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

Adam J. Chicco, Ph.D.

Assistant Professor
Departments of Health and Exercise Science, Food Science & Human Nutrition
Biomedical Sciences, Program in Cell and Molecular Biology
Colorado State University

Barth Syndrome Scientific Conference 2012
St. Pete Beach, FL

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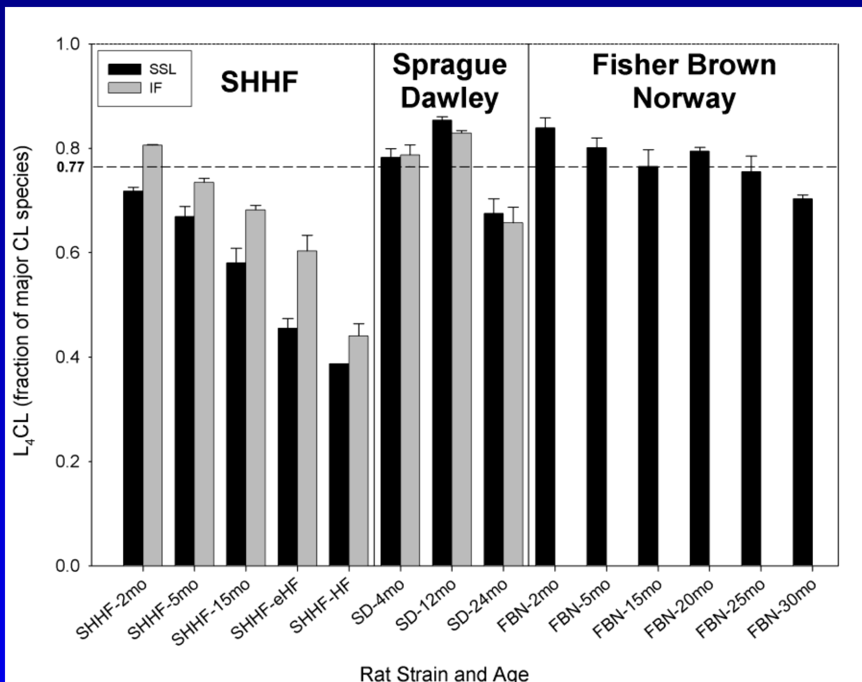
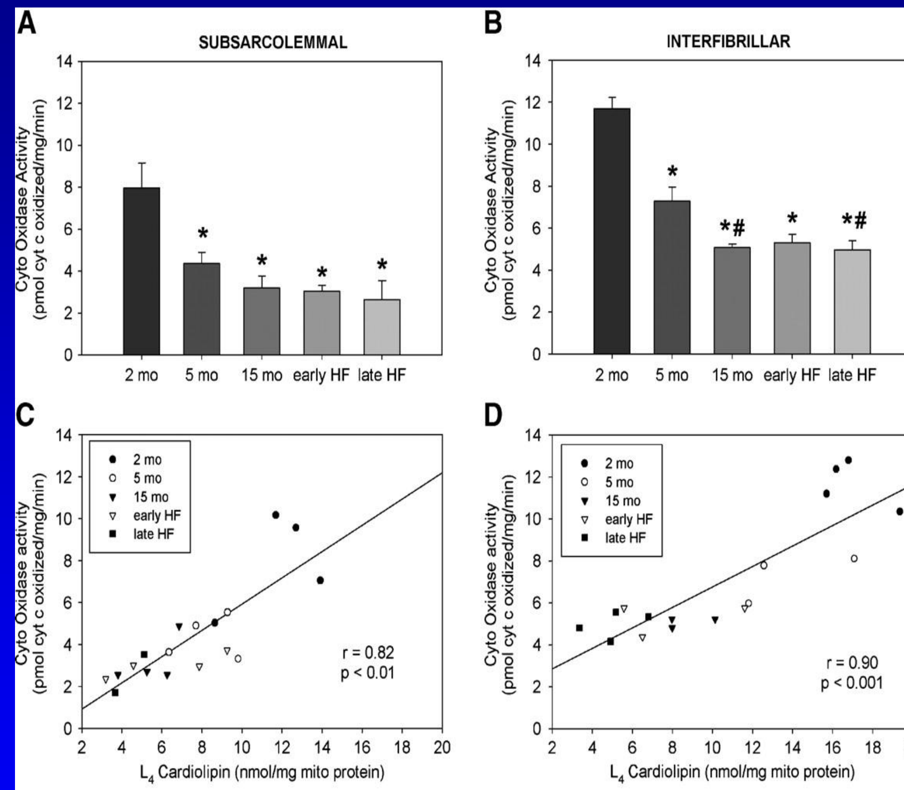
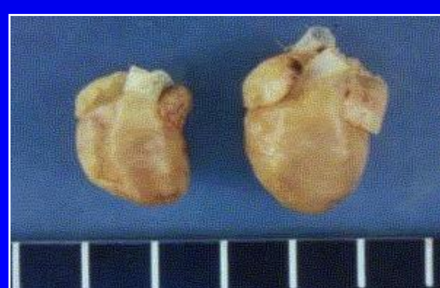
Progressive loss of cardiac L_4CL in Spontaneously Hypertensive HF rats



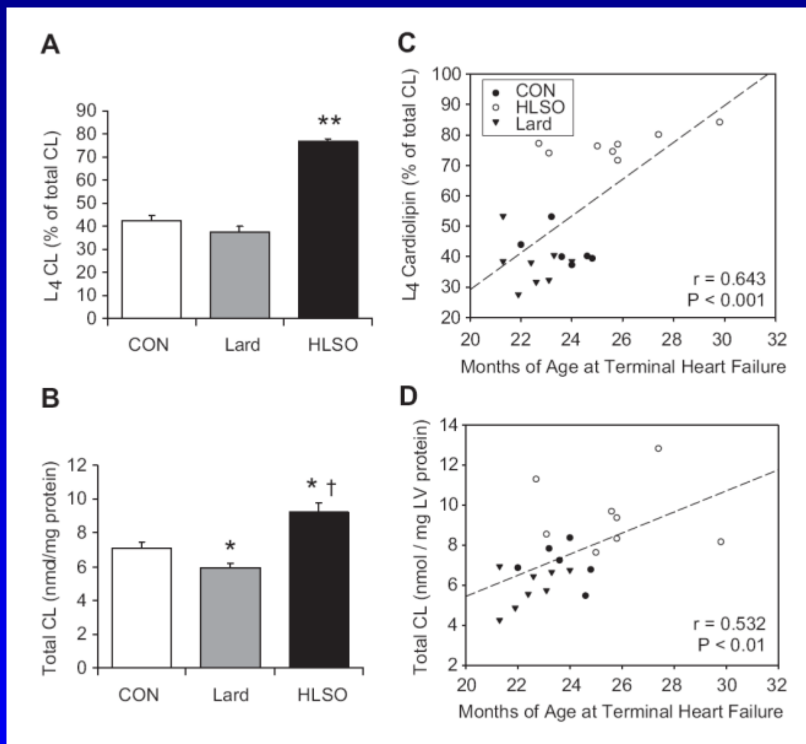




Sparagna, Chicco et al. *J Lipid Res*, 2007

Supplementation with 20% (w/w) *high-18:2 safflower oil* restores L_4CL 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
Hypertension 2008;52;549-555; originally published online Jul 28, 2008;

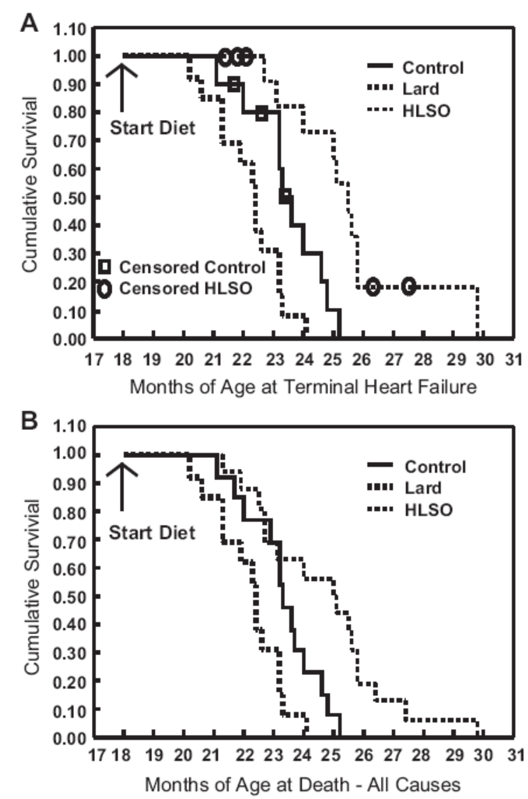
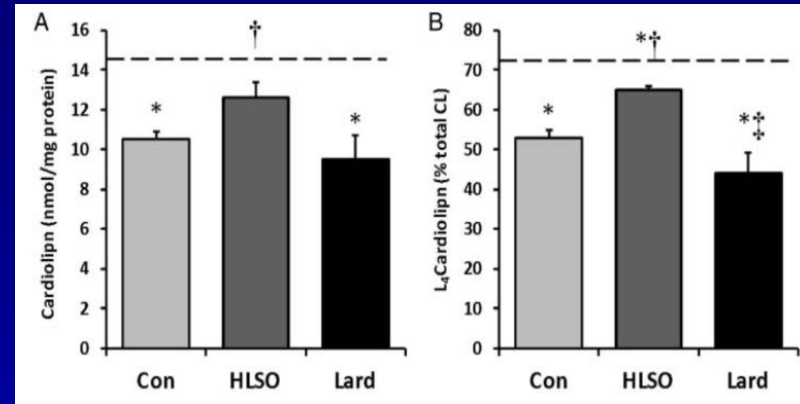


Figure 1. Kaplan–Meier curves illustrating cumulative mortality because of HF (A) and all causes (B) beginning at 18 months. The HLSO diet significantly improved survival resulting from HF and all causes compared with CON and lard, whereas the lard diet increased HF mortality.

