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

Semone B. Myrie[1,2], Samar Ahmad[1], Louise Merkens[3], Jean-Baptiste Roullet[3], Robert D. Steiner[3,4] and Peter J.H. Jones[1,2]

[1]Human Nutritional Sciences, [2]The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba

[3]Pediatrics, [4]Molecular and Medical Genetics, Oregon Health & Science University, 3181 SW Jackson Park Road, Portland

# 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 (eg., microphalyl)
- Cleft palate
- Brain malformations
- Growth & developmental retardation
- Limb anomalies (syndactyly)
- Genital anomalies
- Congenital heart defects
- Feeding difficulties
- Behavioral difficulties

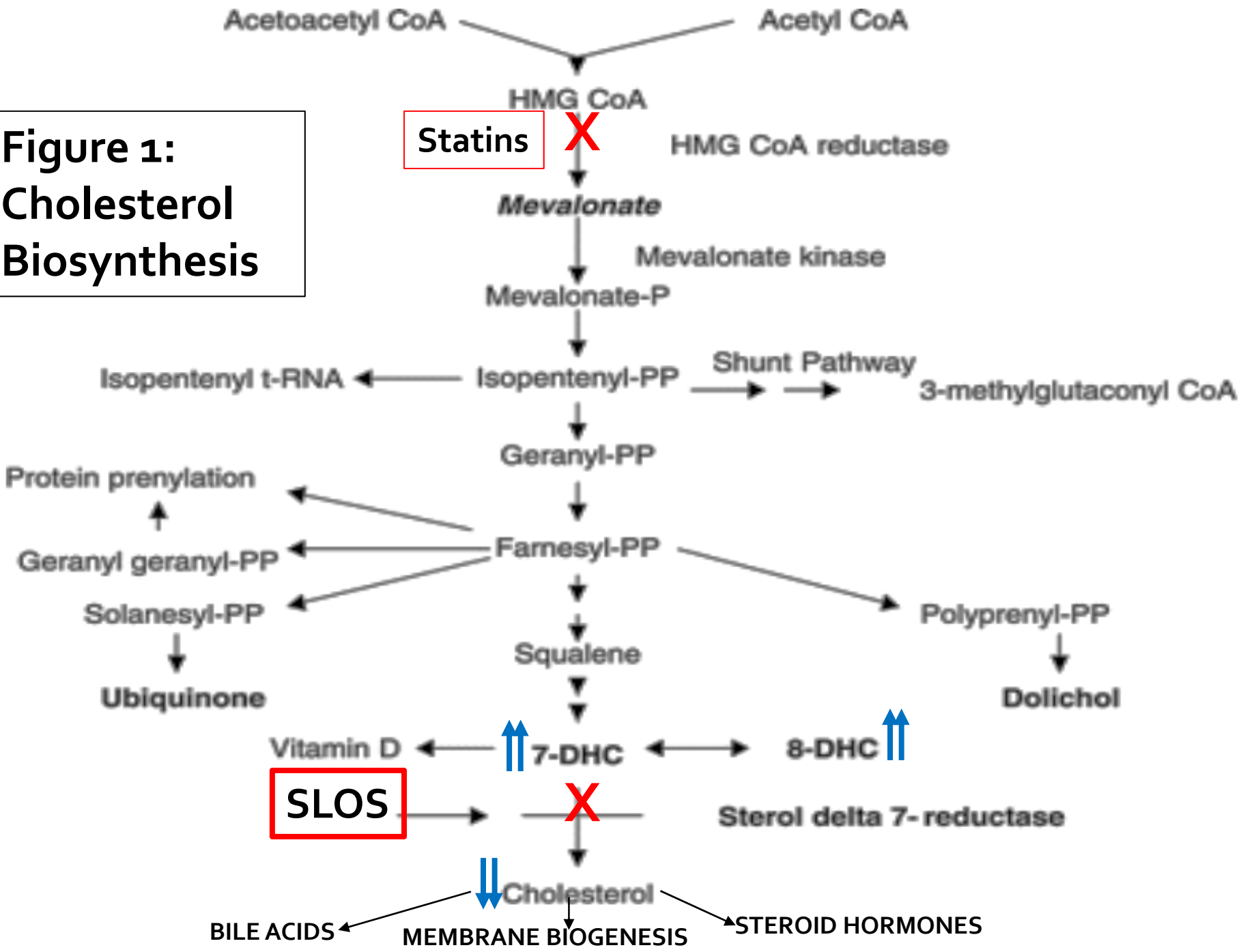

Porter, 2008 **Eur J Hum Genetics ,16: 535-541**

# Biochemical Basis of SLOS

- Defect in cholesterol synthesis (*Iron et al. 1993, Lancet 341: 1414; Tint et al. 1994, NEJM 330: 107-113)*
  - 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-$\Delta^7$- reductase (DHCR7) activity
    - DHCR7 catalyzes the **final** step in cholesterol biosynthetic pathway

