Ethnic Differences in Intramyocellular Lipid Levels and Insulin Resistance in Obese Children and Adolescents

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A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

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ETHNIC DIFFERENCES IN INTRAMYOCYTOLELLAR LIPID LEVELS AND
INSULIN RESISTANCE IN OBESE CHILDREN AND ADOLESCENTS.

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The prevalence of insulin resistance and type 2 diabetes mellitus (T2DM) in obese children and adolescents is growing at an alarming rate, especially in ethnic minorities. It is not clear whether young people of different ethnic backgrounds vary in their metabolic response to excessive adiposity. Differences in lipid partitioning in the abdominal fat compartments have been observed among different ethnic groups. The aim of this study was to evaluate whether there are ethnic differences in intramyocellular lipid (IMCL) levels that are related to differences in insulin sensitivity. Eighty-two obese children and adolescents underwent 1) $^1$H nuclear magnetic resonance (NMR) spectroscopy to non-invasively quantify IMCL levels in their soleus muscle, 2) an oral glucose tolerance test and (in a subset of subjects) a euglycemic-hyperinsulinemic clamp to assess insulin sensitivity, 3) a dual-energy X-ray absorptiometry (DEXA) scan to measure total percent body fat, and 4) magnetic resonance imaging to measure abdominal fat distribution. IMCL levels in Hispanic children and adolescents (1.50 ± 0.64%) were significantly greater than in their Caucasian (1.19 ± 0.40%) and African-American (1.09 ± 0.49%) peers. Visceral fat was significantly lower in African Americans (42.7 ± 18.8cm$^2$) and were similar in Caucasians (70.9 ± 27.5cm$^2$) and Hispanics (77.3 ± 41.9cm$^2$). The three groups were not different with respect to insulin sensitivity. For the entire cohort, IMCL levels were inversely related to insulin sensitivity. There was a significant correlation
between visceral fat and insulin resistance in Hispanics and Caucasians but not in African Americans. In conclusion, these data suggest that there are significant ethnic differences in lipid partitioning in both the muscle and abdominal compartment. These findings may explain ethnic differences in insulin sensitivity and further the understanding of the pathogenesis of insulin resistance and T2DM.
INTRODUCTION

Background

Childhood obesity has reached epidemic proportions and is a paramount public health concern. During the past 30 years, the number of children diagnosed as being overweight has more than doubled. The 1999-2002 National Health and Nutrition Examination Survey (NHANES) data indicate that, among children aged 6 through 19 years in 1999-2002, nearly one third (31.0%) were “at risk for overweight”—defined by a body mass index (BMI) between the 85th and 95th percentiles for age—and 16.0% were overweight—defined as a BMI of ≥95th percentile for age. In contrast, between 1988 and 1994 (NHANES III), approximately 11% of children and adolescents were overweight and 14% had a BMI between the 85th and 95th percentiles. The prevalence of overweight children and adolescents in 2002 (16%) was more than three times the expected prevalence based on prior surveys (5%). Children from racial minority groups are affected disproportionately. Among non-Hispanic black, and Mexican-American children and adolescents, 20.5% and 22.1%, respectively, are overweight, compared with 13.6% among non-Hispanic white children and adolescents. Similar increases in the prevalence of obesity have been observed worldwide. Obesity in children is an important early risk factor for much of adult morbidity and also accounts for many significant health problems in the pediatric population.

Overweight or obesity is the most important risk factor for the development of type 2 diabetes mellitus (T2DM) in youth. Parallel to the obesity epidemic, the prevalence of T2DM has increased alarmingly among children worldwide during the past
decade. The incidence of T2DM in children has increased by 10-fold from 1982 to 1994. The Centers for Disease Control (CDC) predicts that, if current obesity rates continue, one in three newborns born in 2000 will eventually develop diabetes. In African-American and Hispanic youth, T2DM is more common than in people of Caucasian origins.