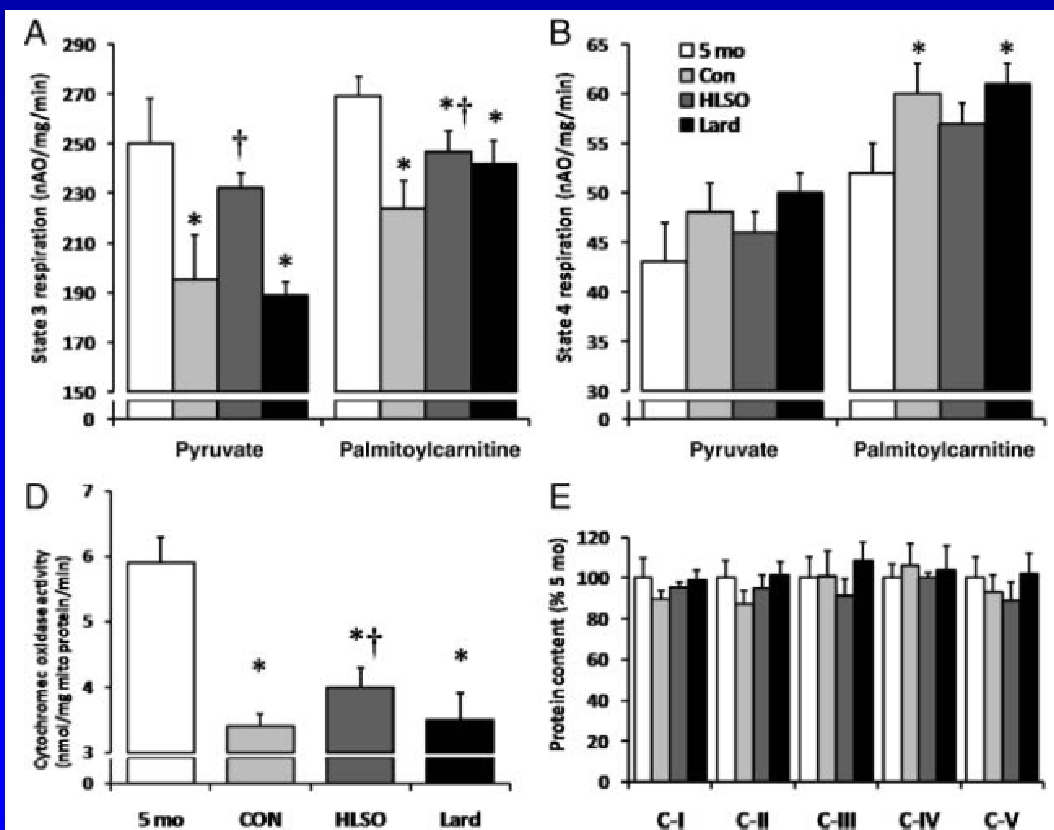
Cardiovascular Research (2012) **94**, 460–468
doi:10.1093/cvr/cvs118

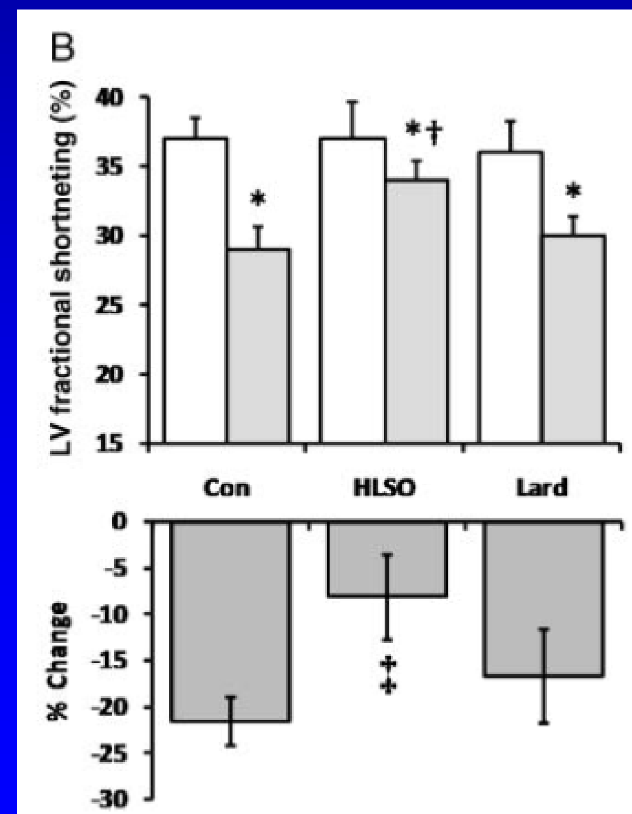
Dietary linoleate preserves cardiolipin and attenuates mitochondrial dysfunction in the failing rat heart

Christopher M. Mulligan[1], Genevieve C. Sparagna[2], Catherine H. Le[3,4], Anthony B. De Mooy[5], Melissa A. Routh[3,4], Michael G. Holmes[6], Diane L. Hickson-Bick[6], Simona Zarini[7], Robert C. Murphy[7], Fred Y. Xu[8], Grant M. Hatch[8], Sylvia A. McCune[2], Russell L. Moore[2], and Adam J. Chicco[1,3,4,5*]



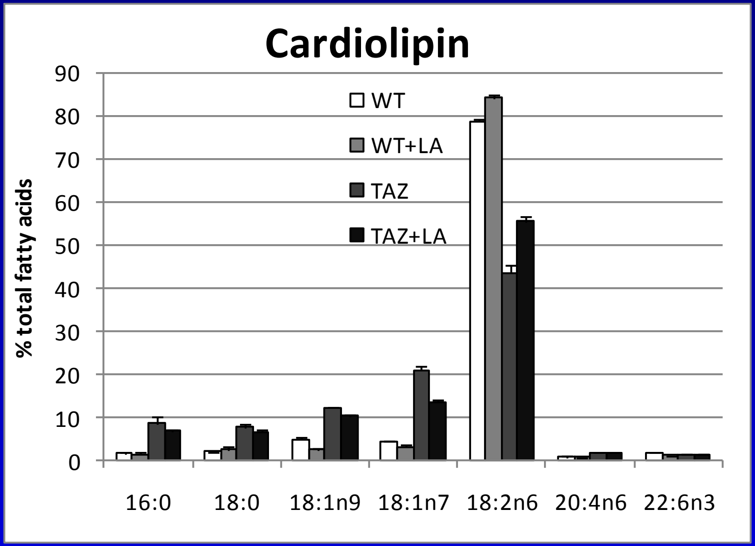
Male 21 mo old SHHF rats (early CHF) fed 10% HLSO or Lard (w/w) 4 weeks





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

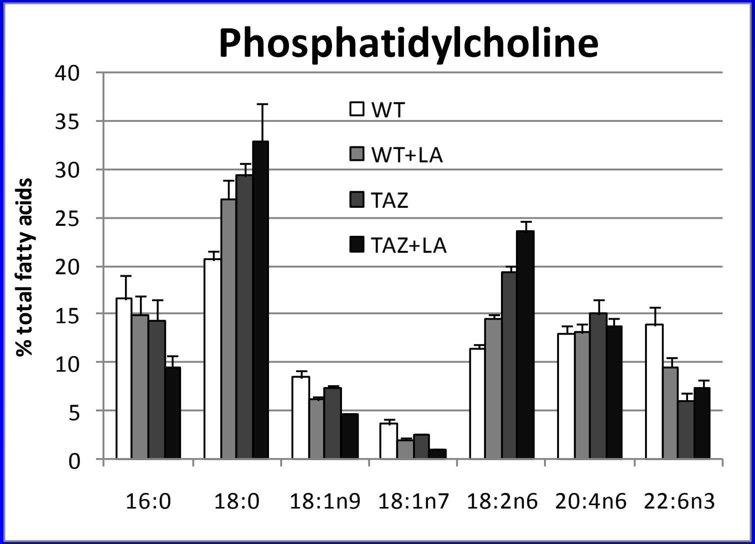


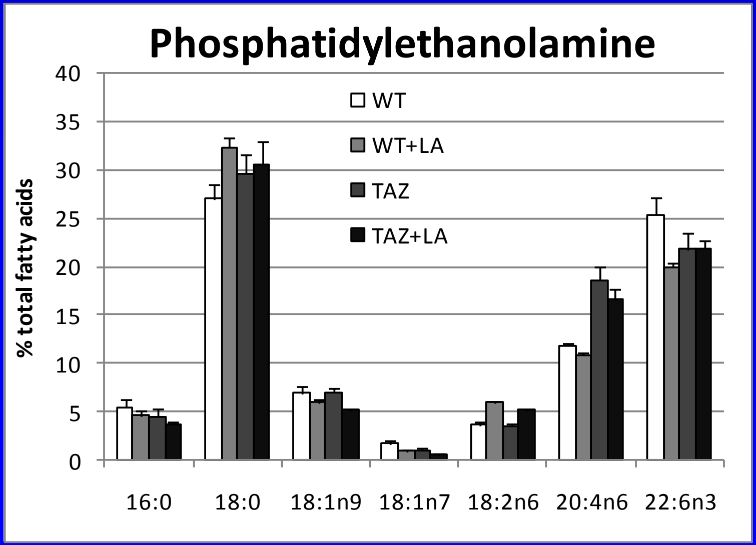
Male taz shRNA mice 4-5 mo of age;
10% HLSO (w/w) mixed in chow for 4 weeks

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

PC 18:2n6: taz > WT

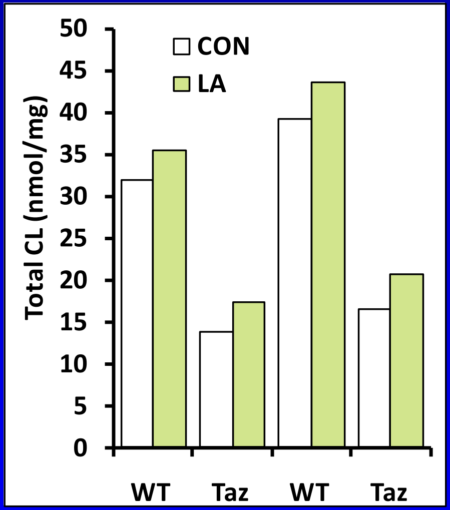
PE 20:4n6: taz > WT





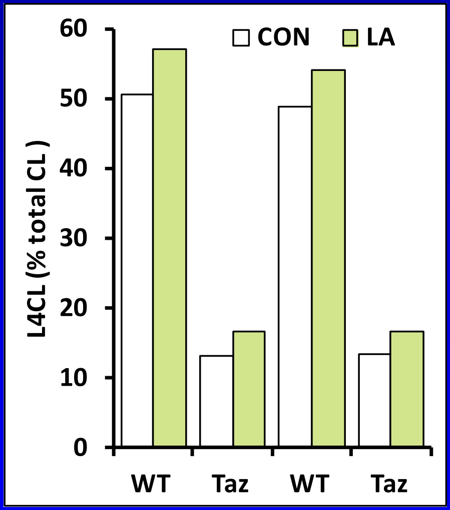
Modest increase in cardiac mito CL content and L4CL% with HLSO supplementation

