Cholesterol pool size in Smith-Lemli-Opitz syndrome children receiving cholesterol supplementation alone or combined with simvastatin

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Smith-Lemli-Opitz Syndrome (SLOS)

- Syndrome of multiple congenital anomalies including intellectual disability and behavioral problems – first described by *Smith et al., 1964 (J Pediatr 64:210-217)*

- Autosomal recessive genetic disorder

- Third most common inborn error of metabolism in the US
  - Observed incidence: 1/20,000-1/40,000
  - Carrier frequency: ~1-2% for Caucasians
Clinical Features

- SLOS phenotypic spectrum is very broad, ranging from mild disorder to lethal malformation syndrome
- Characteristic craniofacial features (e.g., microphaly)
- Cleft palate
- Brain malformations
- Growth & developmental retardation
- Limb anomalies (syndactyly)
- Genital anomalies
- Congenital heart defects
- Feeding difficulties
- Behavioral difficulties

Porter, 2008 Eur J Hum Genetics, 16: 535-541
Biochemical Basis of SLOS

  - Abnormal sterol profile:
    - Elevated 7-dehydrocholesterol (7-DHC): 1000-fold
    - Low plasma cholesterol levels

- Enzyme studies (Shefer et al. 1993, JLR 32: 1441-1448)
  - Deficient 7-dehydrocholesterol-Δ7- reductase (DHCR7) activity
    - DHCR7 catalyzes the final step in cholesterol biosynthetic pathway
Figure 1: Cholesterol Biosynthesis

Statins

SLOS

Bile Acids

Membrane Biogenesis

Steroid Hormones
**Therapeutic Management of SLOS**

- **Diet**: Cholesterol supplementation has become a standard therapy
  - Hypothesis: Improved developmental progress in SLOS children
  - Ameliorate cholesterol deficiency, thus increase plasma and tissue cholesterol levels
  - Lower 7-DHC synthesis by feedback inhibition

- **Medication**
  - HMG-CoA reductase inhibitors (Statins)

- Therapeutic intervention for SLOS is aimed at maximizing *whole body cholesterol pool size* while down-regulating biosynthesis to decrease the buildup of potentially toxic precursors
Cholesterol Miscible Pool and Turnover of Plasma Cholesterol in Man

- 1960’s - measurement of body miscible pool of cholesterol by radio-isotope dilution principles
- Goodman et al: Curve for disappearance of $^{14}$C-cholesterol from plasma over 10 wks
- Studies show that the turnover of plasma cholesterol in humans can be described by a two-pool-model
  - M1: includes cholesterol in liver, plasma, erythrocyte and some in viscera
  - M2: represents cholesterol in all other tissues

Goodman and Noble 1968, J Clin Invest 47: 231-241
Study Objective

- Assessment of whole body cholesterol pool size in SLOS patient receiving supplemental dietary cholesterol alone or combined with simvastatin
SLOS subjects receiving a high cholesterol diet alone (HI; n=11; age: 7±2 yr) or combined with simvastatin (HI+ST; n=4; age: 9±2 yr)

- Admit to hospital inpatient Metabolic unit for 1 week (OHSU)

- **Treatments:**
  - **High cholesterol:** Supplemented with egg yolks (35 mg/kg/d) or crystalline cholesterol (47 mg/kg/d)
  - **Simvastin:** Gradually increased from 0.2 mg/kg to 0.4 mg/kg

- **Stable Isotope cholesterol test**

  - **Baseline blood collection** (t=0 h)
  - **I.V injection of 18O-cholesterol (1.0-1.4 mg/kg BW) or D7- cholesterol (0.9-1.4 mg/kg BW)**
  - **Blood sample collection at:** 12, 24, 48, 72 h and 1, 3, 5, 8, 10 wk
Study Design and Method Analytical Procedures

- Cholesterol extracted from RBC, derivatized with piconyl ester and analyzed with liquid chromatography tandem mass spectrometry (LC/MS/MS)

- Sterols (cholesterol, 7-DHC, 8-DHC) were analyzed by gas chromatography

- Statistical analysis: Unpaired t-test, Mean +/- SEM
Cholesterol enrichment curve in SLOS subject following IV $^{18}$O-Cholesterol.
M1 pool (rapidly exchanging pool) – blood, visceral organs (liver, intestines, pancreas, spleen, kidneys, lung):

- SLOS children M1 pool:
  - Supplemented with chol: 0.33±0.03 g/kg, n=11
  - Cholesterol and statin: 0.52±0.11 g/kg, n=4

- Normal children: None reported to date

- Normal adult M1 pool: 0.35±0.08 g/kg, n=30
The images depict bar charts comparing different parameters between two groups: HI and HI+ST.

1. **Whole body cholesterol pool size (mg/kg body wt)**
   - HI: ~300 mg/kg
   - HI+ST: ~600 mg/kg
   - p-value: 0.04

2. **Plasma cholesterol (mg/dL)**
   - HI: ~80 mg/dL
   - HI+ST: ~100 mg/dL
   - p-value: 0.18

3. **Plasma 7DHC (mg/dL)**
   - HI: ~10 mg/dL
   - HI+ST: ~14 mg/dL
   - p-value: 0.03

4. **Plasma 8DHC (mg/dL)**
   - HI: ~8 mg/dL
   - HI+ST: ~10 mg/dL
   - p-value: 0.02
## PLASMA CHOLESTEROL

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<th>Independent variables</th>
<th>Pearson r</th>
<th>P-value</th>
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<tr>
<td>Weight (kg)</td>
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<td>Age (yr)</td>
<td>0.127</td>
<td>0.665</td>
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<td>Chol intake (mg/kg)</td>
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<td>Chol (mg/dl)</td>
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<td>n/a</td>
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<tr>
<td>7DHC (mg/dl)</td>
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<tr>
<td>8DHC (mg/dl)</td>
<td>-0.692</td>
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## M₁ CHOLESTEROL POOL

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Pearson r</th>
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<td>Weight (kg)</td>
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<td>Age (yr)</td>
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<td>Chol intake (mg/kg)</td>
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<td>Chol (mg/dl)</td>
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<td>7DHC (mg/dl)</td>
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<tr>
<td>8DHC (mg/dl)</td>
<td>-0.107</td>
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Discussion

- Rationale for considering high cholesterol diet with statin as potential therapy
  - Whole body cholesterol pool size enhanced by high cholesterol diet combined with simvastatin
- Simvastatin
  - Inhibits HMGCo-A reductase, blocking cholesterol synthesis as a way to avoid the formation of large amounts of 7-DHC and 8-DHC
- First reported study utilize stable isotope technique to assess cholesterol pool size in children
Acknowledgments

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