Insulin resistance: link to the components of the metabolic syndrome and biomarkers of endothelial dysfunction in youth

Received for publication 31 January 2007 and accepted in revised form 26 April 2007.

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Running title: insulin resistance, metabolic syndrome and adhesion molecules

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ABSTRACT

Objective: We examined the relationship of in vivo insulin sensitivity (IS) to the components of the metabolic syndrome (MetS) and biomarkers of endothelial dysfunction in youth.

Research Design and Methods: Subjects included 216 youths (8-19 years) who participated in a 3-hour hyperinsulinemic-euglycemic clamp.

Results: Independent of race, the frequencies of central obesity, high triglycerides, low HDL, high blood pressure (BP), impaired fasting glucose and impaired glucose tolerance were significantly higher (P <0.05) in the lowest vs. highest quartile of IS. BMI, abdominal adiposity, systolic BP and triglycerides increased, and adiponectin and HDL decreased significantly (P for trend for all <0.05) with decreasing IS in both races. After controlling for BMI, insulin resistance remained associated (P <0.05) with visceral adipose tissue (VAT) in both races (P for trend = 0.01 in Blacks and 0.08 in Whites). In Whites but not Blacks, lower IS was associated (P <0.05) with higher ICAM-1 and E-selectin levels, however these relationships did not remain significant (P >0.05) once VAT is controlled for.

Conclusions: The prevalence of the individual components of MetS increases with decreasing IS in Black and White youth. In Whites but not Blacks insulin resistance is associated with increased circulating endothelial biomarkers. It remains to be determined if lower abdominal adiposity and triglycerides in Blacks underlies the racial differences in risk translation.
INTRODUCTION

The escalating epidemic of childhood obesity is of great public health concern because of the obesity related co-morbid conditions in youth such as high blood pressure (BP) (1; 2), insulin resistance (3; 4), and type 2 diabetes (5). As in adults, the prevalence of the metabolic syndrome (MetS), a cluster of risk factors for cardiovascular disease (CVD) and type 2 diabetes (6), is high in overweight youth (7-9). Despite several definitions for MetS in adults, there are currently no accepted criteria in pediatrics. Previous studies however, have used variations of the adult criteria to report a wide range of MetS prevalence rates in youth (7-10).

Although insulin resistance is proposed to be the underlying mechanism linking the various components of MetS (6), studies exploring the relationship between directly measured insulin resistance and the components of MetS are lacking in pediatrics. Therefore, we examined the individual components of MetS based on quartiles of in vivo insulin sensitivity (IS) in children and adolescents.

Elevated CVD risk factors in childhood are associated with risk of atherosclerotic disease in adulthood (11; 12). During the early stages of atherosclerotic CVD, biomarkers of endothelial dysfunction including intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and E-selectin are increased in response to inflammatory cytokines and play an important role in the formation of atherosclerotic plaque (13). Although the relationship between circulating adhesion molecules and insulin resistance is well documented in adults (14-16), it is unclear if this holds true in youth. Thus, we examined the relationship between IS and the biomarkers of endothelial dysfunction.

RESEARCH DESIGN AND METHODS

Subjects consisted of 99 Black and 117 White youth (8-19 yr) who underwent assessment of in vivo IS at Children’s Hospital of Pittsburgh. Data from some of these subjects have been reported previously (3; 17-20). Exclusion criteria included diagnosed diabetes or any chronic illness and the use of medications that influence glucose and lipid metabolism, and BP. All study participants were recruited through newspaper advertisements in the greater Pittsburgh area, flyers posted in the city public transportation, and posters placed on campus. The investigation was approved by the Institutional Review Board, parental informed consent and child assent were obtained from all participants, and studies were performed in the General Clinical Research Center (GCRC) of Children’s Hospital of Pittsburgh. All participants underwent physical examination, and routine hematological and biochemical tests. Pubertal development was assessed by Tanner criteria. The overweight subjects (BMI ≥95th percentile) (21) underwent an oral glucose tolerance test (OGTT) prior to participation to screen for normal glucose tolerance. Among 105 overweight subjects (50 Blacks and 55 Whites), 24 subjects [10 Blacks (7 PCOS) and 14 Whites (10 PCOS)] had impaired glucose tolerance (IGT) (22). Among 125 girls, 26 subjects had PCOS referred to the endocrine service for evaluation of irregular menses, hirsutism and acne as reported previously (23-25).
Abdominal adiposity

Waist circumference was obtained in 153 subjects at the midpoint between the lowest rib and the iliac crest. A single transverse image of the abdomen (L4-L5) was obtained using computed tomography in 207 as shown by us previously (26).

