Abnormalities of Intermediary Metabolism in Barth Syndrome

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Is Barth Syndrome a Mitochondrial Disease?

1. Muscle biopsies often have normal mitochondrial enzymology and histology
2. Profound muscle fatigue and weakness occur without biochemical signs of mitochondrial dysfunction
3. Severity of growth delay is out of proportion to biochemical signs of mitochondrial dysfunction
4. ATP synthesis is normal in cultured cells and in living tissue
Evidence For a Leucine-Independent Origin of 3-Methylglutaconic Acid

Normal increase after leucine-loading

No increase after prolonged fasting

Normal levels in children with inborn errors of leucine catabolism

3-MGC labels with $^{13}$C-acetate
3-Methylglutaconate Metabolism

Cardiolipin Remodeling Cycle

Tetrainoleoylcardiolipin $\xrightarrow{\text{Phospholipase A}}$ Lyso-phosphatidylcholine

$\text{TAZ} \quad 1$-Linoleoyl-phosphatidylcholine

Monolysocardiolipin

Chemical structures and reactions diagrammed.
Phospholipids in *taz1* Mutant Yeast

**WT**  
**taz1 Δ**

- Cardiolipin
- Monolyso-cardiolipin
- Phosphatidic acid
- Phosphatidylethanolamine
- Phosphatidylserine
- Phosphatidylinositol
- Phosphatidylcholine

M. Greenberg, 2002
Personal communication
<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal:</td>
<td>+/- Dilated cardiomyopathy</td>
</tr>
<tr>
<td></td>
<td>Ventricular non-compaction in some</td>
</tr>
<tr>
<td>Postnatal:</td>
<td>Stable or progressive cardiomyopathy, variable severity and course</td>
</tr>
<tr>
<td></td>
<td>Mild left ventricular thickening</td>
</tr>
<tr>
<td>Childhood:</td>
<td>Increasing risk of ventricular arrhythmia even when cardiac function is normal</td>
</tr>
<tr>
<td>Biopsy:</td>
<td>Endocardial fibroelastosis in some</td>
</tr>
<tr>
<td></td>
<td>Mitochondria are normal or variably abnormal histologically and enzymatically</td>
</tr>
</tbody>
</table>
$^{31}$P-NMR Spectroscopy of Heart Muscle
Barth Syndrome: $^{31}$P-NMR Spectroscopy of Cardiac Muscle

Age: 8 months
Dx: dilated cardiomyopathy
$^{31}$P-NMR Spectroscopy of Cardiac Muscle

![Graph showing PCR/β-ATP ratio over age (months) for different conditions: Lactic Acidosis (white squares), Senger Syndrome (black squares), Barth Syndrome (red circle), and Sibling Controls (black circles).](image)
$^{31}$P-NMR Spectroscopy of Gastrocnemius
Mitochondrial Electron Transport Chain

Mitochondrial Matrix

<table>
<thead>
<tr>
<th>Complex</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<tbody>
<tr>
<td>Mitochondrial Genes</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<tr>
<td>ND1–6, ND4L</td>
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<tr>
<td>Nuclear Genes</td>
<td>36</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>11</td>
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<tr>
<td>NDUFS1, 2, 4, 7, 8 NDUVF1</td>
<td>SDHA, B, C, D</td>
<td>BCS1L</td>
<td>SUPF1, SCO1 SCO2, COX10</td>
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<tr>
<td>Clinical Syndrome</td>
<td>Leigh syndrome Leukodystrophy Cardiomyopathy</td>
<td>Leigh syndrome Paraganglioma Pheochromocytoma</td>
<td>Hepatopathy Encephalopathy</td>
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## Plasma Amino Acids – Normal 4 to 6-Hour Fasting

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<tr>
<th>Amino Acid</th>
<th>µmol/L</th>
<th>Min/Max</th>
<th>Mean</th>
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<tr>
<td>Aspartic Acid</td>
<td>11</td>
<td>1 - 17</td>
<td>9</td>
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<tr>
<td>Citrulline</td>
<td>30</td>
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<td>22</td>
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<tr>
<td>Valine</td>
<td>195</td>
<td>78 - 326</td>
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<td>54</td>
<td>44 - 96</td>
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<td>Methionine</td>
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<td>7 - 43</td>
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Barth Syndrome – Plasma Amino Acids