# Figure 1: Cholesterol Biosynthesis



# 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

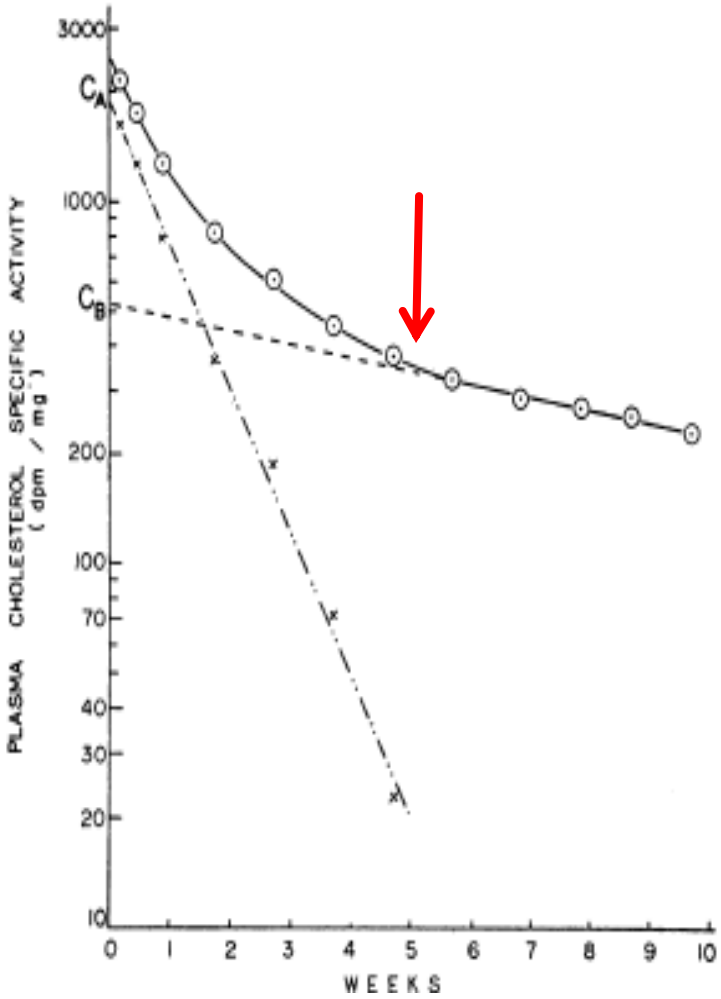
- 1960's - measurement of body miscible pool of cholesterol by radio-isotope dilution principles
- Goodman *et al*: Curve for disappearance of $^{14}$C-cholesterol from plasma over 10 wks
- Studies show that the turnover of plasma cholesterol in humans can be described by a two-pool-model

M1
**Rapid equilibrium**
(hrs-days) with
plasma cholesterol

⇔

M2
**Slow equilibrium**
(days-wks) with
plasma cholesterol

- M1: includes cholesterol in liver, plasma, erythrocyte and some in viscera
- M2: represents cholesterol in all other tissues



