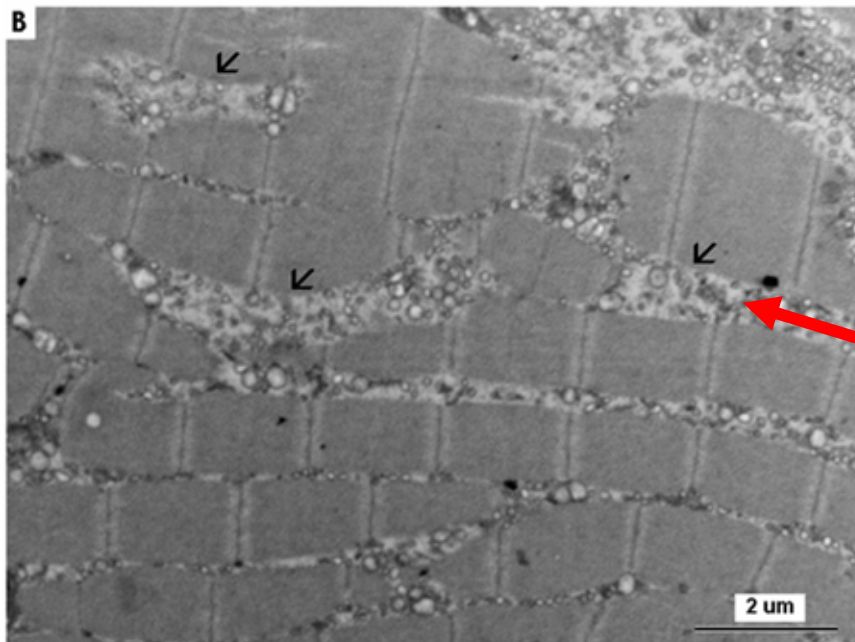
Myofiber-
myofiber
Detachment

Bahuaud et al., 2008



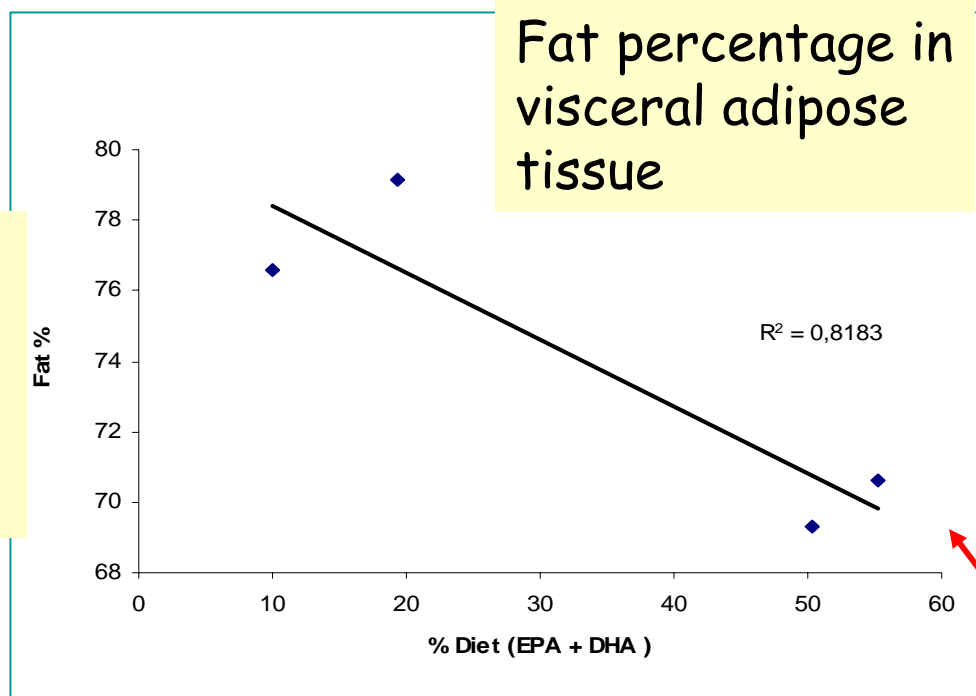
Electron microscopy pictures
of muscle

normal

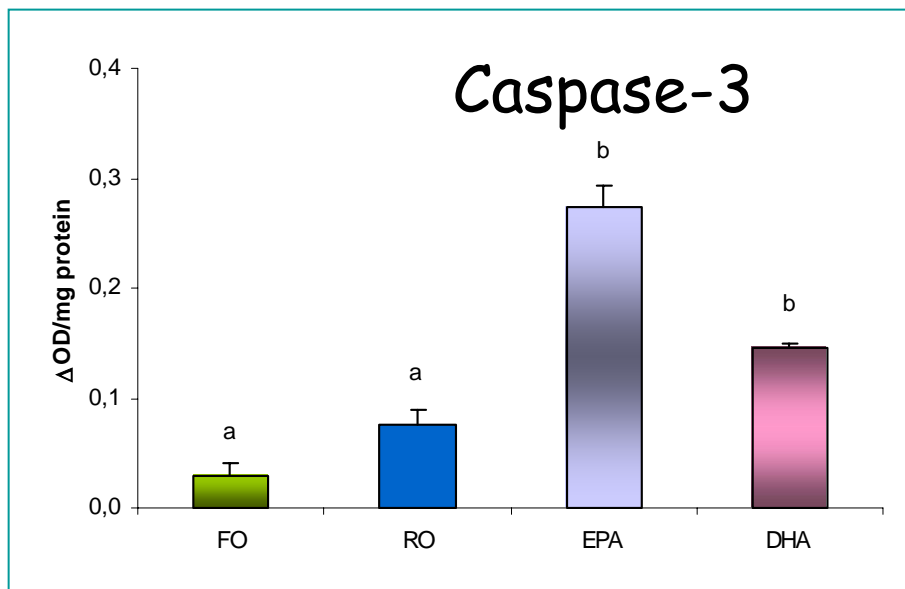


detachment, particularly
observed in the high HUFA
groups