Insulin resistance (IR), defined as an inability of target tissues to increase glucose uptake in response to insulin, is now widely believed to be the first step in the development of T2DM. In 1988, Reaven and colleagues described “the metabolic syndrome”—otherwise referred to as “syndrome X” or the “insulin-resistance syndrome”—as a link between insulin resistance, hypertension, dyslipidemia, T2DM, and other metabolic abnormalities associated with an increased risk of atherosclerotic cardiovascular disease in adults. According to the NCEP and ATPIII panel, individuals meeting at least three of the following five criteria qualify as having the metabolic syndrome: (1) elevated blood pressure, (2) high triglyceride level, (3) low HDL-cholesterol level, (4) high fasting glucose, and (5) central obesity. This syndrome affects approximately 25% of the U.S. adult population, and may be present in as many 30% of obese adolescents. While there are no established criteria for diagnosing the metabolic syndrome in the pediatric population, in a recent study of 439 obese, 31 overweight, and 20 normal-weight children and adolescents, the metabolic syndrome, as defined by modified ATP criteria, was present in 39 and 50 percent of the moderately and severely obese subjects, respectively. This indicates that, among obese children, the prevalence of the metabolic syndrome is high and increases with worsening obesity. Similar to obesity and T2DM, insulin resistance seems to affect children from racial
minorities disproportionately. The ethnicity-related prevalence of T2DM is attributed to a greater degree of obesity and severity of insulin resistance. Several studies have demonstrated decreased insulin sensitivity in healthy African-American and Hispanic children compared with their Caucasian peers. The reason for these racial differences have not yet been elucidated.

The underlying mechanisms relevant to the changes in insulin sensitivity and secretion in the early stages of diabetes in youth are still largely unknown. Increasing our understanding of the mechanisms responsible for the insulin resistance associated with childhood obesity is of utmost importance. The obese pediatric population provides an interesting opportunity to study early metabolic alterations associated with obesity. Unlike in children, obesity in adults is usually long lasting. Therefore, the association of generalized insulin resistance, compensatory hyperinsulinemia, and increased visceral fat may simply reflect adaptations to long-term obesity rather than a causal relationship. Also, the model of childhood obesity is free from long-term effects of life-style conditions, such as smoking, chronic alcohol consumption, and uncontrollable stress, which are known to strongly influence fat distribution and its metabolic correlates.

**Insulin Resistance and Lipid Compartments**

In addition to the association between insulin resistance and generalized obesity, many studies have revealed associations between insulin resistance and distribution of body fat. Using non-invasive methods, such as computed tomography (CT)—which clearly distinguishes fat from other tissues and allows for the measurement of visceral and subcutaneous abdominal fat—has furthered our understanding of the links among body
composition and insulin resistance. Visceral fat accumulation is known to be associated with features of the metabolic syndrome in adults and obese children, although the nature of this association and the relative importance of visceral and subcutaneous abdominal fat in the pediatric population remains a matter of debate. More recently, a different fat deposit has garnered increased attention as an important contributor to the metabolic phenotype responsible for insulin resistance.

Skeletal muscle is the primary site of insulin-stimulated glucose disposal at euglycemia and, as such, is a major locus of insulin resistance. Among the putative mechanisms underlying insulin resistance, increased attention has been paid to elevated circulating levels of free fatty acids (FFAs). In 1967 Denton and Randle were the first to describe the existence of lipid storage in muscles. More recently, FFAs derived from skeletal muscle lipolysis have been recognized as an important endogenous source of energy in addition to the energy storage in adipose tissue. Triglyceride content measured in muscle biopsies obtained from non-diabetic Pima Indians (an ethnic group with a pronounced disposition to obesity and T2DM) was found to be related to insulin resistance, independent of total adiposity. Subsequent studies have shown similar results.

The putative molecular mechanism responsible for the inverse relationship between insulin sensitivity and IMCL focuses on the action of free fatty acids and IMCL metabolites, such as long-chain fatty acyl-CoAs (LCA-CoA), on phosphatidylinositol-3-kinase (PI3K). PI3K is a key signal transducer in insulin-mediated glucose transport facilitated by incorporation of GLUT4 transporters into the muscle cell membrane. Increased plasma FFA and intracellular accumulations of LCA-CoA, as found in obesity,
can activate isoforms of protein kinase C (PKC), which in turn might lead to phosphorylation of serine/threonine sites of the insulin receptor and its substrates (IRS), thereby decreasing their ability to activate PI3K.\(^{32,33}\)

![Figure 1: Increased plasma FFA- and IMCL-related intracellular accumulations of LCA-CoA activate isoforms of protein kinase C (PKC), which in turn lead to phosphorylation of serine/threonine sites of the insulin receptor and its substrates (IRS), thereby decreasing their ability to activate PI3K and inhibiting GLUT4-dependent glucose import.](image)

Until recently, muscle fat deposits were either measured by muscle biopsy or estimated by computed tomography. These techniques are incapable of accurately differentiating between intramyocellular (IMCL) and extramyocellular (EMCL) lipid content.\(^{34}\) Moreover, the invasive nature of muscle biopsy and the relatively high dose of ionizing radiation of CT make these techniques unsafe to perform on the pediatric population. These problems have been overcome by the use of \(^{1}\)H nuclear magnetic resonance (NMR) spectroscopy.\(^{35}\) This technique has been shown to be capable of differentiating between IMCL and EMCL \(^{34}\), as demonstrated in patients with congenital
lipodystrophy who do not have EMCL content. In 1999 Shulman and his group showed a negative correlation between IMCL concentration, as assessed by localized $^1$H-NMR spectroscopy, and whole body insulin sensitivity.

