Impaired fatty-acid metabolism in tafazzin-deficient mice

Zaza Khuchua, PH.D.
Cincinnati Children’s Medical Center
Cincinnati, OH

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Overview

- Background: phospholipids, cardiolipin, Barth syndrome, mouse model, mitochondrial defects in sarcomeric tissue.

- Indirect calorimetry: Energy expenditure, Oxygen consumption, Respiratory Exchange Ratio (RER) in basal and stressed conditions. Cold exposure and forced exercise on treadmill.

- Mitochondrial respiration in taz-deficient neonatal cardiomyocytes. Mitochondrial proteomics.

- Cardiolipin in physical interaction of fatty acid oxidation enzymes with mitochondrial complexes. 2D-Native electrophoresis.
Cellular Phospholipids

CARDIOLIPIN IN HEART AND MUSCLE

Heart

Skeletal muscle

L4CL

CL, nmol mg w.w.

CL, nmol mg w.w.
TAFAZZIN KNOCKDOWN SKELETAL MUSCLE

Acehan et al. JBC 2011
Indirect Calorimetry
Calorimetry
At room temperature

Calorimetry cold (+5°C)

Calorimetry during exercise on treadmill
Definitions & Abbreviations Used in Calorimetry

- Reference O₂ Concentration \((O_2i)\)
- Reference CO₂ Concentration \((CO_2i)\)
- Sample O₂ concentration \((O_2o)\)
- Sample CO₂ concentration \((CO_2o)\)
- Fresh Air Flow

\[
\begin{align*}
\text{VO}_2 &= V_iO_2i - V_oO_2o \\
\text{VCO}_2 &= V_oCO_2o - V_iO_2i \\
\text{Respiratory Exchange Ratio (RER)} &= \frac{\text{VCO}_2}{\text{VO}_2} \\
\text{Heat} &= CV \times \text{VO}_2, \text{ where } CV = 3.815 + 1.232 \times \text{RER}
\end{align*}
\]
If carbohydrate is completely oxidised to CO$_2$ and H$_2$O then the relationships is as follows:

$$6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O + 38\text{ATP}$$

$$\text{RER} = \frac{6CO_2}{6O_2} = 1$$

If fat is completely oxidised to CO$_2$ and H$_2$O then the relationships is as follows:

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 129\text{ATP}$$

$$\text{RER} = \frac{16CO_2}{23O_2} = 0.7$$

**RESPIRATORY EXCHANGE RATIO (RER)**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>RER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>1</td>
</tr>
<tr>
<td>Mixed substrates, Protein</td>
<td>0.7</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7</td>
</tr>
</tbody>
</table>
ENERGY EXPENDITURE
(Resting, Dark cycle)
ENERGY EXPENDITURE
(Resting, Dark cycle)

WT

Taz-KD
FORCED EXERCISE ON THE TREADMILL

![Graph showing the relationship between RER and Work (mJ) for Taz-KD and WT participants.](image-url)
Oxygen consumption on the treadmill

ΔO₂

Taz-KD  WT Control
RER on the treadmill

Exercise intensity
RER on the treadmill

![Graph showing RER over time with three different groups: WT, Taz KD, and TRDM.](image_url)
Tafazzin-deficient mice demonstrate normal rates of energy expenditure at basal resting condition.

When exposed to cold, energy expenditure in Tafazzin-deficient mice is severely impaired due to limited ability to consume oxygen.

When subjected to moderate-intensity workload, Tafazzin-deficient mice exhibit reduced rates of oxygen consumption and fail to adapt to high-energy demands.

How mitochondrial function is affected in Tafazzin-deficient mice?
Metabolic profiling of tafazzin-deficient mouse neonatal cardiomyocytes

OXYGEN CONSUMPTION

GLYCOLYTIC FLUX
Palmitate-stimulated respiration in cardiomyocytes

Palmitate~BSA
200 μM
Lipid metabolism:
• Tri-functional protein, subunit β
• Acyl-Co A thioesterase 2

ETC and Metabolism:
• COX6
• ANT-1&2
• ATP synthase, subunit β
• NADP transhydrogenase
• Malate dehydrogenase

Protein sorting and degradation:
• Lon protease homolog
• HSP60
• HSP70

Ca^{2+} homeostasis:
• SERCA-2a

Structural proteins:
• Myosin-6
• Myosin LC 3
• Myosin regulatory LC 2
• α-Actin
• Tropomyosin α-1

Evidence of Physical association of FAO and OXPHOS complexes.
Y. Wang et al. JBC, 280(39) 2010
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Is CL required for physical interaction of FAO system with OXPHOS complexes?
FATTY ACID OXIDATION

MITOCHONDRIAL TRIFUNCTIONAL PROTEIN
2D-Native gel electrophoresis

Complex-I zymograms

TFP-alpha (LCHAD) western blots

TFP-beta (Thiolase) western blots

WT

Taz-KD

WT

Taz-KD

TFP-α (LCHAD)

C-1 (NDUFS3)
SUMMARY

✧ Under stress conditions, energy expenditure is severely limited in Tafazzin-deficient mice.

✧ Tafazzin-deficiency results in significant reduction of maximal mitochondrial oxygen consumption in neonatal mouse cardiomyocytes, while glycolytic activity is preserved.

✧ Oxidation of fatty acids is impaired in Tafazzin-deficient cardiomyocytes.

✧ In Tafazzin-deficient mitochondria physical interaction between C-I and TFP is destabilized. Evidence suggests that cardiolipin is required for interaction of TFP with ETC complex I.
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