Total CL



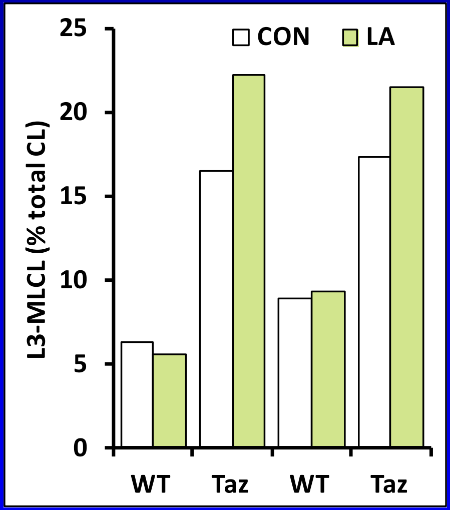
SSM IFM

L4CL (%)



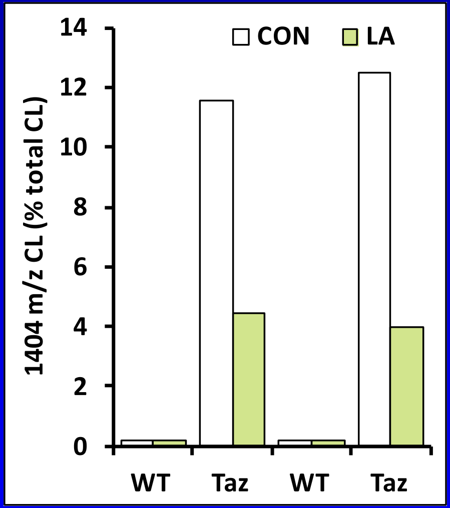
SSM IFM

L3-MLCL (%)



SSM IFM

"Saturated" CL



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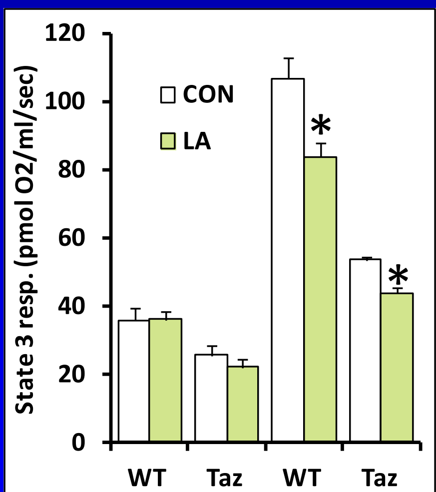
HLSO does not improve mitochondrial OXPHOS capacity or efficiency in *Taz* or WT mice

ADP-Stimulated respiration (State 3)



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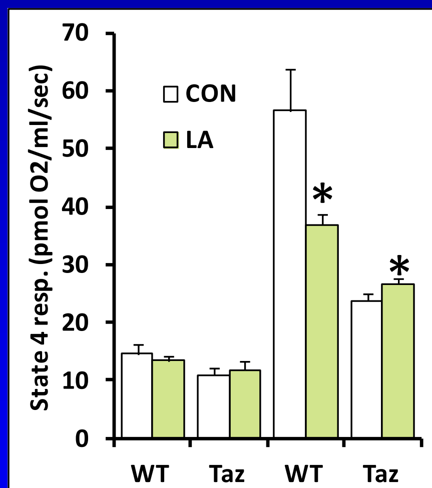
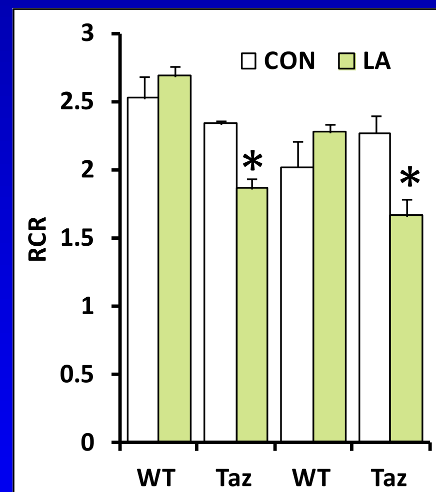
"Uncoupled" respiration (State 4)



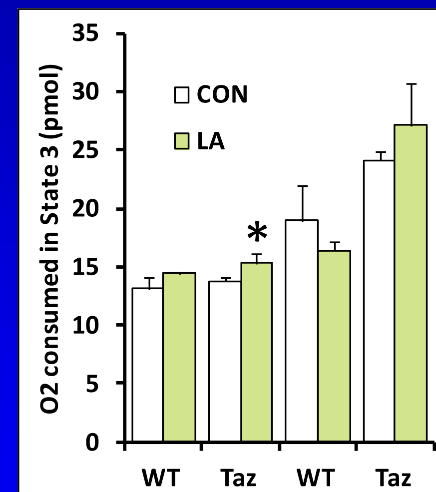
SSM IFM

Respiratory control ratio (State 3/State 4)



SSM IFM

O2 consumed in State 3



SSM IFM

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

Biochimica et Biophysica Acta, 1086 (1991) 139–140

ADONIS 000527609100286N

BBALIP 50330

Rapid Report

Effect of thyroxine on the activity of mitochondrial cardiolipin synthase in rat liver

Karl Y. Hostetler

Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Diego and the VA Medical Center, San Diego, CA (U.S.A.)

Thyroxine stimulates PGPS activity in rat heart mitochondria.
Cao SG, Cheng P, Angel A, Hatch GM.
Biochim Biophys Acta 1995 May 17;1256(2):241-4.

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

G. Paradies *, F.M. Ruggiero, G. Petrosillo and E. Quagliariello

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

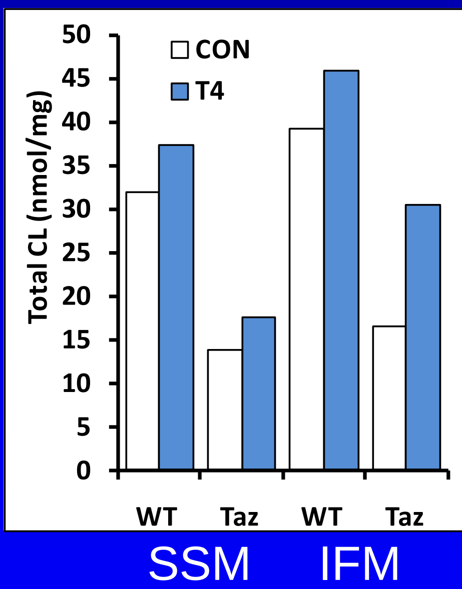
Anthony Martin Gerdes, PhD; Giorgio Iervasi, MD

Circulation. 2010;122:385-393

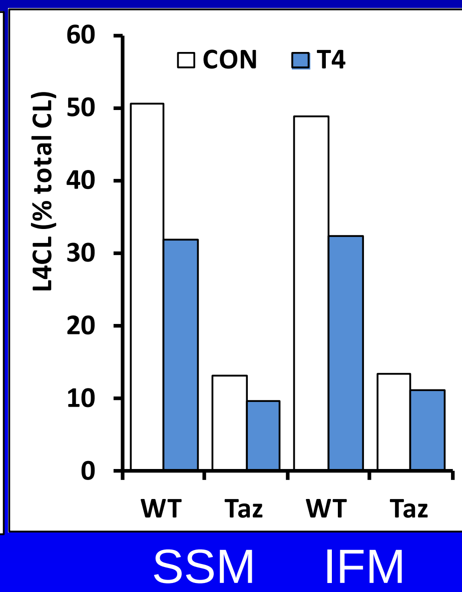
Thyroxine treatment increases total CL, but decreases L4CL% in *Taz* shRNA and WT mice

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

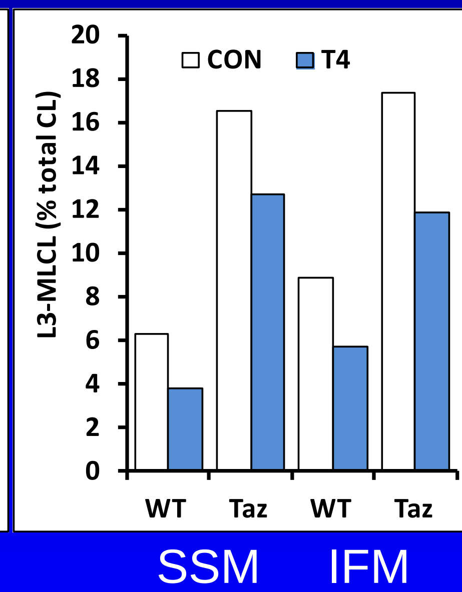
Total CL



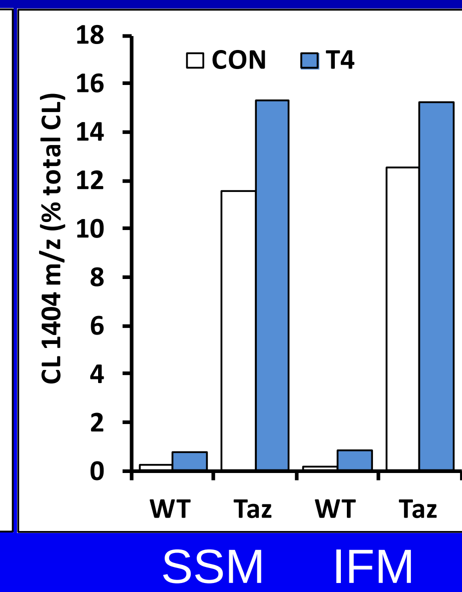
L4CL (%)



L3-MLCL (%)