This noninvasive and safe technique allowed for the determination of IMCL and EMCL levels in the pediatric population. In the first such study in children by Sinha et al., both IMCL and EMCL in the soleus muscle were observed to be greater in obese vs. non-obese adolescents. Moreover, they demonstrated a significant correlation between intracellular lipid and insulin sensitivity independent of total body fat. Weiss et al.—also using NMR—found an excessive accumulation of IMCL in the soleus muscle of obese adolescents with impaired glucose tolerance (IGT) compared to age- and adiposity-matched obese adolescents with normal glucose tolerance (NGT).

**Ethnic Differences in Fat Distribution**

Recently, in an attempt to explain the greater severity of insulin resistance among minorities, numerous studies have analyzed racial differences in regional fat distribution. Paradoxically, it has been found that, despite the correlation between visceral fat accumulation and insulin resistance, African-American children have significantly lower levels of visceral fat than their Caucasian peers. Bacha et al. demonstrated that visceral adiposity has a different impact on the metabolic profile of black obese adolescents when compared to well matched white adolescents. However, differences in visceral fat were not able to explain the more diabetogenic insulin sensitivity profile observed in African Americans. The failure of visceral or subcutaneous adipose tissue to
explain the ethnic difference in insulin sensitivity suggests that the accumulation of lipid in other deposits may account for this variation.

Ama et al.\textsuperscript{42} reported an increased expression of type II muscle fibers in African-American sedentary adult men compared to Caucasians. More recently, similar results were found when comparing obese black and white women, and it was also shown that an over-expression of type II fibers contribute to the pathophysiology of obesity.\textsuperscript{43,44} Type II muscle fibers are deficient in oxidative capacity, particularly in relation to lipid disposal, and an abundance of these fibers can thereby result in the partitioning of lipid toward storage in skeletal muscle, rather than oxidation within the muscle, leading to a positive fat balance, fat mass gain and marked insulin resistance.\textsuperscript{45}

There are no studies in the pediatric population that have examined the role of muscle fiber composition and its relation to obesity and insulin resistance. It is also unknown whether the racial differences observed with regard to muscle fiber composition are already present in childhood or adolescence.\textsuperscript{1} \textsuperscript{1}H-NMR spectroscopy of skeletal muscle is a practical method of accurately assessing the lipid content of that compartment in children and adolescents and thereby ascertaining whether there are racial differences in skeletal muscle composition that can be correlated to the differences in insulin sensitivity.

**Statement of Purpose**

To determine whether ethnic differences in insulin sensitivity are related to a greater intramyocellular accumulation of lipid content in obese adolescents of African-American and Hispanic origins compared to Caucasian adolescents closely matched for overall adiposity. \textsuperscript{1}H-NMR was used to non-invasively quantify IMCL and EMCL in
African-American, Hispanic, and Caucasian obese adolescents. Insulin sensitivity was ascertained by oral glucose tolerance tests (OGTT) in all subjects and the euglycemic-hyperinsulinemic clamp in a subset of subjects.
METHODS

Participants

We studied three well matched groups of subjects: 21 overweight African-American adolescents, 30 overweight Caucasian adolescents, and 31 overweight Hispanic adolescents. They were recruited from a multi-ethnic cohort of obese children and adolescents drawn from the Pediatric Obesity Clinic at Yale-New Haven Hospital. To be eligible for this study, they had to be between 8 and 21 years of age, overweight or obese, (BMI Z-score >1.5), to be taking no medications that can alter glucose metabolism, and to be otherwise healthy. In all participants we did a complete physical examination and took a detailed medical history. Ethnicity was determined by self-report and was based on all four grandparents being of the same ethnic group as the child in the study. The study was approved by the Human Investigational Committee of the Yale School of Medicine. Written informed consent was obtained from the parents, and written assent was given by the participants.