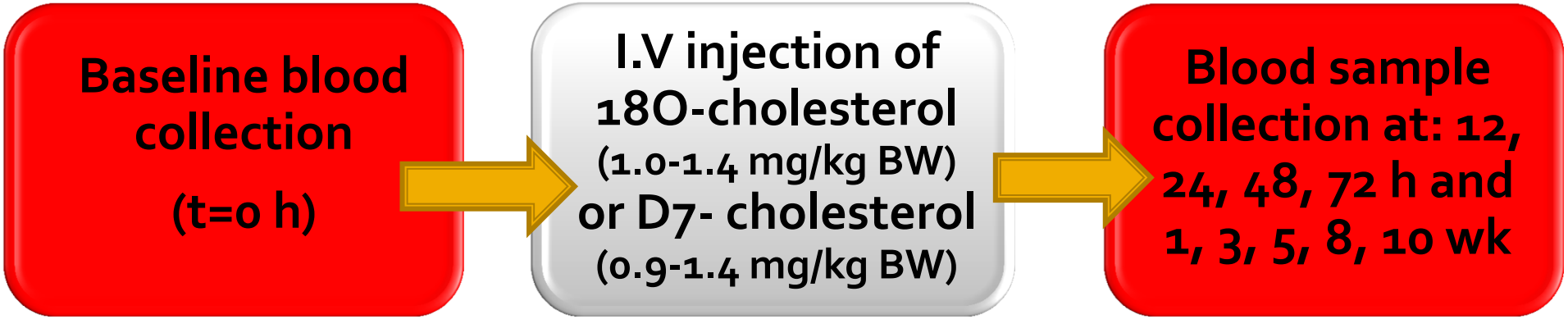

Goodman and Noble 1968, **J Clin Invest 47: 231-241**

# Study Objective

- Assessment of whole body cholesterol pool size in SLOS patient receiving supplemental dietary cholesterol alone or combined with simvastatin

# Study Design and Method
# Subjects and Testing

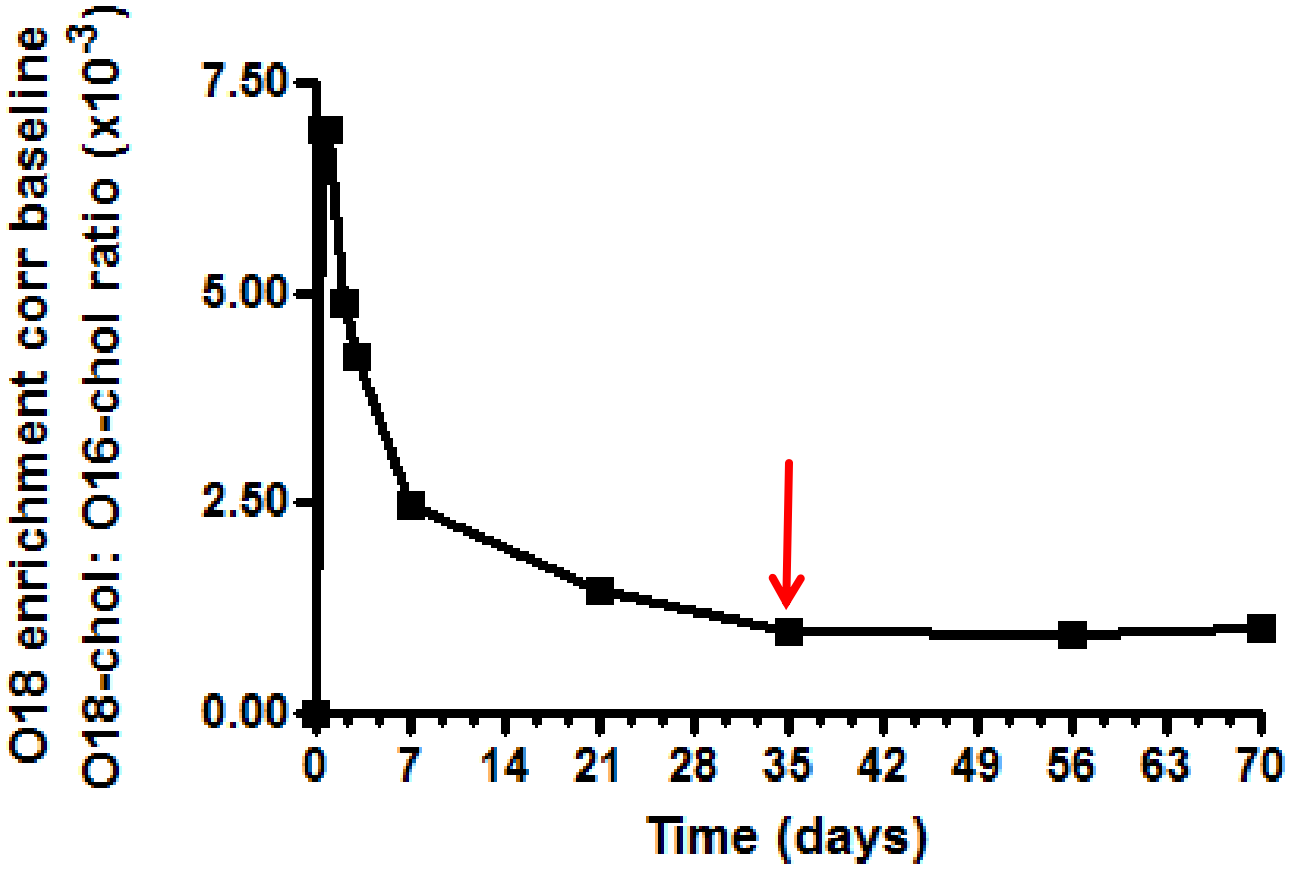
- SLOS subjects receiving a high cholesterol diet alone (HI; n=11; age: 7±2 yr) or combined with simvastatin (HI+ST; n=4; age: 9±2 yr)
- Admit to hospital inpatient Metabolic unit for 1 week (OHSU)
- Treatments:
  - **High cholesterol:** Supplemented with egg yolks (35 mg/kg/d) or crystalline cholesterol (47 mg/kg/d)
  - **Simvastin**: Gradually increased from 0.2 mg/kg to 0.4 mg/kg
- Stable Isotope cholesterol test