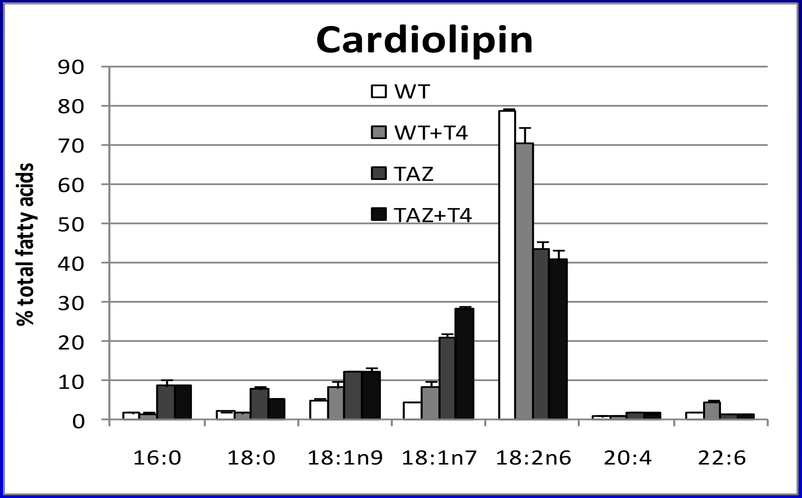


"Saturated" CL



Effect of T4 on mitochondrial PL composition

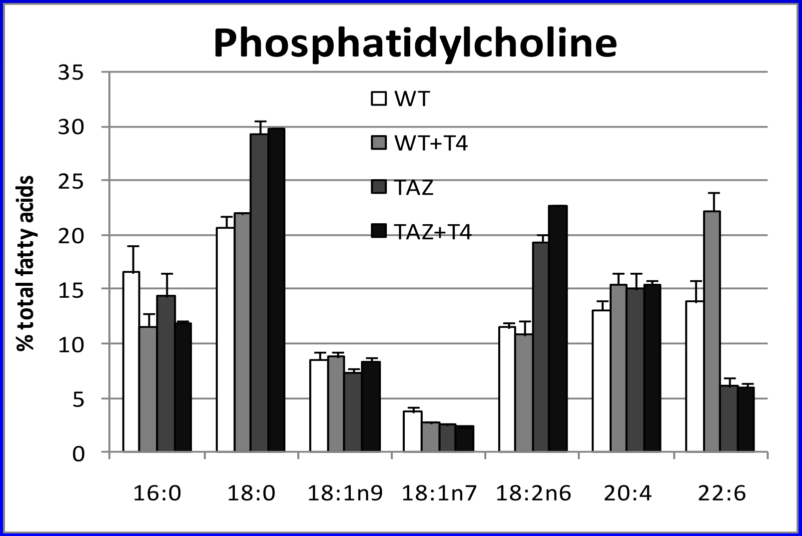
Male WT or *Taz* mice 4-5 mo of age; 0.1% T4 mixed in chow for 4 weeks (n = 6 / group)

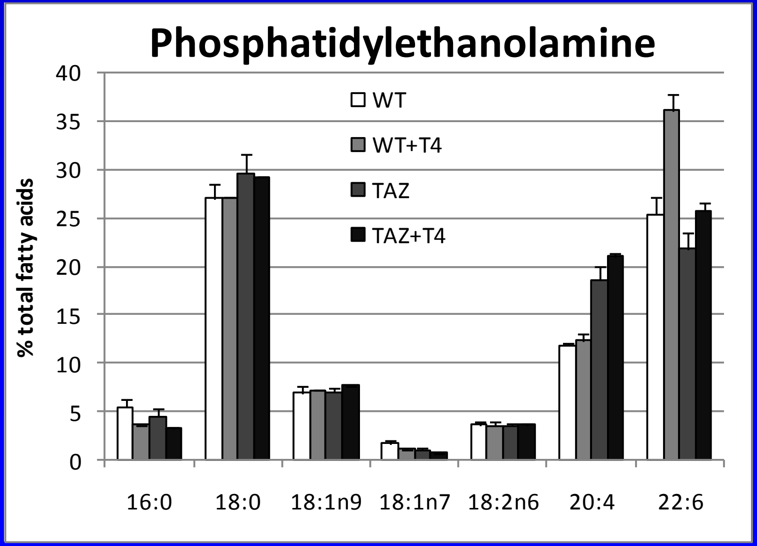


T4 ↑18:1n7, ~↓18:2n6 in CL

T4 ↑18:2n6 in PC, not CL or PE

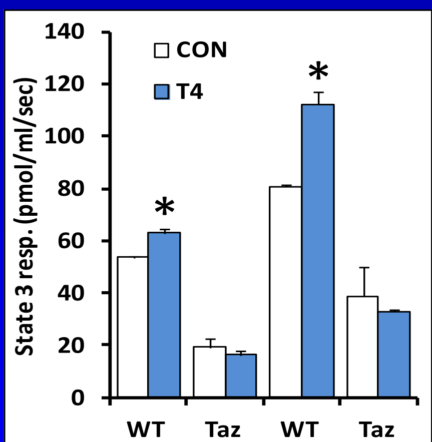
T4 ↑22:6n3 in WT in all PLs, less/no effect in taz





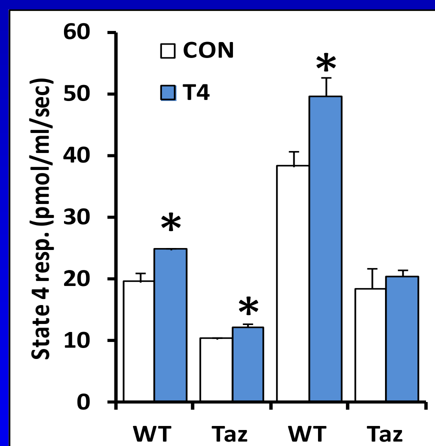
T4 treatment fails to restore mitochondrial respiratory function in *Taz* shRNA mice

ADP-Stimulated respiration (State 3)



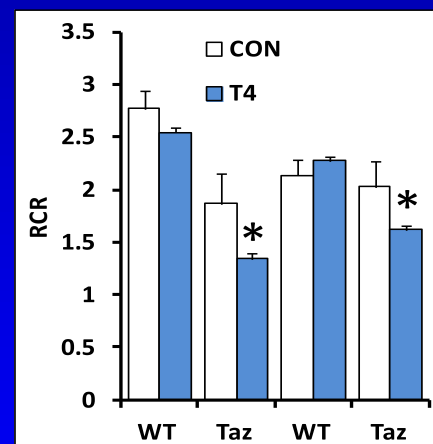
SSM IFM

“Uncoupled” respiration (State 4)



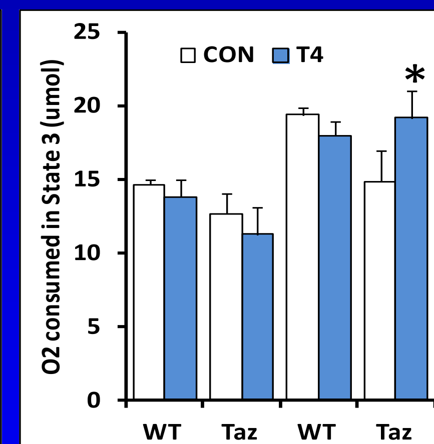
SSM IFM

Respiratory control ratio (State 3/State 4)