The hyperinsulinemic-euglycemic clamp

All subjects were admitted to the GCRC on the afternoon prior to the day of the clamp experiment, after assuring that they have not had any intercurrent illnesses for the preceding 3-4 weeks. The clamp was performed after a 10 to 12 hr overnight fast, where fasting blood was obtained for lipid profile, adiponectin, interleukin-6 (IL-6) and adhesion molecules. Briefly, the 3-hr hyperinsulinemic-euglycemic clamp was performed with plasma glucose clamped at 5.6 mmol/l with a variable-rate infusion of 20% dextrose based on arterialized plasma glucose determinations every 5 min as previously described (19; 25). The insulin-stimulated glucose disposal rate was calculated using the average exogenous glucose infusion rate during the final 30 minutes of the clamp. IS (mg/kg/min per µu/ml x 100) was calculated by dividing insulin-stimulated glucose disposal rate by the steady-state insulin levels during the last 30 min of the clamp as described previously (19).

Definition of the individual components of MetS

We used cutoffs using the modified NCEP/ATP III criteria similar to those published previously (7; 10): high triglycerides (≥110 mg/dl), low HDL (≤40 mg/dl), high BP (systolic or diastolic BP ≥90th for age, sex and height) (27), impaired fasting glucose (IFG, ≥100 mg/dl) (28) or IGT (22), and large waist (≥90th for age, sex and race) (29).

Biochemical Measurements

The measurements of plasma glucose, insulin, lipids and adiponectin are described by us previously (18). IL-6 (highly sensitive solid-phase assay kits), ICAM-1, VCAM-1 and E-selectin were quantified using commercially available double-sandwich enzyme-linked immunoassays (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation were: 6.7% and 8.6% for IL-6; 6.0% and 9.4% for ICAM-1; 4.5% and 9.0% for VCAM-1 and 6.1 and 9.0% for E-selectin, respectively.

Statistical Analyses

Statistical procedures were performed using SPSS 14.0 (SPSS, Inc.,Chicago, IL). Independent t-tests were used to examine subject characteristics. Analysis of covariance, adjusted for age, sex and Tanner stage (pre-pubertal and pubertal), was used to compare metabolic variables between the quartiles of IS groups within each race. Frequencies of the individual components of MetS were compared using the chi-square tests between the quartiles of IS groups. Receiver operating characteristic (ROC) curves were used to obtain the area under the curve (AUC), an indicator of the ability of MetS components to discriminate the subjects with vs. without insulin resistance. Multiple regression analyses were used to examine the independent contribution of IS and VAT to the metabolic markers.

RESULTS

The subject characteristics are shown in Table 1. Independent of race, the frequencies of central obesity, high triglycerides, low HDL, high BP, IFG and IGT were higher (P <0.05) in the lowest versus highest quartile of IS (Figure 1). In
the lowest quartile of IS group, central obesity had the highest prevalence (92.9%) among all components of MetS in Blacks followed by low HDL (62.5%) and IFG + IGT (62.5%). In Whites, central obesity (100%) was the most common feature followed by high triglycerides (69%) and low HDL (65%).

Figures 2 and 3 indicate the individual components of MetS, circulating adhesion molecules, adiponectin and IL-6 levels (after adjusting for age, gender and tanner stage) stratified by quartiles of IS. BMI, systolic BP, triglycerides, and abdominal adiposity (waist circumference and VAT) decreased (\(P\) for trend <0.01) and HDL increased with increasing IS in both races (Figure 2). After further adjustment for BMI, VAT decreased with increasing IS in both races (\(P\) for trend = 0.01 in Blacks and 0.08 in Whites). In the lowest quartile of IS group, VAT (\(P =0.036\)), waist circumference (\(P = 0.027\)) and triglycerides (\(P = 0.088\)) were higher in Whites versus Blacks.

As shown in Figure 3, in Whites but not Blacks ICAM-1, E-selectin and IL-6 levels significantly decreased (\(P\) for trend <0.01) with increasing IS, however these relationships did not remain significant (\(P\) for trend >0.1 for all) after controlling for BMI. Further, adiponectin levels increased significantly (\(P\) for trend <0.01) in a stepwise manner from the lowest to the highest quartiles of IS in both races, and these observations remained significant (\(P\) for trend <0.01 for both races) after further adjustment for BMI.