# Study Design and Method Analytical Procedures

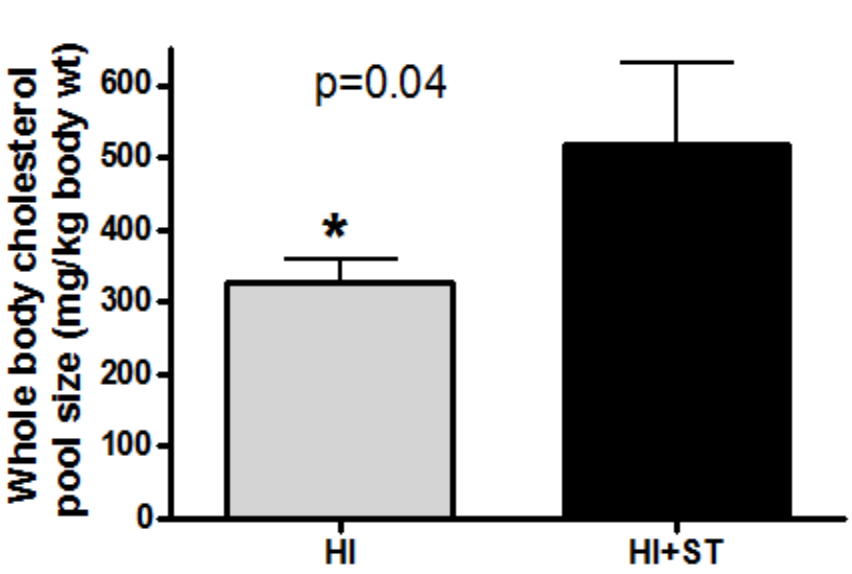
- Cholesterol extracted from RBC, derivatized with piconyl ester and analyzed with liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Sterols (cholesterol, 7-DHC, 8-DHC) were analyzed by gas chromatography
- Statistical analysis: Unpaired t-test, Mean +/- SEM

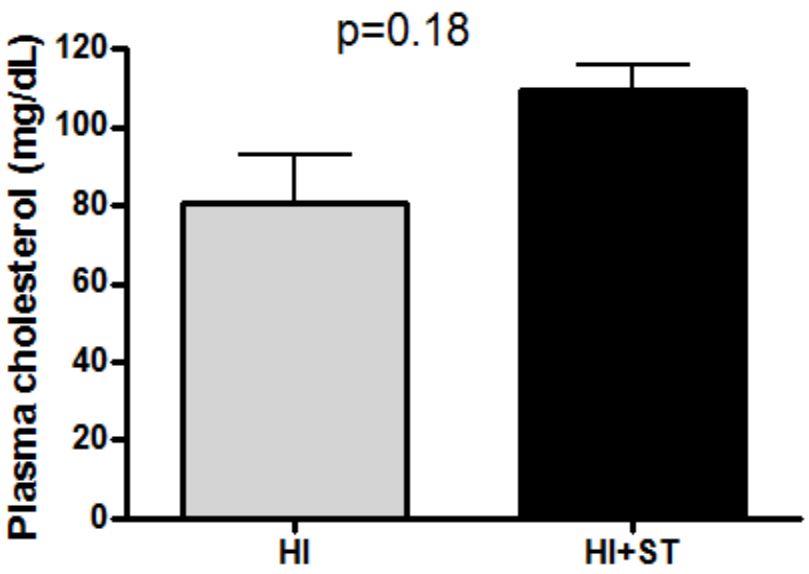
# Cholesterol enrichment curve in SLOS subject following IV [$^{18}$ O]-Cholesterol