SSM IFM

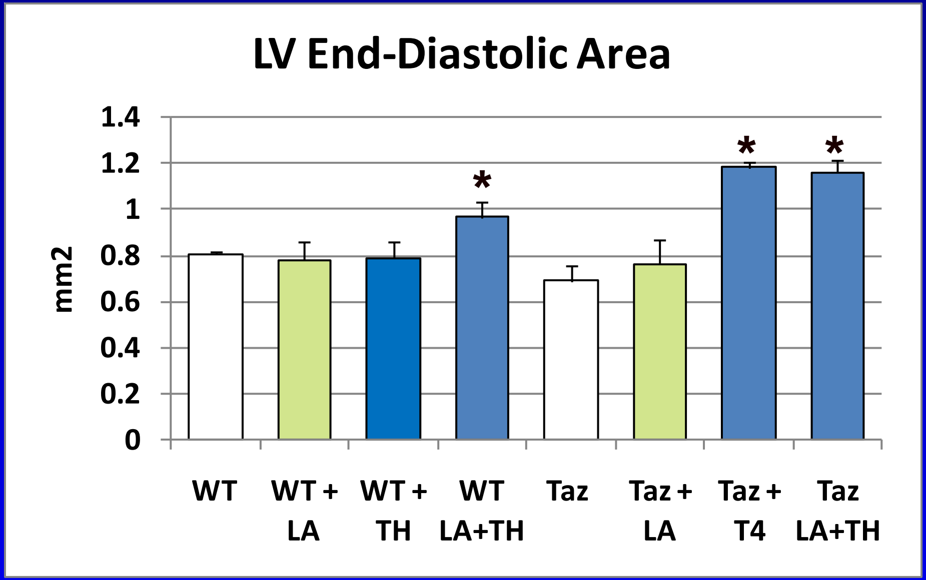
O2 consumed in State 3

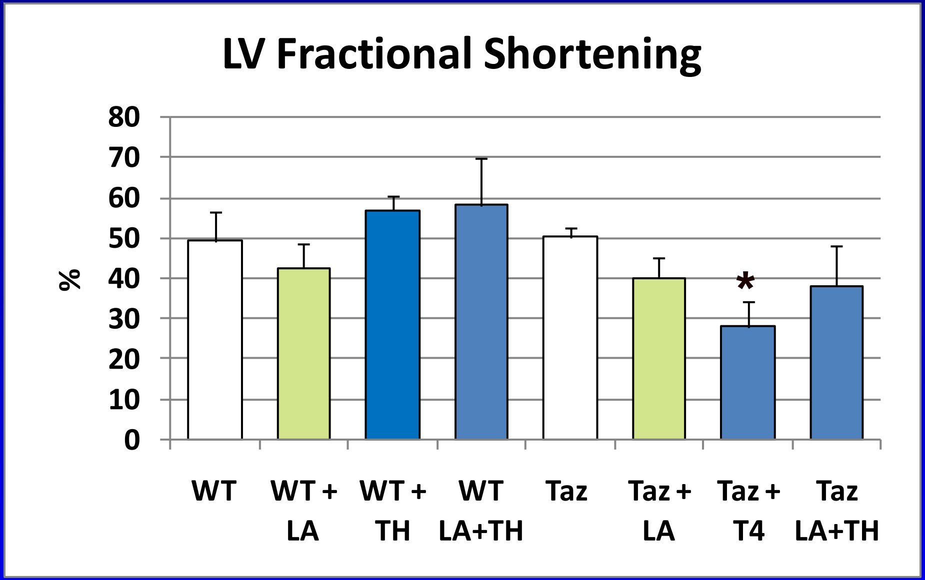


SSM IFM

Pyruvate + Malate as substrates; n = 6 / group

Effect of HLSO and T4 on LV chamber size and contractile function

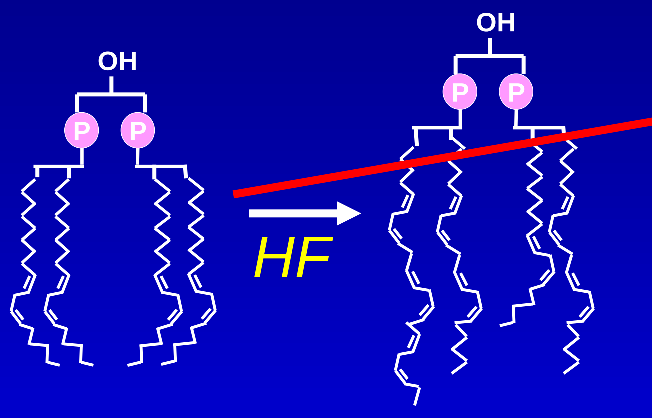


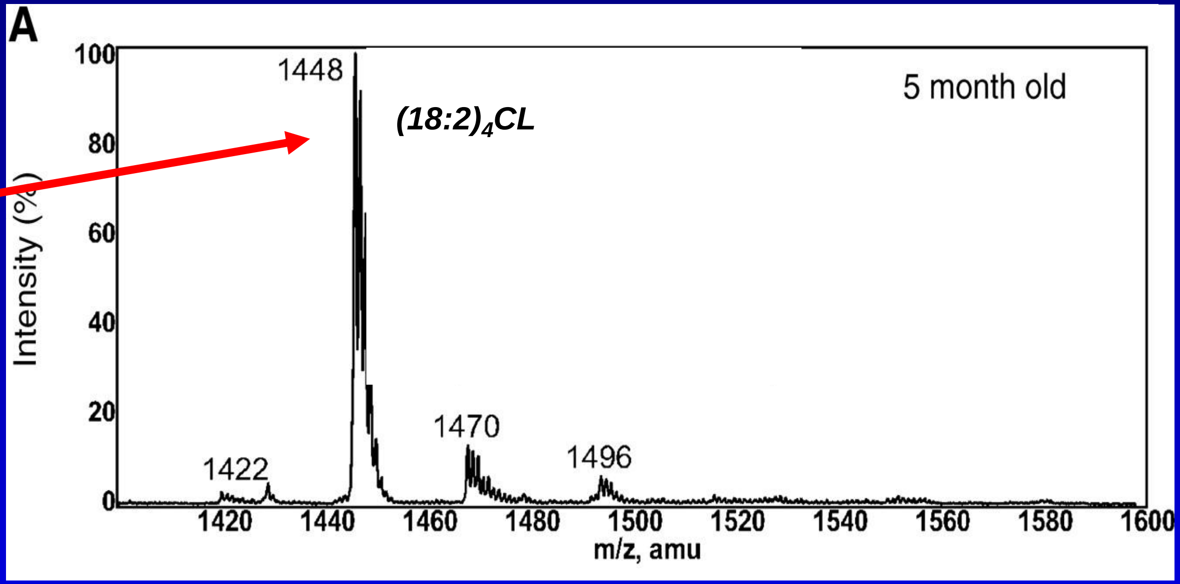


Summary

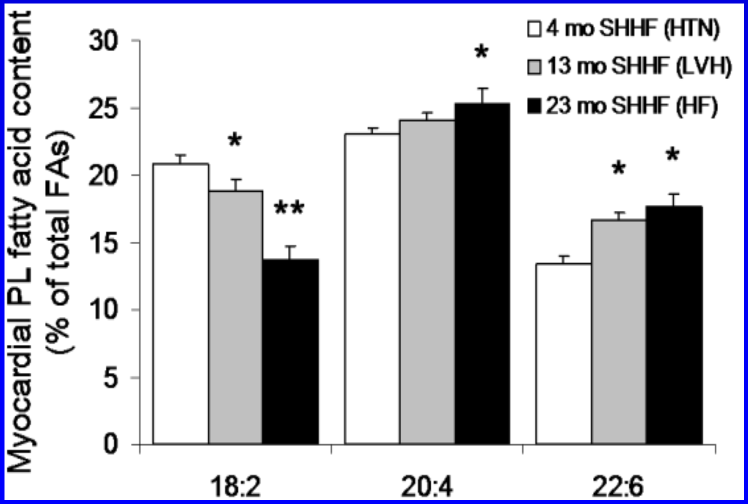
- HLSO suppl. partially restores CL 18:2 content, but fails to improve mitochondrial respiratory function
- T4 tx partially restores total CL levels, but fails to improve mitochondrial respiratory function in *Taz* mice, despite having stimulatory effects in WT mice
- Effect of HLSO+T4 on CL content/composition 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



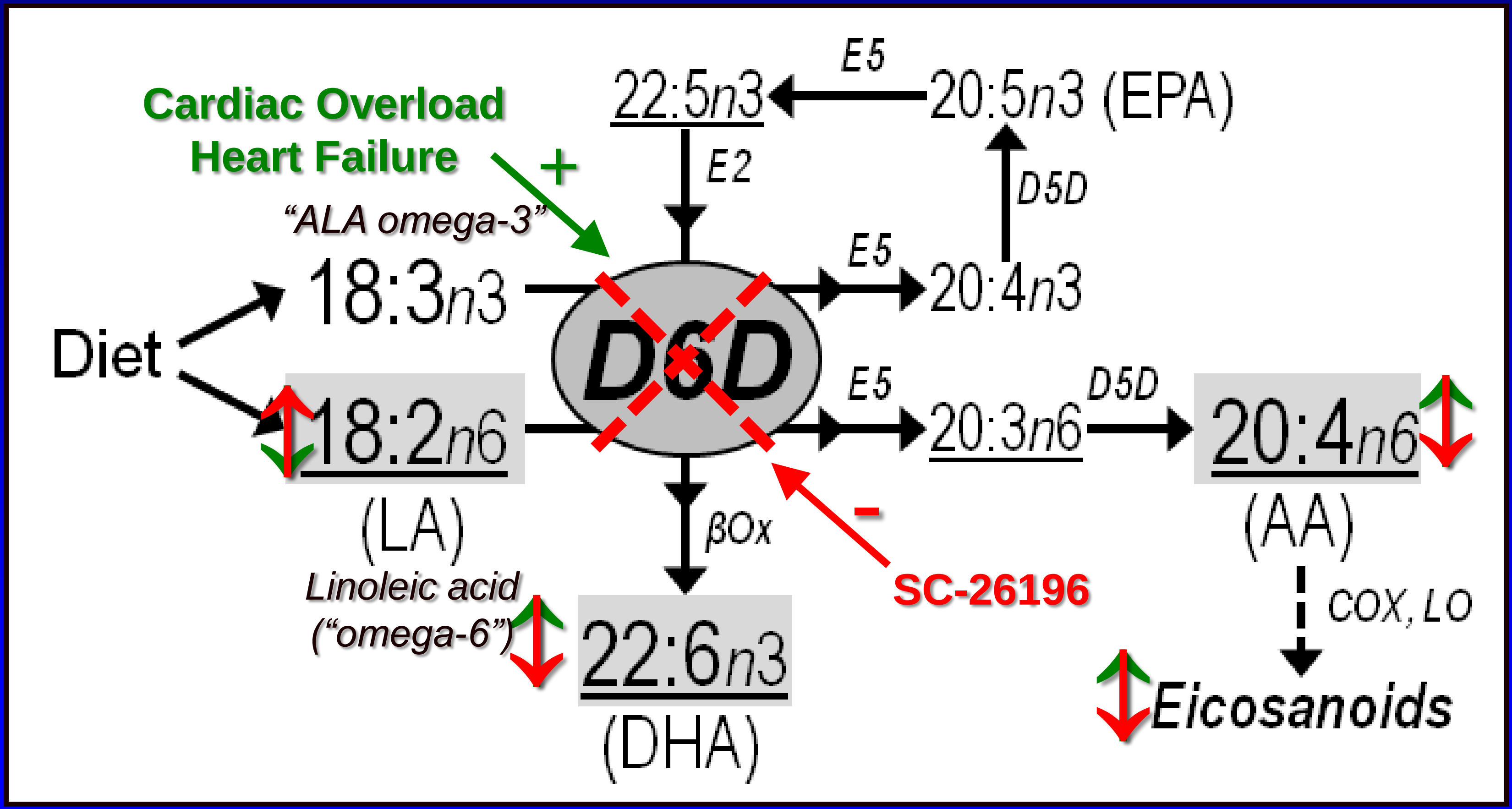


Sparagna et al. *J Lipid Res*, 2005

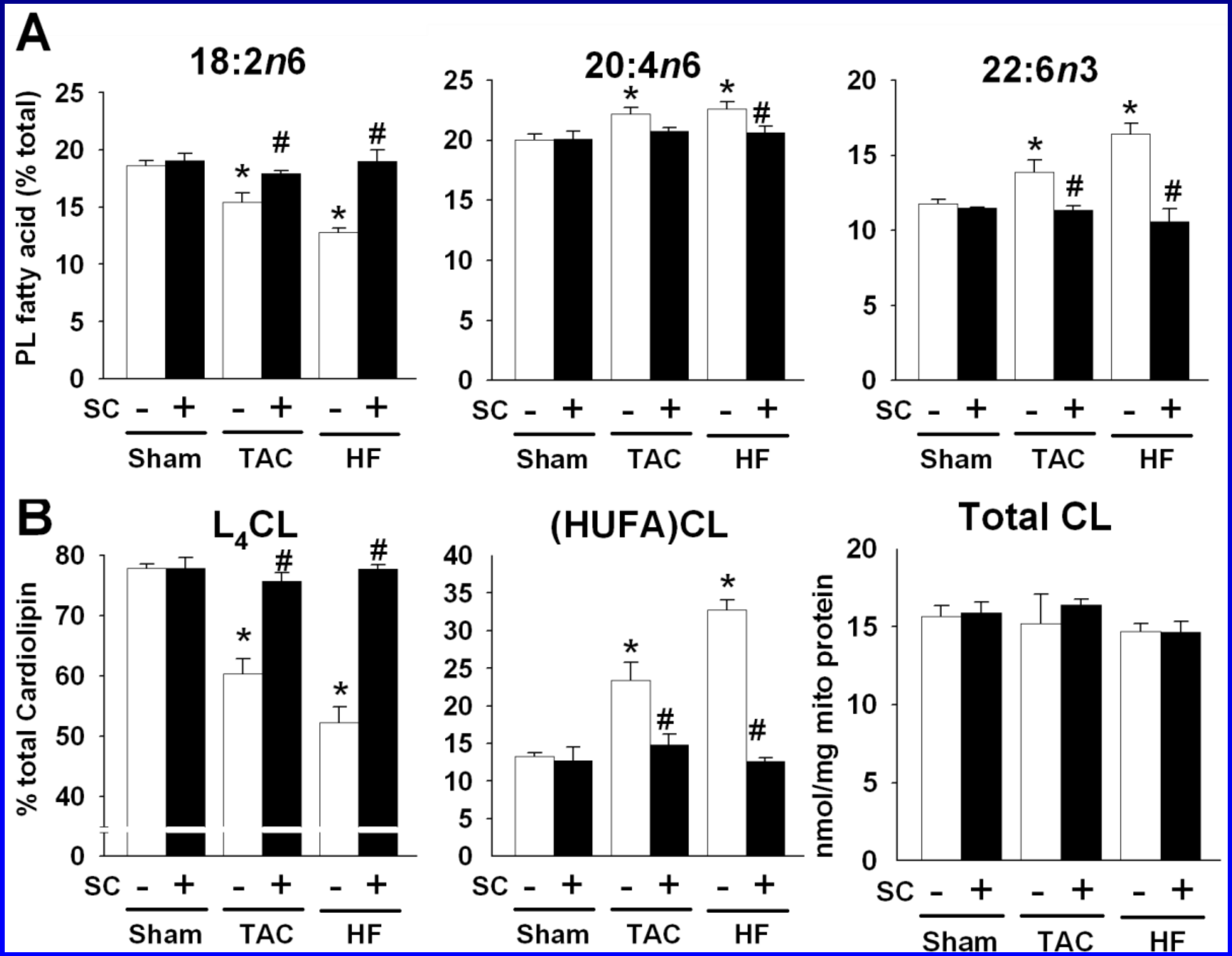


CL remodeling parallels a progressive *loss* of linoleic acid and *increase* in arachidonic acid (20:4n6) and/or DHA (22:6n3) in the global myocardial phospholipid pool

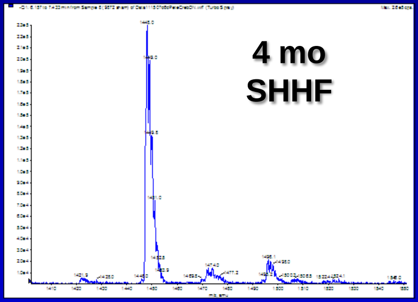
Delta-6 Desaturase: central role in PUFA metabolism…***and*** *phospholipid composition*?

