High mitochondrial densities in the hearts of Antarctic icefishes are maintained by an increase in mitochondrial size rather than mitochondrial biogenesis

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Icefish

- Three species of Antarctic fish
  - *Chaenocephalus aceratus*- Hb/-Mb
  - *Notothenia coriiceps*- Red blooded
  - *Chionodraco rastrosinosus*- Hb/+Mb
- Live from 5-500m deep
Fish Heart

- Fish hearts only have three chambers
- Tissue samples taken from icefish were from the ventricle
Mitochondria and Metabolism

- Mitochondria are responsible for aerobic metabolism
- Increased mitochondrial density results in increased oxidative metabolic (aerobic) capacity
- Mitochondrial biogenesis occurs in response to external stimuli such as exercise
Mitochondrial Biogenesis

- Mitochondria are produced by transcription/translation of genes both from mitochondrial and nuclear genomes.
- Transcriptional coactivator PGC-1α activates NRF-1 and 2.
- NRF-1 and 2 turn on mitochondrial transcription factor A (tfam).
- Tfam is directly responsible for transcribing nuclear encoded mitochondrial proteins.

NUGEMPS - nuclear genes encoding for mitochondrial proteins.
Oxidative Metabolism
Hemoglobin/ Myoglobin

• Hemoglobin- in blood (RBC’s)
• Four cooperative oxygen binding sites

• Myoglobin: in Muscle
• One oxygen binding site
• Higher affinity for $O_2$ than Hb.
Heme group with central Iron atom (Fe)
Hemoglobin/Myoglobin Function

• Problem:
• Oxygen diffuses freely through membranes but does not dissolve well in aqueous solution
• Solution:
• Hemoglobin and Myoglobin both function to lower free oxygen concentrations at a cell’s periphery in order to facilitate diffusion down concentration gradient
• Mitochondrial oxygen consumption ultimately drives this oxygen binding cascade.
Oxygen binding cascade

During Exercise...

Lungs/Gills → Blood → Muscle

HbO₂ → MbO₂

High [O₂] → Low [O₂]

Mitochondria
The icefish paradigm

- Cold, deep environment decreases oxygen-diffusion coefficient
- Lose large oxygen binding proteins to decrease blood viscosity
- Increase heart size, increased capillary size allow for more than two fold increase in blood circulation
- Not energetically favorable
- Increase in mitochondrial density without increase in metabolic capacity
- Why higher mitochondrial density?
- Why wouldn’t you want a higher oxidative capacity in this circumstance?
Methods

• Transmission Electron Microscopy
  – Mitochondrial volume fraction and surface density calculated

• Spectrophotometry
  – Quality of nucleic acids in RNA and DNA assessed

• qRT-PCR
  – Quantitatively evaluate specific gene expression levels of Transcription coactivators and citrate synthase.
Table 3: Mitochondrial densities in Heart Ventricle of notothenioid fishes

<table>
<thead>
<tr>
<th></th>
<th>N. Coriiceps (+Hb/+Mb)</th>
<th>C. Rastrospinosus (-Hb/+Mb)</th>
<th>C. Aceratus (-Hb/-Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume density (%)</td>
<td>18.18 ±0.69</td>
<td>20.10±0.74</td>
<td>36.53±2.07</td>
</tr>
</tbody>
</table>
Expression of PGC-1α and NRF-1

• Higher mitochondrial density in *C. aceratus*
• Expected higher PGC-1α and NRF-1
### Table 5: Amount of RNA and DNA in heart ventricles of Antarctic notothenioids

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</tr>
</thead>
<tbody>
<tr>
<td>Total RNA (mg g⁻¹ wet mass)</td>
<td>1.01±0.16</td>
<td>0.67±0.08</td>
<td>1.13±0.14</td>
</tr>
<tr>
<td>Total DNA (mg g⁻¹ wet mass)</td>
<td>0.33±0.03</td>
<td>0.24±0.02</td>
<td>0.26±0.02</td>
</tr>
</tbody>
</table>
Quantification of mitochondrial DNA copy number

- Expected higher mitochondrial copy number in *C. aceratus*
Citrate synthase

- Enzyme in the first step of the Citric acid cycle
- Housed inside the mitochondrial matrix
- Commonly used as a quantitative enzyme marker for intact mitochondria as well as oxidative capacity

![Citrate synthase diagram](image)
Citrate synthase expression

- Expected higher Expression in *C. aceratus*

- Oxidative metabolic capacity is not likely increased
- Why not measure cytochrome oxidase as well?
Relative expression of mitochondrial biogenic mediating genes

- Used to determine if PGC-1α and NRF-1 are responsible for biogenesis in these fish
- Red adductor muscle and white muscle from *N. coriiceps*
- Both tissues had known significant differences in mitochondrial density but not different size
Increased organelle size: Culprit for increased mitochondrial density

*N. coriiceps*  
*C. aceratus*

Scale bar = 500nm
Conclusions

- Increased Mitochondrial density in *C. aceratus* is due to increased size of organelles
- This occurs without parallel increase in metabolic capacity
- PGC-1α and NRF-1 and 2 do facilitate mitochondrial biogenesis in these fish
- However, this pathway is not the pathway responsible for increased mitochondrial density in this case
Discussion

- Why do myoglobinless fish have higher mitochondrial density than other red-blooded fish?
- Why are the mitochondria so large without increasing metabolic capacity?
Oxygen diffusion and Mitochondrial density

• Decrease diffusion distances?
• Increase amount of soluble medium for oxygen? (hydrophobic bilayer) (Increased storage)

= Oxygen  ○ = Mitochondria
Discussion cont..

• Are there any other possible reasons why mitochondria in these fish would be so large?
• From the data presented in this paper, is Nitric Oxide (NO) a reasonable hypothesis?