![Graph showing plasma amino acids levels in normal controls and Barth Syndrome patients.](image_url)

- Normal Control (N=21)
- Barth Syndrome (N=18)

- Cystine
- Methionine
- Phenylalanine
- Histidine
- Arginine
- Prealbumin

- Normal range marked with a "*".
- Statistically significant difference at p < 0.0001.
Autosomal Recessive Barth Syndrome – Cardiomyopathy-Parkinsonism Syndrome

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<th>Condition</th>
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<tr>
<td>Postnatal growth retardation</td>
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<tr>
<td>Mild chronic neutropenia</td>
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<tr>
<td>Dilated cardiomyopathy – mild to severe</td>
</tr>
<tr>
<td>Sudden death from presumed arrhythmia</td>
</tr>
<tr>
<td>Ataxia - Parkinsonian tremor</td>
</tr>
<tr>
<td>Moderate developmental delay, +/- seizures</td>
</tr>
<tr>
<td>Apparent autosomal recessive inheritance (Xq28 excluded)</td>
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Autosomal Recessive Barth Syndrome
Autosomal Recessive Barth Syndrome: Laboratory abnormalities

- Chronic neutropenia - mild to moderate
- Hypocholesterolemia
- 3-Methylglutaconic aciduria
- Citric acid cycle organic aciduria
- Low plasma arginine levels
- Multifocal white matter lesions on MRI
Autosomal Recessive Barth Syndrome: DNAJC19
Mitochondrial Protein Import Motors

Mitochondrial Protein Import Motors

TIM 17 mRNA Expression Profile
Mitochondrial Protein Import in Yeast Crd1Δ Cardiolipin Mutants

Plasma Citric Acid Cycle Intermediate Levels

Plasma Citric Acid Cycle Intermediates

- Normal Control (N = 22)
- Barth Syndrome (N = 8)

Concentration - μmol/L

Malate | Fumarate | Succinate | 2-Ketoglutarate | Isocitrate | Aconitate | Citrate/10

p=0.0001

p=0.0001
Barth Syndrome

Erythrocyte Total Lipid Fatty Acid Levels
RBC Lipid Fatty Acid Levels vs. Neutrophil Index
Growth in Barth Syndrome
Barth Syndrome: Conclusions & Speculations

Cardiomyopathy is largely nutritional, due to endogenous amino acid depletion.

A major effect of cardiolipin deficiency is impaired import of citric acid cycle enzymes.

TAZ deficiency affects non-mitochondrial lipids.

Neutropenia & growth abnormalities could be caused by abnormal receptor response.
TCA Intermediates in Pediatric Autism

Plasma Citric Acid Cycle Intermediates

- Normal control - N = 25
- Autism - regression age 15 m

Concentration - μmol/L

- Malate
- Fumarate
- Succinate
- 2-Ketoglutarate
- Isocitrate
- Aconitate
- Citrate/10
Mitochondrial Treatment of Regressive Autism

Plasma Citric Acid Cycle Intermediates

- Normal control – N = 30
- 10/23/10 Before Treatment
- 4/18/11
- 11/14/11

Concentration – μmol/L

Malate, Fumarate, Succinate, 2-Ketoglutarate, Isocitrate, Aconitate, Citrate/10
TCA Intermediates in Multiple Sclerosis

Plasma Citric Acid Cycle Intermediates

- Normal control – N = 30
- MS Patients Group A

Concentration – μmol/L

- Malate
- Fumarate
- Succinate
- 2-Ketoglutarate
- Isocitrate
- Aconitate
- Citrate/10
Citric Acid Cycle
TCA Intermediates in Pierpont Syndrome

Plasma Citric Acid Cycle Intermediates

- Normal control – N = 22
- 11/4/10 Pierpont Syndrome
- 10/6/11 Pierpont Syndrome

Concentration – μmol/L

- Malate
- Fumarate
- Succinate
- 2-Ketoglutarate
- Isocitrate
- Aconitate
- Citrate/10