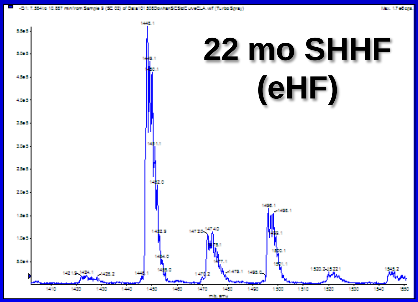


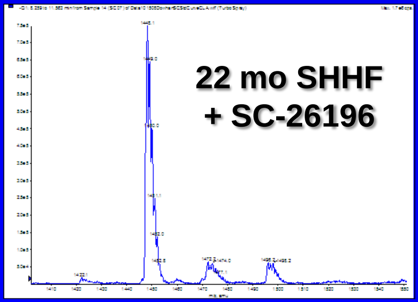
D6D inhibition normalizes phospholipid PUFA profile and restores L_4CL in TAC and HF



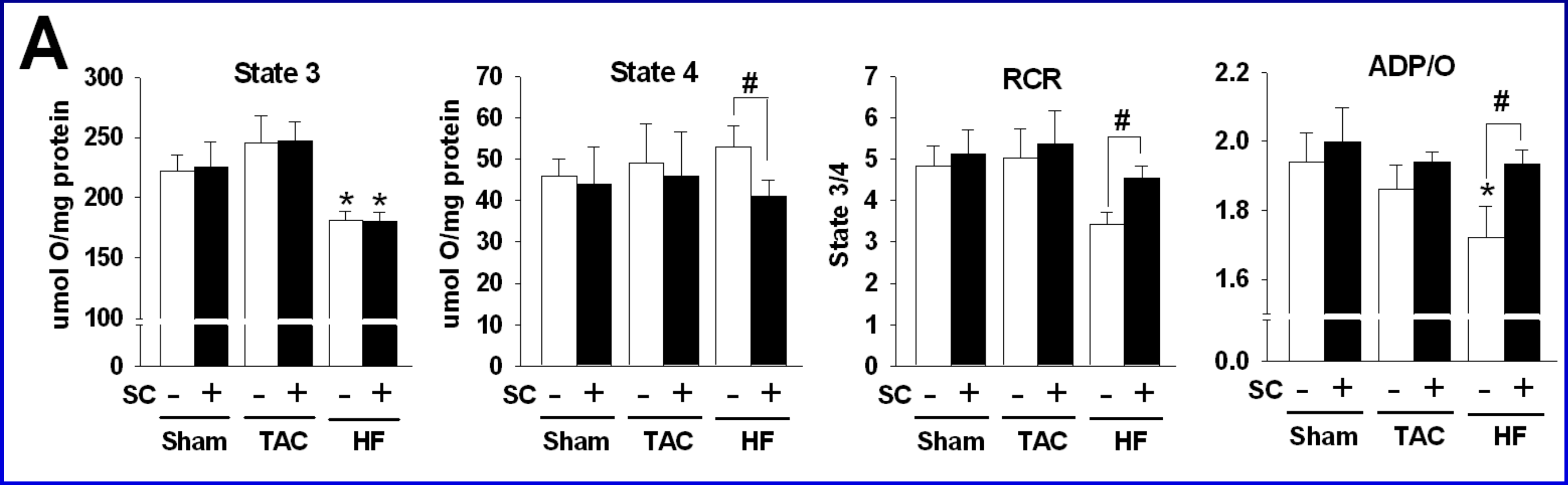
CL Subspecies profile (LC/MS)

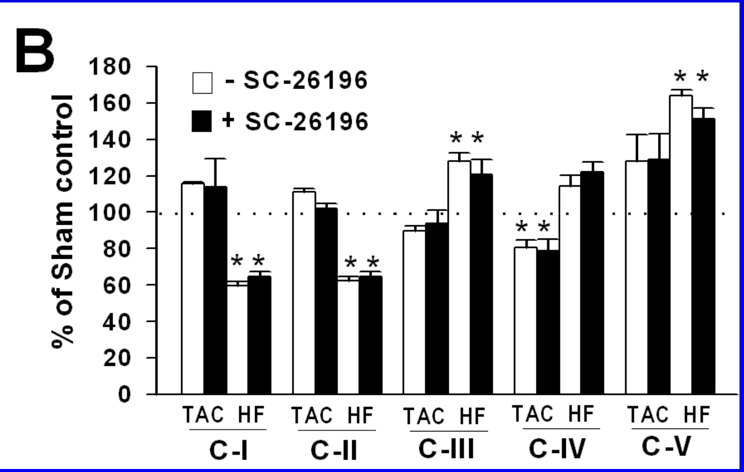






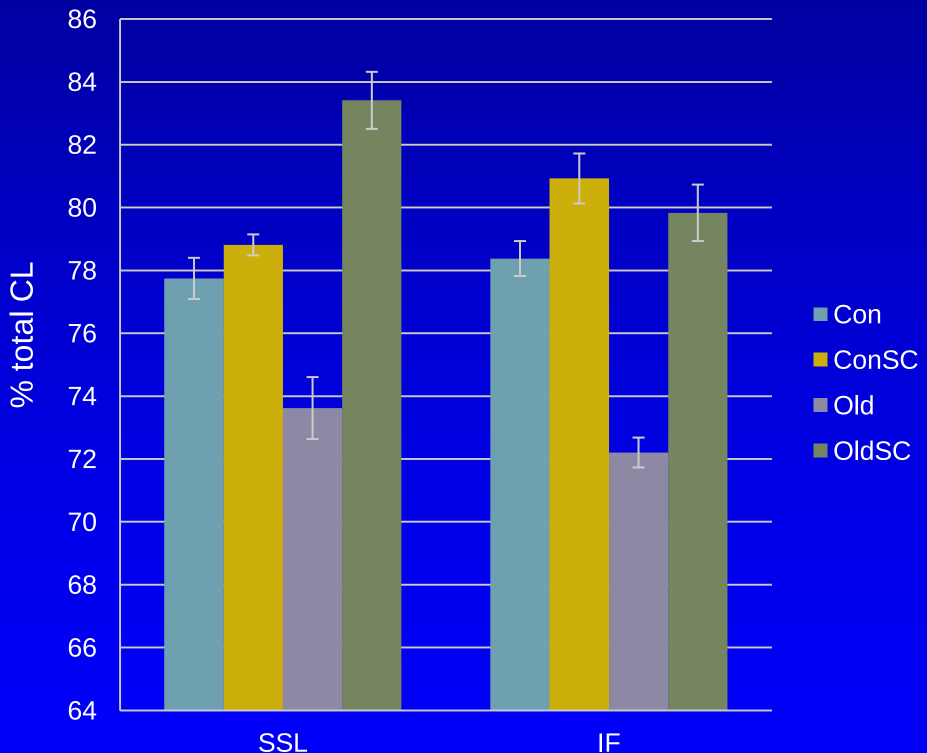
D6D inhibition preserves respiratory efficiency, but fails to restore State 3 respiratory capacity



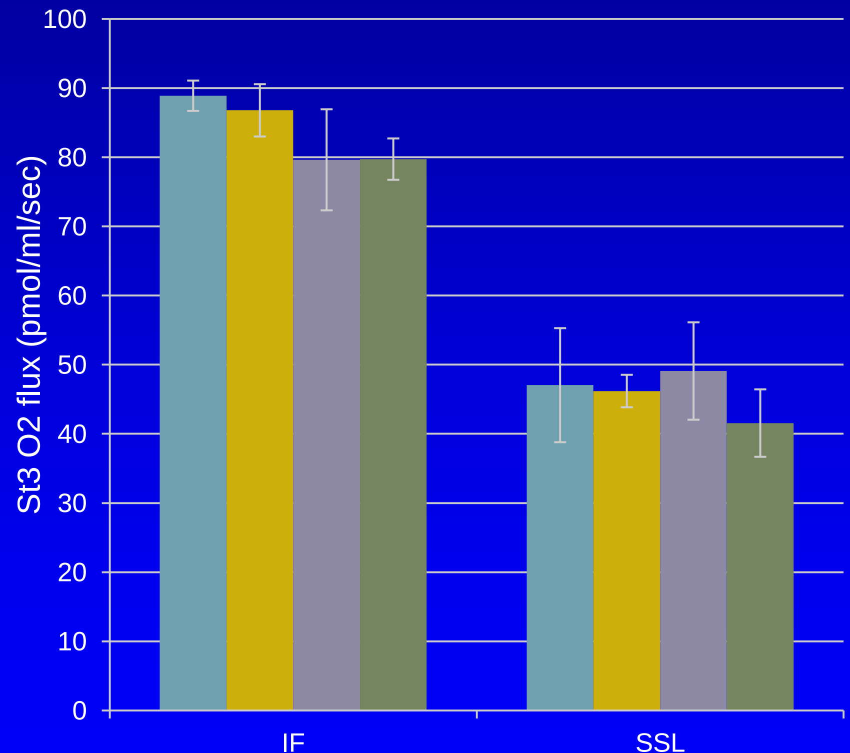


D6D inhibition reverses loss of CL 18:2n6 in aged mouse hearts without significant changes in mitochondrial respiratory function

CL Linoleic acid (%)