Figure 4 indicates the ROC curves of the MetS components as markers of insulin resistance. Independent of race, the greatest area under the ROC curves as markers of insulin resistance were BMI (Blacks: 0.972, Whites: 0.928) and waist circumference (Blacks: 0.950, Whites: 0.943). In multiple regression analyses with insulin sensitivity as the dependent variable and BMI, WC, TG, BP, or HDL, as the independent variables with age, race and gender, the \(R^2\) for BMI and WC were 0.75 and WC 0.74 (data not shown). In the same analyses the \(R^2\) for TG, BP and HDL were 0.33, 0.41 and 0.31, respectively (data not shown).

**Independent contribution of IS and VAT to the MetS components and adhesion molecules**

In Blacks, IS was independently related to triglycerides (partial \(r = -0.29, P <0.05\)) and HDL (partial \(r = 0.29, P <0.05\)), while in Whites VAT was independently associated with these markers (triglycerides: partial \(r = 0.23, P <0.05\), HDL: partial \(r = -0.22, P <0.05\)) after accounting for age, sex and Tanner stage. In both races, VAT was associated (\(P <0.05\)) with systolic BP independent of IS (partial \(r = 0.26\) and \(r = 0.25\) in Blacks and Whites, respectively). In Whites, VAT was associated with ICAM-1 (partial \(r = 0.17, P =0.08\)) and E-selectin (partial \(r = 0.25, P <0.01\)) independent of IS.

**CONCLUSIONS**

Our findings demonstrate the following: 1) the prevalence of the individual components of MetS increases with decreasing *in vivo* IS, 2) adiponectin segregates with the degree of IS, 3) in Whites insulin resistance is significantly associated with higher IL-6 and biomarkers of endothelial dysfunction, ICAM-1 and E-selectin, 4) significant racial differences exist in the level of the individual components of MetS mainly abdominal adiposity and triglycerides, and last but not least, although obesity plays a major role in the clustering of MetS,
insulin resistance is also associated with VAT and adiponectin after controlling for BMI.

Similar to findings in adults, our study in youth demonstrates that all of the components of MetS substantially increase with the degree of insulin resistance independent of race. Given that most of our subjects in the most insulin resistant group met the abdominal obesity criteria and that the greatest area under the ROC curves as markers of insulin resistance were waist circumference, it is reasonable to assume that the relationships between insulin resistance and MetS could be modulated by abdominal adiposity. Indeed, in our multiple regression analyses VAT predicts triglycerides and HDL in Whites, and systolic BP in both races independent of IS. Given that anthropometric measures of BMI or waist circumference provide the highest variance in insulin sensitivity in our study, such measures constitute important clinical markers of metabolic risk in youth, and in the absence of a pediatric definition of MetS, a measure of abdominal obesity (waist circumference) should be considered as an important component of the pediatric MetS definition.

Our findings of high levels of adhesion molecules in White versus Black youth is consistent with findings in adults (30). Our study suggests that racial differences in circulating adhesion molecules are already present during childhood. However, unlike the previous report (30) demonstrating significant sex differences in soluble adhesion molecules in a large sample of middle aged men and women (40-59 years), we observed a sex difference in VCAM-1 levels in Blacks only (boys: 378.9 ± 20.0 vs. girls: 450.7 ± 22.7, \( P = 0.028 \)). In addition, the observation that in Whites the relationship between IS, ICAM-1 and E-selectin levels disappear after controlling for VAT is consistent with Targher et al. (31) who reported a similar finding in Caucasian adults with type 2 diabetes. These findings suggest that excess adiposity may contribute to increased circulating levels of adhesion molecules.

Our findings of significant relationship between directly measured insulin resistance and endothelial markers in Whites extend the previous observations in obese children (32; 33) wherein insulin resistance was evaluated by homeostasis model assessment of insulin resistance. However, the underlying cause for the race-related differences in the levels of some of the adhesion molecules and their relationship or lack of, in the case of Blacks, with IS remains to be determined. If visceral adiposity plays a role in circulating adhesion molecules, it would be tempting to theorize that the race-related differences may stem from the fact that Blacks have significantly lower VAT compared with Whites, a finding consistent with studies in adults (34; 35). In parallel with lower VAT, Blacks have lower triglycerides than Whites similar to previous reports in youths (36) and adults (37). By contrast, Blacks tend to have a greater prevalence of hypertension than Whites (38). These observations raise the concerns about the validity of the current MetS definitions and the theoretical question of whether or not it is reasonable to accept the same cut-off criteria of MetS for triglycerides and BP in African-Americans and Caucasians.

