Dr. Melinda Sothern
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Social Determinants, Pro-inflammatory and Obesity-related Metabolic Biomarkers in Developing Youth

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Physical Activity

Adiposity

Metabolic Syndrome

Type 2 Diabetes

Glucose Tolerance

Insulin Sensitivity

Inflammation?

Obesity, Metabolic Functioning (e.g. BP, Cholesterol, Ectopic Fat, Fat Oxidation)

Postnatal/Infancy

Early Childhood

Maternal Nutrition

Breastfeeding

Prenatal

Mother’s Pregnancy Weight

High/Low Birth Weight Offspring; Fetal Programming
Background

- In adults obesity is considered a pro-inflammatory condition characterized by the presence of mediators of dyslipidemia, hypertension, visceral adipose tissue (VAT), and insulin resistance (IR).
- The production and modulation of these mediators is determined genetically and modified over time by social and environmental determinants.
- VAT and Ectopic (Intrahepatic [IH]) and Intramyocellular [IMCL]) fat increase the secretion of pro-inflammatory adipokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin 8 (IL-8) resulting in cellular damage and IR.
- Mechanisms may originate in the intrauterine environment and work synergistically to promote subtle, non-symptomatic metabolic abnormalities.
- Metabolic abnormalities are exacerbated by early onset obesity and further promoted by social disadvantage and an obesity-promoting environment.
- Studies in developing children are needed to unravel this series of events.

Children are not Little Adults

• Movement is required for cognitive development
• Enjoy unstructured physical activity (play)
• Play fosters healthy emotional development
• Unable to stay focused for long periods of time
• Lower oxygen uptake

• Immature metabolic systems

Sothern, M. Profile of the Overweight Child, in Safe and Effective Exercise for Overweight Youth, CRC Press, 2014
Study of Insulin sensitivity in Louisiana Low, High and Normal Weight Pre-pubertal Youth, 7-9 Years (SILLY)

- **African American**
  - Low/High Birth Weight
  - Normal Birth Weight
  - **N = 200**

- **Caucasian**
  - Low/High Birth Weight
  - Normal Birth Weight
  - **N = 200**

- Social Disadvantage
  - Concentrated Index (CDI)

- Inflammatory Adipokines (IL8, TNF@, IL6, IL1B, etc)

- Insulin Sensitivity
  - (FSIGTT)

- Insulin Resistance
  - (HOMA)

- Intramyocellular (IMCL) and Intrahepatic (IHL) Lipids (1H-MRS)

- Visceral Obesity (MRI; waist)

- Body Fat (DEXA)

- Blood Pressure

- Lipid Profile (TC, HDL, LDL)

- Fat Oxidation via Respiratory Quotient
  - (Indirect Calorimetry)

- Physical Activity
  - (Accelerometry)

Research sponsor: U. S. National Institutes of Health/NICHD (HD41071; HD49046); NIDDK; NIMHD; LSUHSC Jim Finks Endowed Chair in Health Promotion.
Certified Astronaut Hero enrolled in the Silly Study

S.I.L.L.Y.
(Study of Insulin sensitivity in Louisiana Low birth weight Youth)

S.I.L.L.Y. Astronaut ____________ Participant

Your courageous SILLY space adventure will help to prevent diabetes in Earthlings all around the planet!

LSU Health New Orleans
Methods: Social Determinants
Concentrated Disadvantage Index

- Using Census Data from the American Community Survey an index score for each study participant’s residence was created using the following variables:

- Percent of Individuals:
  - Below The Poverty Line
  - Receiving Public Assistance
  - Female-Headed Households
  - Unemployed
  - Less Than Age 18 Years of Age
  - Percent Black
Methods: Insulin Sensitivity

• 180-minute Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT)

• Glucose infused at minute 0; Insulin at minute 20

• Using the minimum model program the following variables are calculated:
  • Insulin sensitivity (Si)
  • Glucose effectiveness (Sg)
  • Acute Insulin Response to glucose (AIRg)
  • Disposition Index (DI)

Methods: Ectopic Fat
($^1$H-MRS [water-suppressed])

- Intramyocellular Lipids (IMCL; soleus muscle)
- Intrahepatic Lipids (IHL)

Larson-Meyer et al, Technology and Therapeutics, 2010
Results: Concentrated Disadvantage Index (CDI)

CDI was negatively associated with:

- $z$-BMI (-0.234, $p=0.023$)
- body fat (-.228, $p=0.028$, $n=95$)
- VAT (-.241, $p=0.042$, $n=74$)

*relationships remained significant in Caucasian children only after adjustment for race

Of the seven CDI variables the strongest predictors in Caucasian children only were:

- female head of households
- unemployed parents
- parents<18 years

Mohler, et al, Obesity, in press, 2014
Inverse correlation of serum pro-inflammatory markers with metabolic parameters in healthy, Black and White pre-pubertal youth

Pairs of markers with significant inverse Spearman's correlation. Logarithmic scale is used for the correlations between TNF-α and Intrahepatic Lipid; adjusted for race and gender.