Graph showing the concentration of TCA intermediates in normal controls and individuals with Pierpont Syndrome.
TCA Intermediates in Adrenoleukodystrophy

Plasma Citric Acid Cycle Intermediates

- Normal Control - N = 22
- Age 8
- Age 10

Concentration - µmol/L

Malate  Fumarate  Succinate  2-Ketoglutarate  Isocitrate  Aconitate  Citrate/10

[Graph showing the concentration of TCA intermediates for different age groups and controls]
TCA Intermediates in Smith-Leml-Opitz Syndrome

Plasma Citric Acid Cycle Intermediates

- Normal control – N = 22
- Smith-Lemli-Opitz Syndrome

Concentration – µmol/L

Malate, Fumarate, Succinate, 2-Ketoglutarate, Isocitrate, Aconitate, Citrate/10
Substrate Fluxes in Leigh’s Disease Fibroblasts

Molecular Genetics and Metabolism 91 (2007) 15–22
Substrate uptake and secretion rates of Leigh affected cells relative to controls.

- **Substrate uptake**
- **Substrate secretion**

The graph shows the relative uptake and secretion rates of various substrates, with Met (methionine) and Arg (arginine) highlighted.

The substrates listed are: glc, lac, pyr, asn, tyr, thr, leu, phe, trp, ile, ala, gln, gly, pro, his, cystin, glu, arg, val, lys, ser, met, acac, fatty acids, ATP.
TCA Intermediates in Leigh’s Disease

Plasma Citric Acid Cycle Intermediates

- Normal control – N = 30
- Leigh’s Disease

Concentration - μmol/L

- Malate
- Fumarate
- Succinate
- 2-Ketoglutarate
- Isocitrate
- Aconitate
- Citrate/10
Citric Acid Cycle

Gluconeogenesis (in liver)

Glucose

Pyruvate

Ketones

Ketogenesis

Fat

Alanine, Serine

in liver

Acetyl-CoA

Acetylcholine

Fatty Acid Synthesis

Glutamine

Arginine

Asparagine

Aspartate

Water

Malate

Fumarate

Succinate

FADH$_2$

Succinate dehydrogenase

FAD

GDP + P$_i$

Succinyl-CoA synthetase

Succinyl-CoA

Methylmalonyl-CoA

Isoleucine, Valine, Threonine, Methionine

Glutamate

Ornithine

Proline
Folate – Methionine Cycle

- SAM Cycle
  - Guanidinoacetate → SAM
  - Acceptor
  - Methylated acceptor → SAH
  - Creatine

- SAM hydrolase
  - SAH → CBS
  - Cystathionine
  - Cystathionase
  - Cysteine
  - Glutathione

- Folate Cycle
  - Methionine → THF
  - Betaine
  - Homocysteine
  - Dimethylglycine
  - ATP

- Complex I
  - Serine → GCE → Glycine
  - Methyl-THF

- MTHFR → SAM

- Trans-sulphuration Pathway
  - SAM → Methionine synthase
  - CBS - Cystathionine-beta-synthase
  - GCE - Glycine cleavage enzyme
  - SAH - S-Adenosylhomocysteine
  - SAM - S-Adenosylmethionine
  - BHMT - Betaine:homocysteine methyltransferase
  - SHMT - Serine-threonine hydroxy-methyltransferase
Red Cell Volumes in Leigh’s Disease

Mean Corpuscular Volume

Mitochondrial Encephalopathy

MCV - fL

Age - Years
Red Cell Volumes in Leigh’s Disease

Mean Corpuscular Volume

MCV - fl

+2 SD

-2 SD

Age - Years

Start Folinic Acid

2 3 4 5 6
Disorders in which Mitochondrial Dysfunction Contributes to the Disease Process

1. ATP deficiency may not be the primary of cellular damage in mitochondrial diseases.

2. In Barth syndrome and many classical mitochondrial diseases, extramitochondrial substrate depletion is a major cause of cellular and organ pathology.

3. Careful analysis of plasma amino acid and TCA cycle intermediates can identify both the essential pathology of a mitochondrial disease and its treatment.
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