Although the mechanism(s) by which VAT is associated with coronary artery disease risk are uncertain, VAT is now recognized as an endocrine tissue that produces inflammatory markers including TNF-alpha, PAI-1 and IL-6, all of which are thought to play an important
role in the development of atherosclerotic CVD (16). In this study, we observed that in Whites VAT is strongly associated with circulating IL-6, ICAM-1 and E-selectin levels. It is plausible that higher VAT in Whites may explain the greater atherogenic risk profile than Blacks which is consistent with our previous finding (36). Future studies are needed to examine if the thresholds for the future development of CVD or type 2 diabetes are comparable between the two racial groups.

We observed that plasma adiponectin level increased significantly with increasing IS independent of race. Given the simplicity of this measure relative to in vivo IS measurement, our findings suggest that adiponectin could potentially be added as a surrogate marker of insulin resistance in the aggregate criteria of MetS in the research setting. However, additional studies are needed to define the tracking of adiponectin levels from childhood to adulthood and its predictive value for future CVD.

In conclusion, although our study has some weaknesses such as 1) waist circumference was not obtained in all of our subjects, and 2) PCOS subjects were included in the analyses, we have demonstrated that insulin resistance is significantly associated with the individual components of MetS in Black and White youth. Although in Whites insulin resistance mediated in part via VAT is associated with the early manifestation of the atherosclerotic process through elevated biomarkers of endothelial dysfunction, this does not seem to be the case in Blacks. It remains to be determined what underlies the racial differences in risk translation. We propose that the inherently lower abdominal adiposity and triglyceride levels in Blacks may play a role. Longitudinal studies are needed to test these hypotheses.

ACKNOWLEDGEMENTS
The authors would like to express their gratitude to the study participants and their parents, and to the GCRC nurses and Resa Stauffer for technical assistance. This research was supported by RO1-HD-27503, K24-HD-01357, MO1-RR-00084 and Eli Lilly and Company.
REFERENCES


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Table 1. Subject characteristics

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<th>Characteristic</th>
<th>Blacks</th>
<th>Whites</th>
<th>P values</th>
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<tr>
<td>n = 99</td>
<td>n = 117</td>
<td></td>
<td></td>
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<tr>
<td><strong>Sex</strong></td>
<td>39M/60F</td>
<td>52M/65F</td>
<td></td>
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<tr>
<td>Prepubertal, Tanner I (n, %)</td>
<td>20 (20.2%)</td>
<td>21 (17.9%)</td>
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</tr>
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<td>Pubertal, Tanner II-V (n, %)</td>
<td>79 (79.8%)</td>
<td>96 (82.1%)</td>
<td>NS</td>
</tr>
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<td>Age (yr)</td>
<td>12.3 ± 0.2</td>
<td>12.9 ± 0.2</td>
<td>NS</td>
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<td>BMI (kg/m²)</td>
<td>26.9 ± 0.9</td>
<td>26.5 ± 0.9</td>
<td>NS</td>
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<td>BMI z-score</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>NS</td>
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<td>Waist circumference (cm) *</td>
<td>80.8 ± 2.6</td>
<td>82.5 ± 2.3</td>
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<tr>
<td>Visceral AT (cm²) †</td>
<td>39.5 ± 3.5</td>
<td>51.6 ± 4.1</td>
<td>0.029</td>
</tr>
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<td>Subcutaneous AT (cm²) †</td>
<td>281.5 ± 23.9</td>
<td>296.6 ± 22.8</td>
<td>NS</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>84.6 ± 4.6</td>
<td>120.0 ± 6.3</td>
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<td>HDL (mg/dl)</td>
<td>46.5 ± 1.2</td>
<td>44.1 ± 0.9</td>
<td>NS</td>
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<td>LDL (mg/dl)</td>
<td>94.1 ± 2.9</td>
<td>97.3 ± 2.6</td>
<td>NS</td>
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<td>VLDL (mg/dl)</td>
<td>16.9 ± 0.9</td>
<td>23.9 ± 1.3</td>
<td>&lt;0.001</td>
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<td>A.M. Systolic BP (mmHg)</td>
<td>108.8 ± 1.1</td>
<td>108.3 ± 1.1</td>
<td>NS</td>
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<td>A.M. Diastolic BP (mmHg)</td>
<td>63.4 ± 0.8</td>
<td>60.6 ± 0.7</td>
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<td>Fasting glucose (mg/dl)</td>
<td>95.9 ± 0.7</td>
<td>95.9 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>31.5 ± 2.3</td>
<td>30.1 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>6.5 ± 0.5</td>
<td>7.2 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Adiponectin (µg/ml) ‡</td>
<td>9.9 ± 0.6</td>
<td>10.8 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/ml) ‡</td>
<td>1.9 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>NS</td>
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<tr>
<td>VCAM-1 (ng/ml) ‡</td>
<td>422.1 ± 16.1</td>
<td>519.7 ± 14.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml) ‡</td>
<td>270.7 ± 10.0</td>
<td>309.8 ± 8.0</td>
<td>0.002</td>
</tr>
<tr>
<td>E-selectin (ng/ml) ‡</td>
<td>64.9 ± 2.6</td>
<td>62.2 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>PCOS (n, %)</td>
<td>12 (12.1%)</td>
<td>14 (12.0%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Prevalence (n, %) ††</strong></td>
<td>20 (33.9%)</td>
<td>35 (37.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Large waist circumference *</td>
<td>19 (19.2%)</td>
<td>49 (41.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High triglycerides</td>
<td>34 (34.3%)</td>
<td>41 (35.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Low HDL</td>
<td>17 (17.2%)</td>
<td>13 (11.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>IFG and IGT</td>
<td>27 (27.3%)</td>
<td>35 (29.9%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means ± SE. AT, adipose tissue; BP, blood pressure.