Inverse correlation of serum pro-inflammatory markers with metabolic parameters in healthy, Black and White pre-pubertal youth

Overweight Children are not like Healthy Weight Children

- Physically compromised during weight-bearing aerobic exercise
- Biomechanically disadvantaged during walking and running
- Emotionally compromised due to teasing
- Metabolically compromised due to impaired insulin sensitivity and low fat oxidation

Sothern, M. Profile of the Overweight Child, in Safe and Effective Exercise for Overweight Youth, CRC Press, 2014
Results: Insulin Sensitivity is Inversely Related to Body Fat in Pre-pubertal Youth

TOTAL BODY FAT VS LOG-TRANSFORMED INSULIN SENSITIVITY

(r=0.42, p=0.04)

Obese Children are Metabolically Compromised

Bennett, et al, Obesity, 2012; Larsen-Meyer, et al, Diabetologia, 2011; Research supported by NICHD # HD49046 and #HD41071; NIDDK/NORC; NIMHD; After adjustment for body fat: IHL only (p<0.01)
The SILL Y Study - Results
(Sothern, et al, IASSO, 2012)

The best model for predicting Insulin Resistance in healthy children prior to entering puberty is below:

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>$R^2$</th>
<th>Mean Squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Birth Weight</td>
<td>0.62</td>
<td>2.26</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>• Low Density Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Diastolic and Systolic Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fat within Liver Cell (IHL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Abdominal Fat (VAT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Total Physical Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Insulin sensitivity by FSIVGTT was significantly correlated with IHL ($r=0.47; p=0.006$) and VAT ($r=0.42, p=0.04$).
Summary

• In adults, adolescents and pre-pubertal children IHL, independent of body fat, is a marker of obesity-related metabolic dysfunction through a mechanism of altered fatty acid transport away from adipose tissue toward liver and muscle tissue (Fibrini, et al, Hepatology, 2010; Bennett, et al, Obesity, 2011)

• Metabolic antecedents of IR, especially ectopic fat, occur in children before puberty. Thus, IHL and VAT are determinants of impaired insulin sensitivity; Kirchhoff, et al, JDSD, 2007; Larsen-Meyer, et al, Diabetologia, 2011)

• IHL is tightly associated with VAT, which is known to secrete pro-inflammatory adipokines, leading to inflammation, oxidative damage and impaired insulin receptor signaling (Fibrini, et al, NAS, 2009; Gade, et al, Clin Lab Science, 2011).
Conclusion

• Conversely, in healthy pre-pubertal children:
  
  • IHL is negatively associated with pro-inflammatory markers, TNFa and IL8. Thus, the role of inflammation is unclear (Zabaletta, et al, 2014).

  • Social disadvantage is negatively associated with inflammation in Caucasian youth prior to puberty; more research is needed (Mohler, Obesity, 2014)

• Low or high pregnancy weight, birth weight, lack of breastfeeding, poor nutrition and physical activity behaviors may collectively increase the risk for obesity, poor metabolic function and insulin resistance during early childhood.

• **Pregnancy weight, birth weight, breastfeeding, nutrition & physical activity can all be modified.**
The Obesity Trinity

- Tobacco use during pregnancy,
- Formula vs. Breastfeeding
- Frequent Pregnancies......
  - Resulted in fetal-programmed obese baby-boomers, maternal obesity, obese infant-toddlers, obese children/adolescents, maternal obesity and so on......

Solutions:
- Implement intense nutrition, physical activity and behavioral counseling/education during first visit to the Ob/Gyn and continuing until the child enters puberty
- Establish high-quality weight management programs for obese adolescent girls to ensure healthy pregnancies

(Sothern, M. Childhood Obesity, 2011)
### Objectively Measured Physical Activity Level (Accelerometry)

<table>
<thead>
<tr>
<th></th>
<th>Minutes (Mean + SD)</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>Sedentary</td>
<td>488.0 + 20.7</td>
<td>476.6 + 26.6*</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>27.7 + 9.6</td>
<td>29.9 + 13.3</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>17.1 + 8.4</td>
<td>22.7 + 10.4*</td>
</tr>
<tr>
<td></td>
<td>Vigorous</td>
<td>7.2 + 4.7**</td>
<td>10.8 + 6.3*</td>
</tr>
<tr>
<td></td>
<td>Total PA</td>
<td>52.0 + 20.9</td>
<td>63.4 + 26.6*</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>540</td>
<td>540</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Sedentary</td>
<td>482.8 + 40.4</td>
<td>480.3 + 36.1</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>27.7 + 16.2</td>
<td>29.8 + 17.3</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>19.1 + 16.1</td>
<td>19.1 + 12.6</td>
</tr>
<tr>
<td></td>
<td>Vigorous</td>
<td>10.4 + 11.2**</td>
<td>10.8 + 8.4</td>
</tr>
<tr>
<td></td>
<td>Total PA</td>
<td>57.2 + 40.5</td>
<td>59.7 + 36.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>540</td>
<td>540</td>
</tr>
</tbody>
</table>