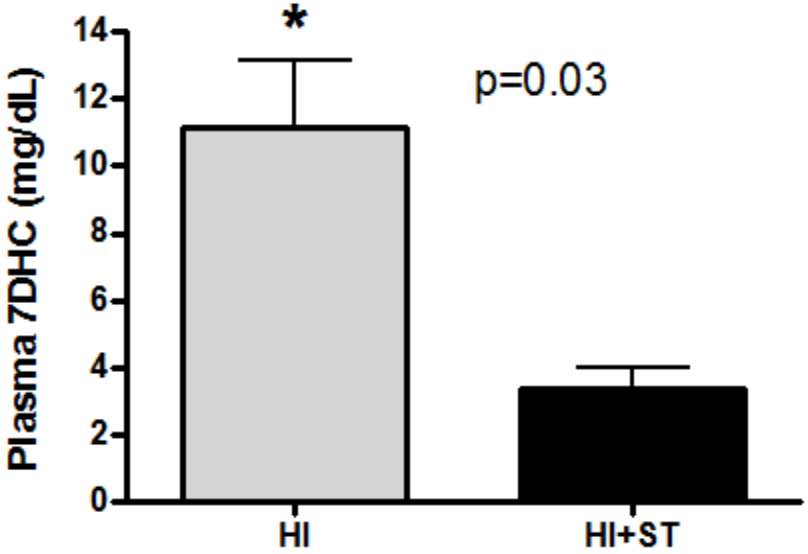


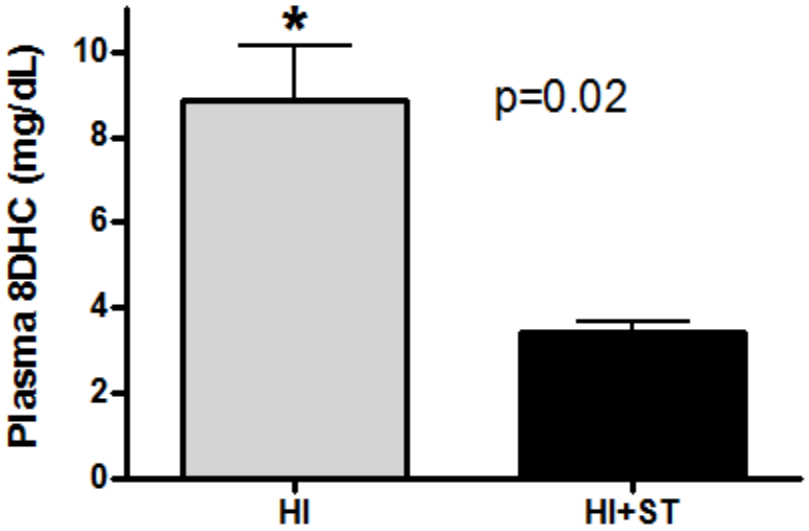
# Cholesterol Pool Size

- 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
  (Samuel *et al*, 1978 J Lipid Res 19: 94-1020)


Whole body cholesterol pool size (mg/kg body wt)
600
500
400
300
200
100
0
p=0.04
*
HI
HI+ST


Plasma cholesterol (mg/dL)
120
100
80
60
40
20
0
p=0.18
HI
HI+ST


Plasma 7DHC (mg/dL)
14
12
10
8
6
4
2
0
*
p=0.03
HI
HI+ST


Plasma 8DHC (mg/dL)
10
8
6
4
2
0
*
p=0.02
HI
HI+ST

# Effects of Cholesterol Supplementation in SLOS Children

## PLASMA CHOLESTEROL

| Independent variables | Pearson r | P-value |
|---|---|---|
| Weight (kg) | 0.277 | 0.359 |
| Age (yr) | 0.127 | 0.665 |
| Chol intake (mg/kg) | 0.892 | <0.0001 |
| Chol (mg/dl) | n/a | n/a |
| 7DHC (mg/dl) | -0.652 | 0.016 |
| 8DHC (mg/dl) | -o.692 | 0.009 |

## M1 CHOLESTEROL POOL

| Independent variables | Pearson r | *P-value* |
|---|---|---|
| Weight (kg) | 0.514 | 0.072 |
| Age (yr) | 0.563 | 0.045 |
| Chol intake (mg/kg) | 0.051 | 0.868 |
| Chol (mg/dl) | 0.128 | 0.676 |
| 7DHC (mg/dl) | -0.179 | 0.541 |
| 8DHC (mg/dl) | -0.107 | 0.715 |

# 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
  - Animal studies suggests that simvastatin up-regulates residual DHCR7 activity (Wassif et al. 2005, Mol Genet Metab 85:96-107; Correa-Cerro et al 2006, Hum Mol Genet 15:839-851)
- First reported study utilize stable isotope technique to to assess cholesterol pool size in children

# Acknowledgments


- Patients and families
- Sterol & Isoprenoid research consortium
- Rare Diseases Clinical Research network
  - RDCRN Travel award

- Funding:
  - NIH R01 HL073980,
  - NIH U54 HD061939