Metabolic Studies

Oral Glucose Tolerance Test (OGTT):

All subjects underwent an OGTT; the subjects consumed a diet containing at least 250 g of carbohydrates per day for three days before the study and refrained from vigorous physical activity. They were evaluated at 8 a.m., after a 12-hour, overnight fast. Their weight and height were measured, and their BMI was calculated. Baseline blood samples were obtained from subjects while they were fasting, with the use of an
indwelling venous line for measurement of levels of glucose, insulin, lipids, adiponectin, leptin, and C-reactive protein. An oral glucose-tolerance test was then performed with the administration of 1.75 g of glucose per kilogram of body weight (maximal dose, 75 g).\footnote{46}

**Euglycemic Clamp:**

In 47 patients whole-body insulin sensitivity was measured by a two-step euglycemic clamp.\footnote{47} The subjects were instructed by a registered dietitian to follow a weight maintenance diet consisting of at least 250 g carbohydrate per day for 7 days before the study and to refrain from physical activity. The children arrived at the Yale Children's Clinical Research Center at 7:30 a.m. after an overnight fast of 10-12 h. Two intravenous catheters—one for blood sampling and one for infusion of glucose, insulin, and tracers—were inserted, one in the antecubital vein of each arm after local infiltration with lidocaine. The arm used for blood sampling was kept in a heated box for arterialisation of blood. Whole-body insulin sensitivity was measured by a two-step euglycemic clamp by infusing insulin as a primed continuous infusion at 8 mU m\(^{-2}\) min\(^{-1}\) and 80 mU m\(^{-2}\) min\(^{-1}\). Each step lasted 2 h. A primed-continuous infusion of 6,6-deuterium-labelled glucose at a rate of 11·11 μmoles m\(^{-2}\) min\(^{-1}\) and a continuous infusion of \(^2\)H\(_5\)-glycerol at a rate of 0·21 μmoles m\(^{-2}\) min\(^{-1}\), were used to quantify insulin’s effects on glucose and glycerol turnover. To maintain the plasma enrichment of \(^2\)H-glucose constant at baseline value throughout the clamp, we used the Hot GINF method.\footnote{48} Arterialised blood samples were collected every 5-10 min during the last 30 min of the baseline period and during each insulin infusion period for measurement of glucose and
glycerol enrichments, hormones, and substrates. Indirect calorimetry was used at baseline and during the last 30 min of each step of the clamp to estimate net rates of carbohydrate and lipid oxidation. Non-oxidative glucose metabolism was calculated by subtracting the amount of glucose oxidized from the whole-body glucose uptake. Insulin sensitivity (M) was determined by milligrams glucose uptake per kilogram per minute and expressed as M/lean body mass (M_{lbm}).

**Imaging Studies**

**^1H-NMR Spectroscopy:**

Localized ^1H-NMR spectra of the soleus muscle were acquired on a 2.1 T Biospec system (Bruker Instruments, Inc, Billerica, MA, USA). The clinical status of the participants was concealed from the investigator who collected and analyzed the data. All participants were instructed not to undertake any physical activity for 7 days before the test. All 82 subjects underwent this study.

**MRI:**

Abdominal MRI studies were performed on a Siemens Sonata 1.5-Tesla system. The pulse sequence was a T1-weighted Fast Low Angle Shot Gradent Echo (FLASH). Slices were acquired using a 400cm field of view (TE:4.76, TR:100, 4 excitations, 90° flip angle, matrix: 256x128, bandwidth:140). The mid-axial section was positioned to pass through the L4/L5 disc space. Images were imported into the Yale Bioimage Suite software package. Visceral, subcutaneous, deep subcutaneous, and superficial subcutaneous fat areas were determined. Thresholding was applied to separate fat from
soft tissue. This procedure was done in 75 of the participants (29 Caucasian, 21 African-American, and 25 Hispanic).

**DEXA:**

Total body composition was measured by dual-energy X-ray absorptiometry with a Hologic scanner (Boston, MA, USA). The clinical status of the participants was concealed from the investigator who collected and analyzed these data.

**Analytical Procedures and Calculations**

Plasma and urine glucose concentrations were measured by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Brea, CA, USA). Plasma insulin, C-peptide, leptin, and adiponectin concentrations were measured by double-antibody radioimmunoassays. Plasma fatty acids were assayed by a colorimetric method. Analysis of enrichments of $^2$H-glucose and $^2$H$_5$ -glycerol in plasma and infusates by gas chromatography and mass spectrometry was done as described elsewhere.$^{49}$

Parameters reflecting insulin sensitivity were derived from the OGTT. Estimated insulin sensitivity was calculated using the Matsuda index$^{50}$ (whole-body insulin sensitivity index [WBISI]), which has been validated by comparison with euglycemic-hyperinsulinemic clamp studies in obese children and adolescents.$^{51}$ The index was calculated according to the following formula:

$$WBISI = 10000 \sqrt{\frac{\text{fasting glucose} \times \text{fasting insulin}}{\text{mean glucose} \times \text{mean insulin}}}.$$
During the insulin clamp study, the amount of glucose required to maintain euglycemia provides an index of insulin-stimulated glucose metabolism. The glucose infusion rates were calculated during the last 30 min of the low and high insulin clamp and expressed as μmol glucose per kg of lean body mass per min. Endogenous hepatic glucose production and glycerol turnover at baseline and during the two steps of the insulin clamp, along with the clamped glucose disposal rates, were calculated as previously reported.\textsuperscript{49} In the basal state, we calculated an index of hepatic insulin resistance as the product of endogenous glucose production and the fasting insulin concentrations.\textsuperscript{47,39}