* Significant as compared to its associated pre-intervention value; ** Significant as compared to the associated intensity level of the other group; Level of significance: p < 0.05; Matched pairs (83%)
Acknowledgements

**Louisiana State University Health Sciences Center (LSUHSC)**
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LSUHSC Department of Pediatrics, Children’s Hospital of New Orleans
EXTRA SLIDES FOR QUESTIONS
# Results: The SILLY Study Participants Characteristics

<table>
<thead>
<tr>
<th>Sex</th>
<th>66 males/58 females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8.04 ± 0.77 yrs</td>
</tr>
<tr>
<td>Race</td>
<td>77 Caucasian/46 Non-White</td>
</tr>
<tr>
<td>BMI z-score Range</td>
<td>-1.56 – 2.88</td>
</tr>
<tr>
<td>Body Fat Range</td>
<td>3.05 – 53.38 kg</td>
</tr>
<tr>
<td>Weight Range</td>
<td>18.8 – 107.9 kg</td>
</tr>
<tr>
<td>Non-Obese</td>
<td>94</td>
</tr>
<tr>
<td>Obese</td>
<td>29</td>
</tr>
</tbody>
</table>

Bennett, Sothern, et al, Obesity, 2012
Can Pre-pubertal youth with metabolic dysfunction be de-programmed

- The fetal period represents the only time when the number of muscles fibers can increase (Zhu, 2006).

- In 10-16 year olds, significant, positive correlations were observed between physical activity and both fasting insulin and insulin sensitivity (Schmitz, 2002).

- In overweight 9-15 year olds, 12 weeks of aerobic training improved insulin sensitivity and glucose metabolism **WITHOUT CHANGES IN BODY FAT** (Nassis, 2005).

- The improvement in insulin sensitivity may be due to an increased ability to oxidize fat in the muscles after physical training.
Mass Resonance Spectroscopy

Intrahepatic Lipid (IHL):

- Water-suppressed Press Box using Body coil
- One 20 x 20 x 20 mm box in vascular-free area of liver
- Time domain fitting
- Peanut oil phantom

Statistical Analysis

Additive linear model analysis using multiple regression procedures to determine Best Model (max $R^2$ and min mean squares) to explain variance in the relationship between insulin sensitivity and markers for the metabolic syndrome.

### Dependent / Response Variable
- Insulin Sensitivity (Si)

### Independent / Predictor Variables
- **Ectopic Fat** (IHL, IMCL)
- **Fat Oxidation** (RQ)
- **Lipids** (HDL, LDL, TC, TRI)
- **Blood Pressure** (SBP, DBP)
- **Visceral Adipose** (VAT)

### Covariates
- Race
- Total Body Fat
- Birth Weight
- Mother’s Pregnancy Weight
- Breastfeeding History
- **Physical Activity** (accelerometer and self report [TPA])
Results

After adjusting for total body fat and race, the best model consisted of IMCL, RQ, DBP, SBP, LDL, and HDL explaining 72% of the variance in Insulin Sensitivity (Si)

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Predictor Variables</th>
<th>R²</th>
<th>Mean Squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>IMCL, RQ, DBP, SBP, LDL, HDL</td>
<td>0.72</td>
<td>43.3</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>
Results

After considering additional covariates (i.e. birth weight, mother’s pregnancy weight, breast-feeding history, and self-reported physical activity), IMCL was removed from the model.

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Predictor Variables</th>
<th>$R^2$</th>
<th>Mean Squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>RQ, DBP, SBP, LDL, HDL</td>
<td>0.77</td>
<td>39.2</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>
Results

We then included visceral adipose tissue (VAT) by MRI and considered total physical activity (TPA) by accelerometry, birth weight (BRWT) and mother’s highest pregnancy weight as additional explanatory factors. The best models for predicting fasting insulin and insulin resistance by HOMA are as follows (IASO, 2010, in review):

<table>
<thead>
<tr>
<th>Dependent</th>
<th>Predictor Variables</th>
<th>$R^2$</th>
<th>Mean Squares</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Insulin</td>
<td>DBP, SBP, BRWT, IMCL, VAT, TPA</td>
<td>0.64</td>
<td>2.26</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Insulin Resistance</td>
<td>LDL, DBP, SBP, BRWT, IHL, VAT, TPA</td>
<td>0.62</td>
<td>2.26</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