* N = 59 in Blacks and N = 94 in Whites. † N = 95 in Blacks and N = 112 in Whites. ‡ N = 97 in Blacks.
§ N = 90 in Blacks and N = 110 in Whites. †† N = 98 in Blacks. # N = 92 in Blacks.

Based on the modified NCEP/ATP III criteria; large circumference (≥90th percentile for age, sex and race); high triglycerides (≥110 mg/dl); low HDL (≤40 mg/dl); high BP (systolic or diastolic BP ≥90th percentile for age, sex and height) (27); impaired fasting glucose (IFG, ≥100 mg/dl) (28) or IGT (22).
FIGURE LEGENDS

**Figure 1.** The prevalence of the individual components of MetS by quartiles of insulin sensitivity (IS) in Black (A) and White (B) youth. IFG, impaired fasting glucose ($\geq 100$ mg/dl) (28); IGT, impaired glucose tolerance (22). Mean IS in the lowest quartile: Blacks, $1.2 \pm 0.1$ mg/kg/min per $\mu$U/ml; Whites, $1.3 \pm 0.1$ mg/kg/min per $\mu$U/ml. Mean IS in the highest quartile: Blacks, $13.5 \pm 0.5$ mg/kg/min per $\mu$U/ml; Whites, $15.8 \pm 0.8$ mg/kg/min per $\mu$U/ml.

**Figure 2.** Individual components of MetS and abdominal adiposity in Black (left panel) and White (right panel) youth according to quartiles of IS. *Significantly different ($P < 0.05$) between Blacks versus Whites in the lowest quartile of IS group. Data are shown as estimated marginal means after adjusting for age, gender and Tanner stage. Standard errors (±) shown in parentheses in the bars.

**Figure 3.** Circulating biomarkers of endothelial dysfunction, adiponectin and IL-6 in Black (left panel) and White (right panel) youth according to quartiles of IS. *Significantly different ($P < 0.05$) between Blacks versus Whites in the lowest quartile of IS group. Data are shown estimated marginal means after adjusting for age, gender and Tanner stage. Standard errors (±) shown in parentheses in the bars.

**Figure 4.** ROC curves of individual components of MetS as markers of insulin resistance. Insulin resistance was defined based on cutoffs of IS (lower 10$^{th}$ percentile) derived from the non-overweight group (cutoffs, Black: $4.6$ mg/kg/min per $\mu$U/ml, Whites: $4.45$ mg/kg/min per $\mu$U/ml). AUC, area under the curve.
Figure 1

A. Blacks

B. Whites
Figure 2
Figure 3

Quartiles of Insulin Sensitivity

A. ICAM-1 (ng/ml)

B. VCAM-1 (ng/ml)

C. E-selectin (ng/ml)

D. IL-6 (pg/ml)

E. Adiponectin (µg/ml)

Blacks

Whites

P <0.01

NS
Figure 4

A. Blacks

B. Whites