**Statistical Analysis**

Data are represented as mean ± SD. Parameters that were not normally distributed were log-transformed for analysis. Multiple pair-wise comparisons of subjects were performed using ANOVA with post-hoc Holm correction for multiple comparisons between pairs. Adjustment of comparisons for potential confounders was performed using analysis of covariance with main effects for age, sex, %fat and other relevant covariates where appropriate. Pearson or Spearman correlation analysis was used when applicable to examine bivariate relationships. A p value of <0.05 was considered statistically significant. All analyses were performed using SAS/STAT for Windows.
RESULTS

Anthropometric Characteristics of the Cohort

A total of 82 obese children and adolescents (30 Caucasian, 21 African-American, and 162 Hispanic) participated in this study (Table 1). Fewer boys than girls participated in the study (12 Caucasian (40%), 9 African-American (43%), and 15 Hispanic (48%)), but the distribution of sex was not significantly different across race (p = 0.80). The three groups were similar with regard to age, weight, height, BMI and BMI z-score. Of note, assessment of whole body composition by DEXA revealed similar percent fat, lean body mass, and total body fat mass among the three groups.

Metabolic Profile of the Cohort

The metabolic profile of the participants was in most aspects very similar across race (Table 2). After adjusting for age, sex, and percent body fat, there was no significant race main effect, or race*sex interaction in fasting glucose and insulin, two-hour glucose, insulin, and WBISI. A total of 47 subjects (14 Caucasian, 13 African-American, and 20 Hispanic) underwent the euglycemic clamp. As reported in Table 2, there was no significant race main effect or race*sex interaction in insulin sensitivity (M/lean body mass). For the lipid profile, total cholesterol, HDL, and LDL were similar. African Americans had significantly lower triglyceride levels than Hispanics (p = 0.04) and also lower levels than Caucasians, but this did not reach significance (p = 0.13). Of note, Hispanics had higher levels of free fatty acids than Caucasians (p = 0.01) and African Americans (p = 0.01). African Americans had higher levels of leptin than Caucasians (p = 0.05) and Hispanics (p = 0.02). Adiponectin levels were similar across race.
Table 1: Anthropometric Characteristics (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>African-American</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>Gender</td>
<td>12 (40%) Male 18 (60%) Female</td>
<td>9 (42.9%) Male 12 (57.1%) Female</td>
<td>15 (48.4%) Male 16 (51.6%) Female</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>14.13 ± 2.15 (10-19)</td>
<td>14.19 ± 2.16 (11-21)</td>
<td>14.16 ± 2.46 (10-21)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>95.81 ± 20.10 (55.3-194.7)</td>
<td>101.85 ± 18.79 (58.3-135.0)</td>
<td>100.24 ± 20.47 (60.8-136.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.77 ± 10.09 (150.0-194.7)</td>
<td>165.71 ± 9.46 (147.5-182.0)</td>
<td>163.61 ± 9.78 (142.5-184.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.29 ± 6.22 (23.7-50.5)</td>
<td>36.96 ± 5.84 (26.8-46.8)</td>
<td>37.23 ± 5.95 (24.2-51.29)</td>
</tr>
<tr>
<td>BMIz</td>
<td>2.27 ± 0.32 (1.47-2.71)</td>
<td>2.39 ± 0.26 (1.90-2.89)</td>
<td>2.43 ± 0.36 (1.57-3.08)</td>
</tr>
<tr>
<td>%Fat</td>
<td>40.13 ± 13.12 (27.3-55.1)</td>
<td>39.05 ± 5.06 (26.6-48-6)</td>
<td>40.51 ± 5.16 (26.5-47.4)</td>
</tr>
<tr>
<td>Total Fat Mass (kg)</td>
<td>38.39 ± 13.12 (16.5-76.3)</td>
<td>40.55 ± 10.73 (18.2-61.6)</td>
<td>39.38 ± 9.76 (24.6-62.3)</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>54.21 ±10.84 (32.0-77.9)</td>
<td>58.58 ± 10.22 (38.0-76.9)</td>
<td>56.26 ± 12.13 (37.4-82.3)</td>