State 3 respiration

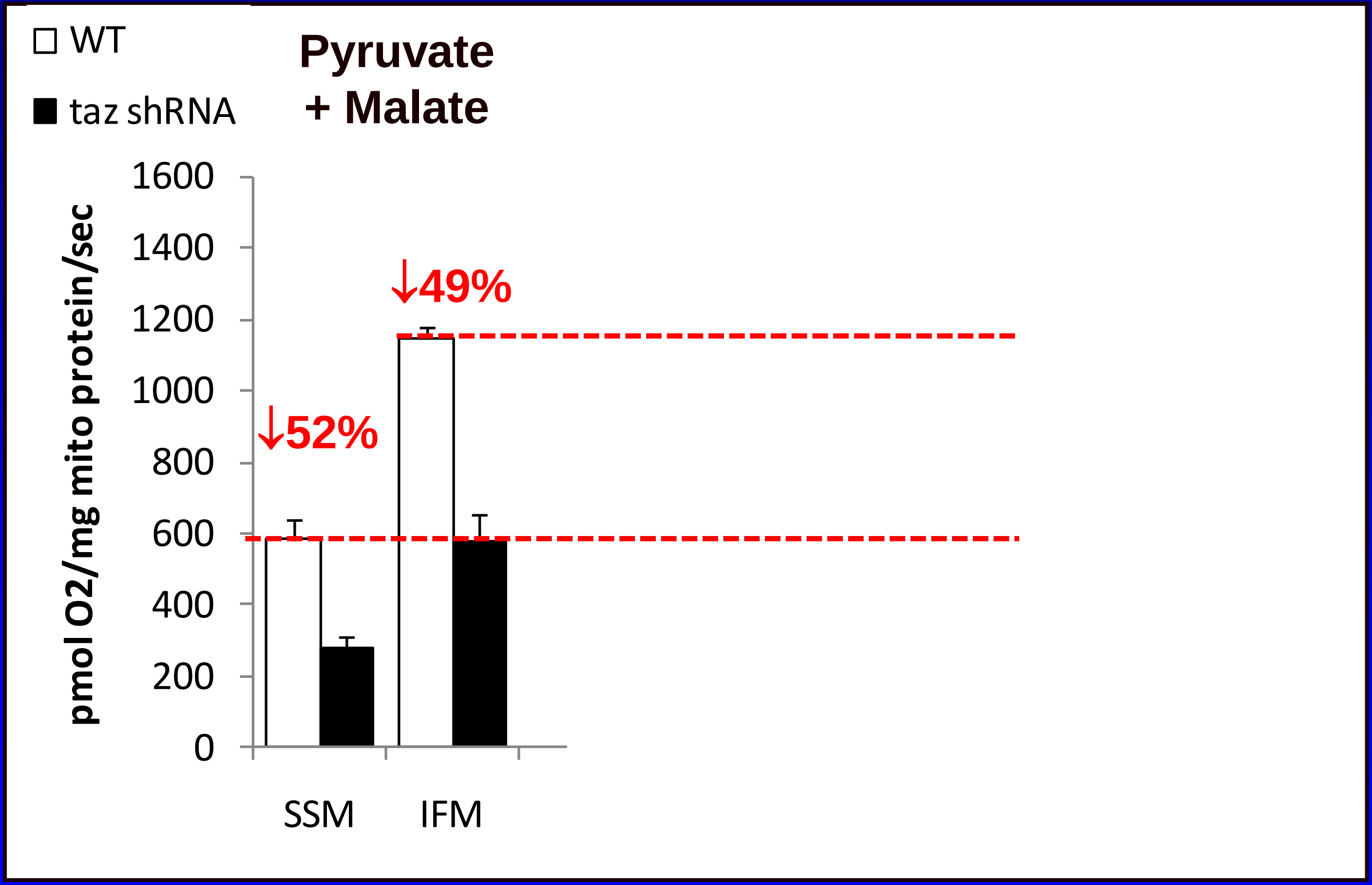


4 mo and 24 mo old C57Bl/6 mice treated with the D6D inhibitor SC-26196 (100mg/kg/d in chow) for 4 weeks (n = 6 / group)

Conclusions

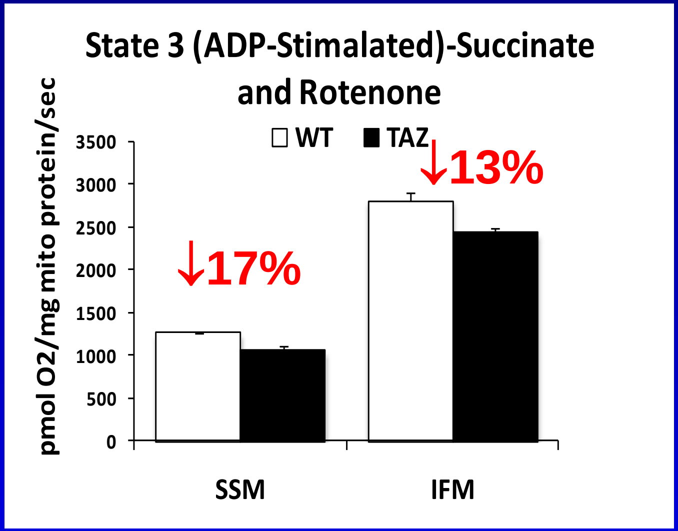
- 18:2n6 enrichment of cardiac CL is not a major regulator of mitochondrial respiratory (dys)function in the heart…at least not in aging/HF rodent models
- Lack of T4 benefit suggests that augmenting CL biosynthesis/mito biogenesis may not rescue *Taz*-deficient mito respiratory phenotype either
- Perhaps the pathologic effects of *Taz* deficiency extend beyond alterations in CL content/composition
- *How exactly does Taz deficiency impair cardiac mitochondrial respiratory function?*

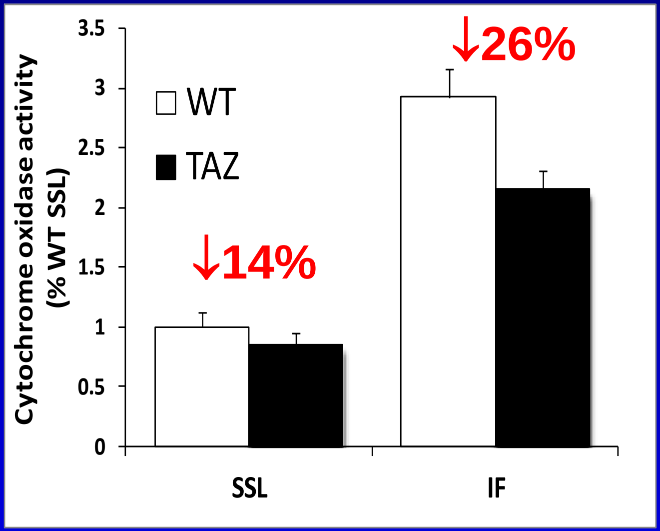
Substrate specificity of respiratory dysfunction in *Taz* shRNA cardiac mitochondria

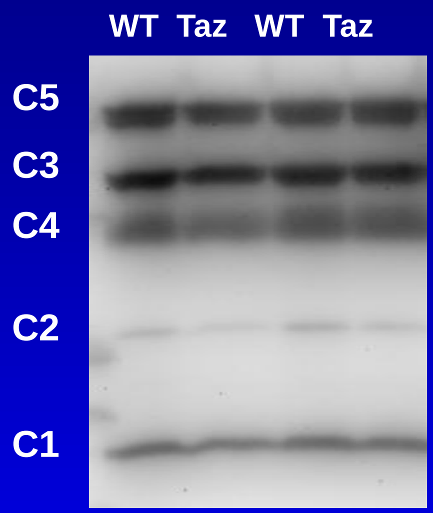


4-5 month old male WT and Taz shRNA mice (n = 8-12/group)

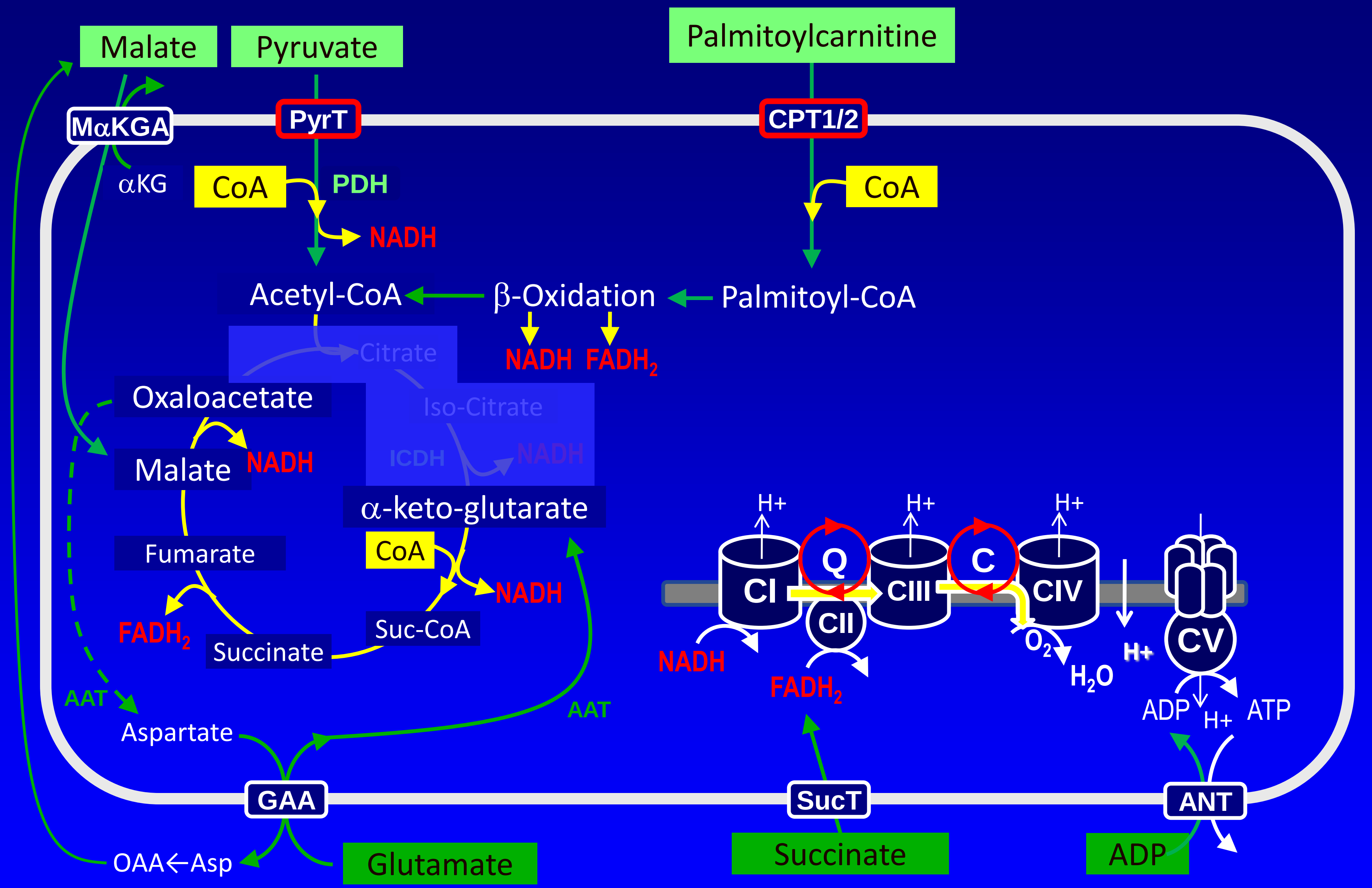
Respiratory chain dysfunction does not contribute significantly to OXPHOS impairment in *Taz* deficiency