Insulin sensitivity by FSIVGTT was significantly correlated with IHL ($r=0.47; p=0.006$) and VAT ($r=0.42, p=0.04$).
## Results – Obese vs. Non-obese

<table>
<thead>
<tr>
<th></th>
<th>Non-Obese</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IMCL</strong> (n=71)</td>
<td>0.46 ± 0.24</td>
<td>0.68 ± 0.35</td>
</tr>
<tr>
<td><strong>IHL</strong> (n=62)</td>
<td>0.57 ± 0.45</td>
<td>1.3 ± 1.16</td>
</tr>
<tr>
<td><strong>Insulin Sensitivity</strong></td>
<td>2.43 ± 0.63</td>
<td>1.77 ± 0.8</td>
</tr>
<tr>
<td><strong>Total Cholesterol</strong></td>
<td>150.8 ± 24.062</td>
<td>171.21 ± 25.405</td>
</tr>
<tr>
<td><strong>HDL</strong> (n=61)</td>
<td>50.739 ± 10.806</td>
<td>45 ± 8.392</td>
</tr>
<tr>
<td><strong>LDL</strong> (n=61)</td>
<td>86.978 ± 20.479</td>
<td>104.47 ± 19.063</td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
<td>107.18 ± 8.917</td>
<td>111.69 ± 9.587</td>
</tr>
<tr>
<td><strong>Diastolic BP</strong></td>
<td>63.074 ± 5.195</td>
<td>64.476 ± 5.482</td>
</tr>
</tbody>
</table>

ANOVA; adjusted for race and sex; *p<0.05; **p<0.01; ***p<0.001
Results – Obese vs. Non-Obese

- When compared to their non-obese counterparts, obese children exhibited the following:
  - Higher IMCL (p=0.005) and IHL (p=0.004)
  - Lower Si (p=0.01)
  - Higher LDL (p=0.009) and lower HDL cholesterol (p=0.0006)
  - Higher systolic blood pressure (p=0.04)

Bennett, Sothern, et al, Obesity, 2012
Social Determinants, Pro-inflammatory and Obesity-related Metabolic Biomarkers in Developing Youth

Objectives:
During the pre-pubertal stage of development in healthy Black and White youth:

- Explore the contribution of social determinants, and pro-inflammatory and metabolic bio-markers to the development of obesity and related co-morbidities.

- Discuss pre-and-post-natal nutrition and physical activity behavioral and environmental factors, which contribute to metabolic health.
Recruitment and Screening (as of 3/3/10)

Subjects Recruited:
- Phone Calls Received: 654

Phone Screening:
- Eligible for Medical Screening: 337
- ADHD Medication: 51
- Asthma Medication: 12
- Premature Birth: 60
- Other: 76
- Not Interested: 206

Medical Screening Visit:
- Enrolled: 197
  - Advanced Maturation: 41
  - Scheduled for 2nd Visit: 156
Summary: Mechanisms of Obesity and Metabolic Disease in Pre-pubertal African American and Caucasian Youth

- IHL, independent of VAT, is a marker of obesity-related metabolic dysfunction through a mechanism of altered fatty acid transport away from adipose tissue toward liver and muscle tissue (Fibrini, et al, Hepatology, 2010; Bennett, Sothern, et al, Obesity, 2011)


- IHL is tightly associated with VAT, which is known to secrete pro-inflammatory adipokines, leading to inflammation, oxidative damage and impaired insulin receptor signaling (Fibrini, et al, NAS, 2009; Gade, et al, Clin Lab Science, 2011).

- However, in pre-pubertal children IHL is positively associated with pro-inflammatory markers, TNFa and IL8 (Zabaletta, Sothern, et al, 2014).

- Social disadvantage is negatively associated with inflammation in Caucasian youth (Mohler, Sothern, Obesity, 2014)
Summary and Conclusion

• In an ethnically mixed cohort of 187 healthy, exclusively pre-pubertal (Tanner <2) obese and non-obese children we identified several early (pre-pubertal) markers and mechanisms that explain impaired insulin sensitivity.

• Metabolic antecedents of IR, especially ectopic fat, occur in children before puberty. Thus, IHL and VAT are determinants of impaired insulin sensitivity.

• Thus, as proposed the metabolic abnormalities increasing the propensity to obesity and type 2 diabetes originate earlier than previously proposed.

• Pre-and-post natal factors may promote the development of obesity and insulin resistance via a mechanism of impaired fat oxidation and lipid metabolism, which leads to excess fat in liver cells.

• Low or high pregnancy weight, birth weight, lack of breastfeeding, poor nutrition and physical activity behaviors may collectively increase the risk for obesity, poor metabolic function and insulin resistance during adolescence.

• Pregnancy weight, birth weight, breastfeeding, nutrition & physical activity can all be modified.