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</table>
Table 2: Metabolic Profiles (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>African-American</th>
<th>Hispanic</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose 0 (mg/dl)</strong></td>
<td>89.8 ± 6.5 (70-102)</td>
<td>92.5 ± 7.1 (76-106)</td>
<td>90.9 ± 6.4 (78-102)</td>
<td>Not significant^b</td>
</tr>
<tr>
<td><strong>Insulin 0 (mg/dl)</strong></td>
<td>30.2 ± 16.5 (8-85)</td>
<td>30.9 ± 13.3 (11-56)</td>
<td>37.8 ± 20.1 (12-97)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td><strong>Glucose 120 (mg/dl)</strong></td>
<td>113.9 ± 14.5 (77-133)</td>
<td>112.0 ± 19.0 (72-136)</td>
<td>112.7 ± 20.4 (39-137)</td>
<td>Not significant^b</td>
</tr>
<tr>
<td><strong>Insulin 120 (mg/dl)</strong></td>
<td>143 ± 88 (31-376)</td>
<td>161.7 ± 153.9 (21-624)</td>
<td>191 ± 217 (45-1209)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>WBISI</td>
<td>2.00 ± 0.86 (0.54-3.89)</td>
<td>2.05 ± 1.09 (0.63-4.62)</td>
<td>1.73 ± 1.02 (0.32-4.30)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>M_lean (mg/kg min)</td>
<td>0.091 ± 0.047 (0.03-0.17)</td>
<td>0.092 ± 0.046 (0.03-0.21)</td>
<td>0.092 ± 0.053 (0.03-0.22)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>Tot. Chol. (mg/dl)</td>
<td>158 ± 28 (103-214)</td>
<td>161 ± 40 (99-243)</td>
<td>150 ± 28 (96-218)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41 ± 9 (23-63)</td>
<td>49 ± 12 (33-77)</td>
<td>37 ± 6 (26-51)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>91 ± 27 (47-150)</td>
<td>95 ± 34 (50-188)</td>
<td>89 ± 23 (37-141)</td>
<td>Not significant^b, c</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>120 ± 81 (15-394)</td>
<td>80 ± 51 (22-263)</td>
<td>117 ± 53 (49-276)</td>
<td>C vs. AA: 0.13 C vs. H: not sig. AA vs. H: 0.04 ^c</td>
</tr>
<tr>
<td>FFA (meq/dl)</td>
<td>456 ± 125 (150-670)</td>
<td>436 ± 133 (295-800)</td>
<td>570 ± 150 (334-888)</td>
<td>C vs. AA: not sig. C vs. H: 0.01 AA vs. H: 0.01 ^c</td>
</tr>
<tr>
<td>Leptin (ng/dl)</td>
<td>28.4 ± 16.9 (8-85)</td>
<td>33.6 ± 15.5 (11-67)</td>
<td>27.0 ± 13.9 (6-59)</td>
<td>C vs. AA: 0.05 C vs. H: not sig. AA vs. H: 0.02 ^c</td>
</tr>
<tr>
<td>Adiponectin (μg/dl)</td>
<td>8.04 ± 4.29 (1.4-18.4)</td>
<td>7.91 ± 3.42 (2.6-14.4)</td>
<td>6.76 ± 3.06 (2.9-16.6)</td>
<td>Not significant^b, c</td>
</tr>
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^a Adjusted for age, sex, and percent fat.
^b No significant race main effect, or race*sex interaction.
^c Variable log transformed in model due to skewed distribution
**Muscle and Abdominal Lipid Partitioning**

As shown in figure 2, IMCL was significantly higher in the Hispanic group (1.50 ± 0.64%) than in the African-American (1.09 ± 0.49%) and the Caucasian (1.19 ± 0.40%) groups (p = 0.01 and p = 0.04 respectively). The difference between the African-American and Caucasian groups was not significant. EMCL levels were not significantly different between the Caucasian (1.51 ± 0.95%), African-American (1.78 ± 0.95%) and Hispanic groups (1.91 ± 0.79%) (Figure 3).

Visceral fat was significantly lower in the African-American group (42.7 ± 18.8cm$^2$) than in the Caucasian (70.9 ± 27.5cm$^2$) and the Hispanic (77.3 ± 41.9cm$^2$) groups (p = 0.0006 and p < 0.0001, respectively) (Figure 4). Subcutaneous fat was higher in the African-American group (596 ± 186cm$^2$) than in the Caucasian (547 ± 191cm$^2$) and Hispanic (543 ± 160cm$^2$) groups (p = 0.04 and p = 0.06, respectively) (Figure 5). The Caucasian and Hispanic groups were similar with regards to visceral and subcutaneous fat.
Figure 2: IMCL

![Graph showing IMCL data for different ethnic groups with significance levels indicated by p-values of 0.04 and 0.01.]

Figure 3: EMCL

![Graph showing EMCL data for different ethnic groups.]