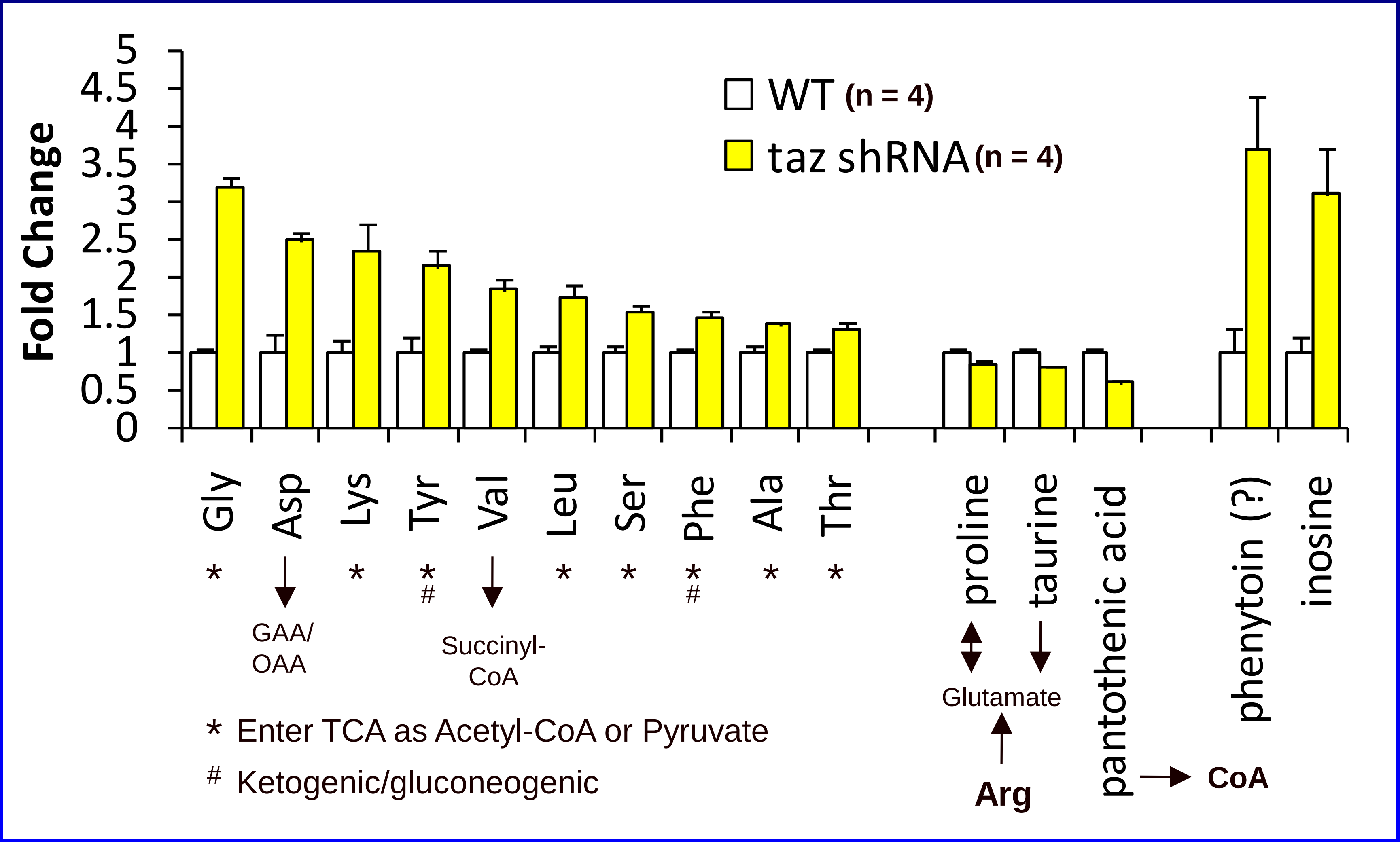


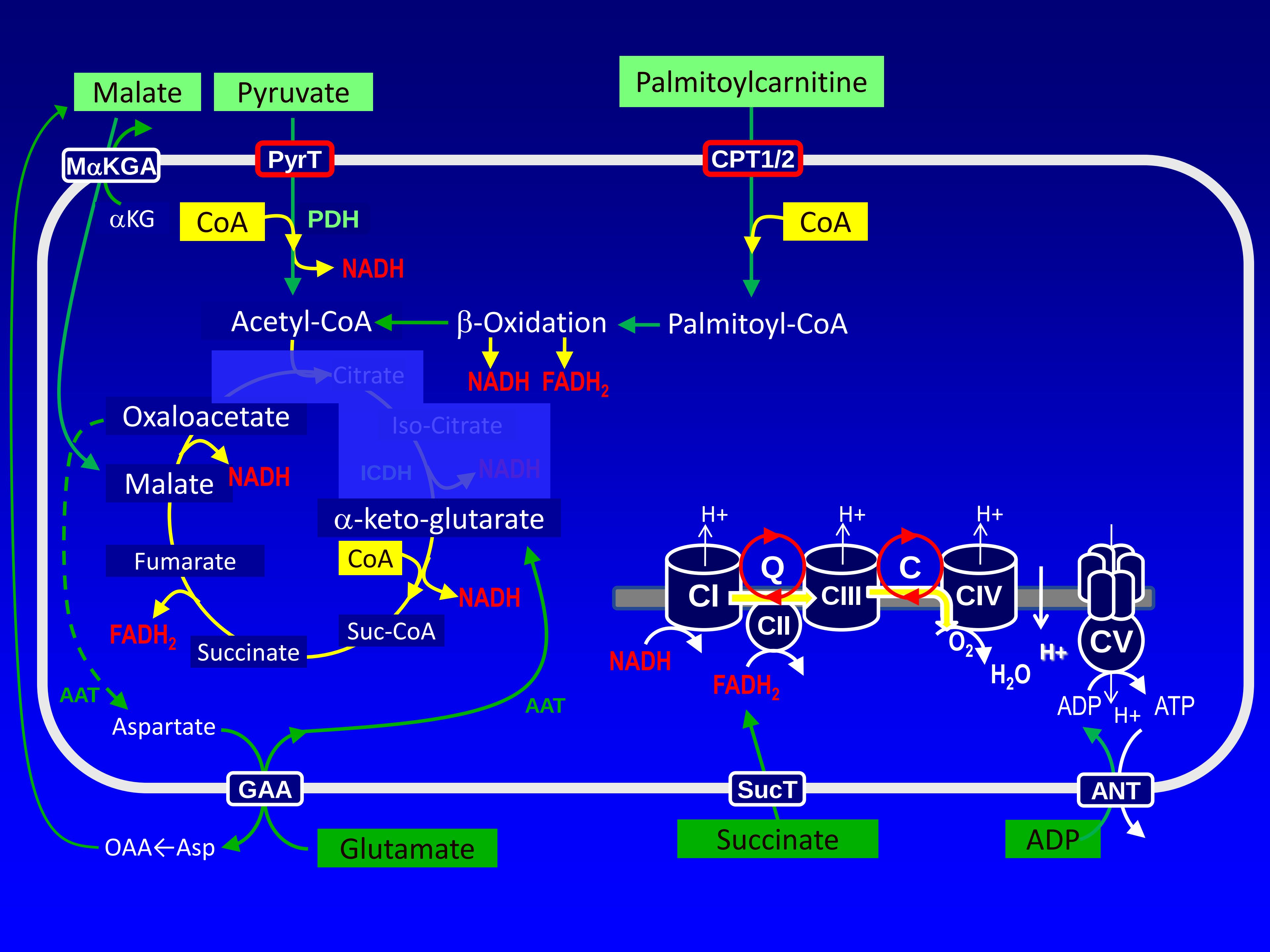
Taz deficiency must impair mitochondrial membrane transport of pyruvate and fatty acids and/or otherwise limit their oxidation by TCA cycle enzymes



Malate
Pyruvate
Palmitoylcarnitine
MαKGA
PyrT
CPT1/2
αKG
CoA
PDH
NADH
CoA
Acetyl-CoA
β-Oxidation
Palmitoyl-CoA
Citrate
NADH
FADH2
Oxaloacetate
Iso-Citrate
Malate
NADH
ICDH
NADH
α-keto-glutarate
Fumarate
CoA
NADH
FADH2
Suc-CoA
Succinate
H+
H+
H+
Q
C
CI
CIII
CIV
CII
CV
NADH
FADH2
O2
H2O
H+
ADP
H+
ATP
AAT
Aspartate
AAT
GAA
SucT
ANT
OAA←Asp
Glutamate
Succinate
ADP

Metabolomic profile of *Taz* shRNA vs. WT hearts




Malate
Pyruvate
Palmitoylcarnitine
MαKGA
PyrT
CPT1/2
αKG
CoA
PDH
NADH
CoA
Acetyl-CoA
β-Oxidation
Palmitoyl-CoA
Citrate
NADH
$FADH_2$
Oxaloacetate
Iso-Citrate
Malate
NADH
ICDH
NADH
α-keto-glutarate
Fumarate
CoA
NADH
$FADH_2$
Suc-CoA
Succinate
H+
H+
H+
Q
C
CI
CIII
CIV
CII
CV
NADH
$FADH_2$
O_2
H_2O
H+
ADP
H+
ATP
AAT
AAT
Aspartate
GAA
SucT
ANT
OAA←Asp
Glutamate
Succinate
ADP

Notable effects of B_5/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-methyglutaconyl-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,[a] Maria D. Prioli,[b] Daniela Toniolo,[c] PierAntonio Pellegrino,[d] Susanna Catuogno,[d] and Alberto B. Burlina[d,*]

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