Targeting cardiolipin content and composition in the Taz shRNA mouse model of Barth syndrome

Investigating the therapeutic effects of dietary linoleate supplementation and thyroxine treatment

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Progressive loss of cardiac L\(_4\)CL in Spontaneously Hypertensive HF rats

Supplementation with 20% (w/w) high-18:2 safflower oil restores L₄CL and improves survival in aged SHHF rats

20% HLSO or Lard diets beginning at 18 mo of age until moribund

Linoleate-Rich High-Fat Diet Decreases Mortality in Hypertensive Heart Failure Rats Compared With Lard and Low-Fat Diets

Adam J. Chicco, Genevieve C. Sparagna, Sylvia A. McCune, Christopher A. Johnson, Robert C. Murphy, David A. Bolden, Meredith L. Rees, Ryan T. Gardner and Russell L. Moore

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Figure 1. Kaplan-Meier curves illustrating cumulative mortality because of HF (A) and all causes (B) beginning at 18 months. The HLSO diet significantly improved survival resulting from HF and all causes compared with CON and lard, whereas the lard diet increased HF mortality.
Dietary linoleate preserves cardiolipin and attenuates mitochondrial dysfunction in the failing rat heart

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Male 21 mo old SHHF rats (early CHF) fed 10% HLSO or Lard (w/w) 4 weeks
Will dietary HLSO supplementation enrich cardiolipin with 18:2n6 and improve cardiac mitochondrial respiratory function in Taz shRNA mice?
Effect of HLSO supplementation on mito PL composition in WT vs. Taz mice

Male taz shRNA mice 4-5 mo of age; 10% HLSO (w/w) mixed in chow for 4 weeks

↑18:2n6, ↓18:1 in WT and taz (all PLs)

PC 18:2n6: taz > WT

PE 20:4n6: taz > WT
Modest increase in cardiac mito CL content and L4CL% with HLSO supplementation

**Total CL**

**L4CL (%)**

**L3-MLCL (%)**

**“Saturated” CL**

(SSM, IFM)

(SSM, IFM)

(SSM, IFM)

(SSM, IFM)
HLSO does not improve mitochondrial OXPHOS capacity or efficiency in *Taz* or WT mice

Pyruvate + Malate as substrates; n = 6-8/group
Will stimulation of CL biosynthesis increase mito CL content and improve mitochondrial respiratory function in the presence of Taz deficiency?
Thyroxine (T4) stimulates CL biosynthesis and mitochondrial respiratory function

Thyroxine stimulates PGPS activity in rat heart mitochondria.

Enhanced cytochrome oxidase activity and modification of lipids in heart mitochondria from hyperthyroid rats

G. Paradies *, F.M. Ruggiero, G. Petrosillo and E. Quagliaiello
Department of Biochemistry and Molecular Biology and CNR Unit for the Study of Mitochondria and Bioenergetics, University of Bari, Bari (Italy) Biochimica et Biophysica Acta, 1225 (1994) 165-170

Effect of hyperthyroidism on the transport of pyruvate in rat-heart mitochondria

Giuseppe Paradies and Francesca Maria Ruggiero
Department of Biochemistry and Molecular Biology and C.N.R. Unit for the Study of Mitochondria and Bioenergetics, University of Bari, Bari (Italy) Biochimica et Biophysica Acta, 935 (1988) 79-86

Thyroid Replacement Therapy and Heart Failure

Anthony Martin Gerdes, PhD; Giorgio Iervasi, MD
Circulation. 2010;122.385-393
Thyroxine treatment increases total CL, but decreases L4CL% in Taz shRNA and WT mice

Male WT or Taz mice 4-5 mo of age; 0.1% T4 mixed in chow for 4 weeks (n = 6 / group)
Effect of T4 on mitochondrial PL composition

Male WT or Taz mice 4-5 mo of age; 0.1% T4 mixed in chow for 4 weeks (n = 6 / group)

- T4 ↑18:1n7, ~↓18:2n6 in CL
- T4 ↑18:2n6 in PC, not CL or PE
- T4 ↑22:6n3 in WT in all PLs, less/no effect in taz
T4 treatment fails to restore mitochondrial respiratory function in *Taz* shRNA mice

**ADP-Stimulated respiration (State 3)**

**“Uncoupled” respiration (State 4)**

**Respiratory control ratio (State 3/State 4)**

**O2 consumed in State 3**

Pyruvate + Malate as substrates; n = 6 / group
Effect of HLSO and T4 on LV chamber size and contractile function

**LV End-Diastolic Area**

**LV Fractional Shortening**

- WT
- WT + LA
- WT + TH
- WT + LA + TH
- Taz
- Taz + LA
- Taz + T4
- Taz + LA + TH

* indicates significance.
Summary

- HLSO suppl. partially restores CL 18:2 content, but fails to improve mitochondrial respiratory function.
- T4 tx partially restores total CL levels, but fails to improve mitochondrial respiratory function in Taz mice, despite having stimulatory effects in WT mice.
- Effect of HLSO+T4 on CL content/composition and mito respiration is pending, but tx augments cardiac dilatation and contractile dysfunction in Taz mice.
- What if we could restore L4CL to ‘normal’ levels without HLSO or T4 treatment?
Cardiolipin accumulates long-chain PUFAs in cardiac overload, heart failure and senescence.