Caucasian
African American
Hispanic
Figure 4: Visceral Fat

![Visceral Fat Graph]

Figure 5: Subcutaneous Fat

![Subcutaneous Fat Graph]
Relationships between Lipid Partitioning and Insulin Sensitivity

Pearson correlation analysis revealed a significant relationship between WBISI and IMCL of the entire cohort ($r = -0.27; p = 0.02$) (Figure 6). In the subset that underwent the euglycemic clamp, $M_{\text{lbm}}$ exhibited a similar correlation with IMCL ($r = -0.37; p = 0.01$). Correlations between WBISI and IMCL within the individual groups did not reach significance and were not significantly different from each other.

There was also a significant correlation between WBISI and visceral adiposity when examining the entire cohort ($r = -0.38; p = 0.001$). (Figure 6). This correlation was also significant for the Caucasian ($r = -0.41; p = 0.03$) and Hispanic ($r = -0.41; p = 0.04$) groups but not for the African-American group ($r = -0.29; p = 0.21$). In the subset of the cohort that underwent the euglycemic clamp, there was a significant correlation between $M_{\text{lbm}}$ and visceral fat ($r = -0.56; p < 0.0001$).
Figure 6: WBISI vs. IMCL
Figure 7: WBISI vs. Visceral Fat
DISCUSSION

Our study is the first to compare IMCL levels—determined by $^1$H-NMR spectroscopy—across race in a pediatric population. We also examined racial differences in the correlation between IMCL levels and insulin sensitivity. Given the higher incidence of insulin resistance and diabetes in minority populations, we hypothesized that obese Hispanic and African-American youth would have higher IMCL levels than their Caucasian counterparts. Our study showed that our hypothesis held true for Hispanics but not for African Americans. The Hispanic children and adolescents in our study had significantly higher IMCL levels (after adjusting for age, sex, and percent fat) than their well-matched Caucasian and African-American counterparts. African-American children, on the other hand, had the lowest IMCL levels, although the difference between African Americans and Caucasians did not reach significance.

Our study also reaffirmed that African-American children have very different composition of their abdominal lipid compartments than their Caucasian and Hispanic peers. The African-American group in our study had significantly lower visceral fat deposits and significantly higher subcutaneous fat deposits than the Caucasian and Hispanic groups. This has already been shown by several other studies.\textsuperscript{22, 40, 41} Consistent with the lower levels of visceral fat, as Gower \textit{et al.} have shown\textsuperscript{17}, we found African Americans to have lower levels of serum triglycerides than Caucasians and Hispanics. Lower triglyceride levels despite higher levels of insulin resistance among African Americans have also been described in adults.\textsuperscript{52, 53}

It is possible that factors similar to those that protect African Americans from accumulating visceral fat\textsuperscript{41, 54} may also be responsible for their lower IMCL levels.
However, in our study, while African Americans tended to have lower IMCL levels than Caucasians, this difference failed to reach significance. It is possible that this is due to insufficient power, but it may also represent the fact that, with regard to lipid deposits in myocytes (as opposed to in the abdominal compartment), African Americans are similar to their Caucasian counterparts. Our study shows that neither increased visceral fat nor increased IMCL levels are responsible for the higher rates of insulin resistance that have been observed in the African-American population. Of note, despite their lower visceral fat and lower IMCL levels, African Americans in our study had similar levels of insulin resistance to the other groups. It seems clear that the fat compartments we analyzed are not directly responsible for the insulin resistance observed in African Americans. It is likely that either a different lipid compartment has a stronger role in the development of insulin resistance in African Americans, or perhaps that the pathogenesis of insulin resistance and T2DM in African Americans is less closely linked to the absolute amount of adipose tissue in any compartment.

In Hispanics, on the other hand, IMCL levels were significantly higher than in the other groups. This indicates that Hispanics have a higher propensity to distribute excess body fat in the muscle compartment than Caucasians and African Americans. Similar racial differences have been observed in the occurrence of nonalcoholic fatty liver disease (NAFLD). A recent population-based study of obese adolescents found that NAFLD is more common in Hispanics than in Caucasians, and is the least common in African Americans. It is unclear why Hispanics are more susceptible to lipid accumulation in their liver and muscle compartments. It may be due to genetic differences in metabolic processing and storage of excess fat, but could also be secondary to
environmental/cultural differences such as diet and exercise. It is very likely that the increased susceptibility of Hispanics to deposit lipid in the muscle compartment plays an important role in their increased risk of developing insulin resistance, T2DM, and other metabolic dysfunction. In accordance with this are the significantly higher levels of plasma free fatty acids we found in Hispanics as compared to Caucasians and African Americans. These elevated levels of free fatty acids may be important factors in the development of increased IMCL levels, which in turn may potentiate and perpetuate the elevated flux of free fatty acids, and thereby contribute to the deregulation of glucose metabolism in the pathogenesis of insulin resistance.56, 57