Visceral adipose tissue

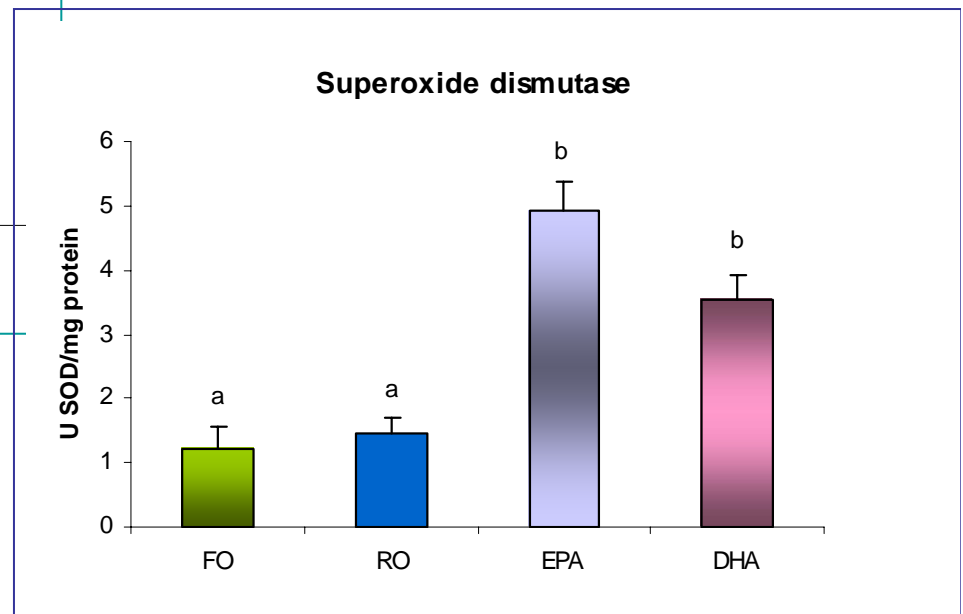
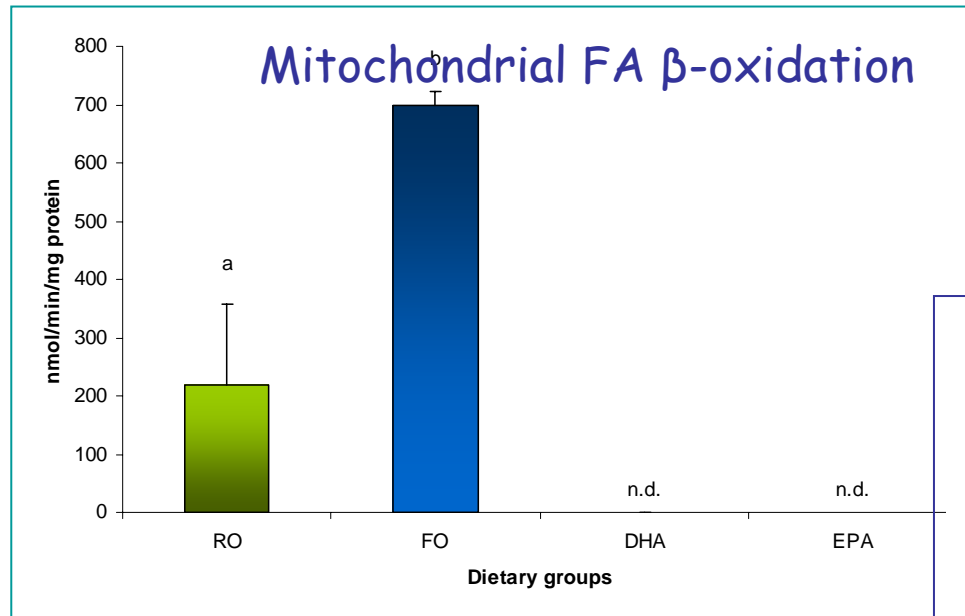


Lower Fat%



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%

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



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