CL remodeling parallels a progressive *loss* of linoleic acid and *increase* in arachidonic acid (20:4n6) and/or DHA (22:6n3) in the global myocardial phospholipid pool.

Sparagna et al. *J Lipid Res*, 2005
Delta-6 Desaturase: central role in PUFA metabolism... and phospholipid composition?
D6D inhibition normalizes phospholipid PUFA profile and restores L₄CL in TAC and HF

**Figure A**

- **18:2n6**
  - SC: 20, Sham: 22, TAC: 24, HF: 19
  - SC: 20, Sham: 22, TAC: 24, HF: 19
  - SC: 20, Sham: 22, TAC: 24, HF: 19
  - SC: 20, Sham: 22, TAC: 24, HF: 19

- **20:4n6**
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10

- **22:6n3**
  - SC: 10, Sham: 15, TAC: 20, HF: 5
  - SC: 10, Sham: 15, TAC: 20, HF: 5
  - SC: 10, Sham: 15, TAC: 20, HF: 5
  - SC: 10, Sham: 15, TAC: 20, HF: 5

**Figure B**

- **L₄CL**
  - SC: 70, Sham: 80, TAC: 90, HF: 60
  - SC: 70, Sham: 80, TAC: 90, HF: 60
  - SC: 70, Sham: 80, TAC: 90, HF: 60
  - SC: 70, Sham: 80, TAC: 90, HF: 60

- **(HUFA)CL**
  - SC: 30, Sham: 40, TAC: 50, HF: 20
  - SC: 30, Sham: 40, TAC: 50, HF: 20
  - SC: 30, Sham: 40, TAC: 50, HF: 20
  - SC: 30, Sham: 40, TAC: 50, HF: 20

- **Total CL**
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10
  - SC: 15, Sham: 20, TAC: 25, HF: 10

**CL Subspecies profile (LC/MS)**

- 4 mo SHHF
- 22 mo SHHF (eHF)
- 22 mo SHHF + SC-26196
D6D inhibition preserves respiratory efficiency, but fails to restore State 3 respiratory capacity.

**Panel A**
- **State 3**: umol/O/mg protein
- **State 4**: umol/O/mg protein
- **RCR**: RCR
- **ADP/O**: ADP/O

**Panel B**
- SC-26196 vs Sham

**Notes**
- SC: Sham
- TAC: TAC
- HF: HF
- *: Significance
- #: Significant difference

**Graphs**
- Bar graphs showing the comparison between treatments and controls.
D6D inhibition reverses loss of CL 18:2n6 in aged mouse hearts without significant changes in mitochondrial respiratory function.

4 mo and 24 mo old C57Bl/6 mice treated with the D6D inhibitor SC-26196 (100mg/kg/d in chow) for 4 weeks (n = 6 / group)
Conclusions

• 18:2n6 enrichment of cardiac CL is not a major regulator of mitochondrial respiratory (dys)function in the heart...at least not in aging/HF rodent models

• Lack of T4 benefit suggests that augmenting CL biosynthesis/mito biogenesis may not rescue Taz-deficient mito respiratory phenotype either

• Perhaps the pathologic effects of Taz deficiency extend beyond alterations in CL content/composition

• *How exactly does Taz deficiency impair cardiac mitochondrial respiratory function?*
Substrate specificity of respiratory dysfunction in *Taz* shRNA cardiac mitochondria

4-5 month old male WT and Taz shRNA mice (n = 8-12/group)
Respiratory chain dysfunction does not contribute significantly to OXPHOS impairment in Taz deficiency.

Taz deficiency must impair mitochondrial membrane transport of pyruvate and fatty acids and/or otherwise limit their oxidation by TCA cycle enzymes.
Metabolomic profile of Taz shRNA vs. WT hearts

* Enter TCA as Acetyl-CoA or Pyruvate
# Ketogenic/gluconeogenic

WT (n = 4)
taz shRNA (n = 4)
Notable effects of B₅/CoA deficiency

- Impaired oxidation of glucose and fatty acids
- Enhanced taurine excretion (in rats)
- Growth failure and adrenal insufficiency
- Impaired cholesterol biosynthesis/ hypocholesterolemia

leucine→3-methylglutaconyl-CoA →HMG-CoA

Dilated cardiomyopathy due to type II X-linked 3-methylglutaconic aciduria: successful treatment with pantothenic acid

I Östman-Smith, G Brown, A Johnson, J M Land

Br Heart J 1994;72:349–353

Long-term treatment of Barth syndrome with pantothenic acid: a retrospective study

Simone Rugolotto, Maria D. Prioli, Daniela Toniolo, Pierantonio Pellegrino, Susanna Catuogno, and Alberto B. Burlina

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