Interestingly, while several studies have shown that African-American and Hispanic adolescents are more insulin resistant than Caucasian adolescents17, in our study insulin sensitivity as measured by the OGTT and the euglycemic clamp (in a subset of 47 subjects) was very similar between the three groups. However, this finding is not surprising considering that our study consisted of a very homogenous obese group that did not include any leans. Bacha et al22 made a similar observation when comparing insulin sensitivity between Caucasian and African-American obese adolescents, ascribing the similarity in insulin sensitivity to the overriding effect of obesity-related insulin resistance that masked race-related differences in insulin sensitivity.

When looking at the relationship between IMCL levels and insulin sensitivity in the entire cohort, our study showed an inverse relationship between IMCL levels and insulin sensitivity. This inverse relationship has already been shown elsewhere.37, 39 When looking at the correlation between IMCL and WBISI within each group, the correlations did not reach significance. However, the trend seems to imply an inverse
relationship in the Caucasian and Hispanic group, whereas in the African-American group, there does not seem to be a clear relationship between the two variables (see Figure 6). Similarly, when looking at the relationship between visceral fat and insulin sensitivity of the entire cohort, we again confirmed the inverse relationship between the two variables. For visceral fat, this inverse relationship was significant for the Caucasian and Hispanic but not for the African-American group (see Figure 7). However, the difference in correlations among the ethnic groups again did not reach significance. Our observations support the above proposed theory that, while lipid deposits in the visceral and myocellular compartments seem to play important roles in the pathogenesis of insulin resistance in both Caucasians and Hispanics, they do not appear to have the same relationship in African Americans. Adequately powered studies are warranted to address this important issue.

While our study would have benefited from a larger sample size, its strength lies in the robustness of our methods. $^1$H-NMR spectroscopy of the soleus muscle is currently the gold standard for determining IMCL levels in children and adults. We used MRI to analyze the abdominal fat compartment, and DEXA for the assessment of total body fat, LBM, and percent fat. A large subset of our subjects underwent the euglycemic clamp, while all subjects had OGTTs for the assessment of insulin sensitivity. We included only NGTs in our study to ensure that our subjects were well matched with respect to their metabolic profiles. Our groups were very well matched with respect to most anthropometric variables (see Table 1); as mentioned above, we had relatively more boys in the Hispanic group, but this difference was not significant. The robust nature of our methods required a large time commitment from our subjects and therefore limited
the number of subjects we were able to enroll. The other limitation of our current study is that our cohort consisted mainly of extremely obese subjects without the inclusion of overweight or lean subjects. With a well matched cohort representing the entire spectrum of adiposity, from lean to very obese, we perhaps would have been able to bring out the differences in insulin sensitivity that have been observed among the different ethnic groups and potentially single out the importance of IMCL in the pathogenesis of insulin resistance.

In conclusion, in a cohort of obese children and adolescents, we demonstrated that Hispanics have higher IMCL levels and plasma free fatty acids than do Caucasians and African Americans. The IMCL levels of African Americans tended to be lower than those of Caucasians but not significantly different. Visceral fat and plasma triglycerides were significantly lower in African Americans and were similar in Caucasians and Hispanics. The three groups were not different with respect to insulin sensitivity. The inverse relationship between IMCL levels and insulin sensitivity held true for the entire cohort but did not reach significance in the individual ethnic groups. The correlation between high IMCL levels and insulin resistance seemed to be stronger in Hispanics and Caucasians than in African Americans, but our study did not have sufficient power to elucidate this. We also confirmed the correlation between visceral fat and insulin resistance in Hispanics and Caucasians but not in African Americans. In addition, our data raises several important questions to be answered in subsequent studies. We showed that, in the Hispanic population, intramyocellular lipid may be an important mediator and marker for insulin resistance. The mechanism that predisposes Hispanics to deposit higher levels of lipid in this compartment than their peers in other ethnic groups needs to
be further elucidated. We also need to further our understanding of the mechanism by
which high IMCL levels contribute to the pathogenesis of insulin resistance and T2DM.
On the other hand, we need to understand by what mechanism African Americans
prevent lipid deposition in the abdominal and muscle compartment and why they are
nevertheless at a higher risk of developing insulin resistance and T2DM compared to
Caucasians. Further molecular analysis and detailed metabolic studies supplemented by
imaging in large cohorts of lean and obese children will be necessary to answer these
important questions.
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