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## **Insulin Resistance, Lipids, and the Metabolic Syndrome Trait Cluster: Impact of Race and Very Low Calorie Diet**

Veeradej Pisprasert  
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INSULIN RESISTANCE, LIPIDS, AND THE METABOLIC SYNDROME TRAIT  
CLUSTER: IMPACT OF RACE AND VERY LOW CALORIE DIET

by

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A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,  
in partial fulfillment of the requirements for the degree of  
Doctor of Philosophy

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2013

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2013

# INSULIN RESISTANCE, LIPIDS, AND THE METABOLIC SYNDROME TRAIT CLUSTER: IMPACT OF RACE AND VERY LOW CALORIE DIET

VEERADEJ PISPRASERT

NUTRITION SCIENCES

## ABSTRACT

Insulin resistance (IR) is central to the pathogenesis of cardiometabolic disorders. Early detection, along with appropriate interventions, is paramount to reduce the morbidity and mortality of IR-related diseases. The gold standard measurement for IR is the hyperinsulinemic-euglycemic clamp, which is markedly sophisticated for clinical evaluation but costly and invasive for clinical practice. Several alternative surrogate indicators of IR have been identified and used by clinicians, including insulin sensitivity indices derived from plasma glucose and insulin values, as well as other indicators that are independent of direct insulin measurements. In addition, lipid panel and lipoprotein subclass profile have emerged as markers of IR, and the cluster of abnormalities that define Metabolic Syndrome also have been used to predict IR and related outcomes in clinical settings.

Race can confound the utility of IR indicators; however, there is no data to demonstrate which indicators may more accurately represent insulin dynamics and homeostasis in different populations. Although it is known that intervention strategies can improve IR, it is not clear whether the effect of interventions in insulin-related outcomes can be accurately captured by traditionally used methods. Thus, three specific aims were investigated in this project. First, we determined the predictability of commonly used insulin sensitivity indices compared to the hyperinsulinemic-euglycemic clamp in European Americans (EA) and African Americans (AA), demonstrating differences between groups and that the most reliable indices were the

Matsuda index and the simple index assessing insulin sensitivity using oral glucose tolerance test. Second, we evaluated the relationship between IR and lipoprotein subclass profile between EAs and AAs, showing an association between IR and lipid subclass profile beyond the conventional lipid panel. Third, we determined changes in lipoprotein subclass profile and insulin sensitivity after a short-term very low calorie diet, verifying concomitant favorable changes in the Matsuda index and lipoprotein subclass profile after the intervention. Taken together, our findings demonstrated that the Matsuda index and lipoprotein subclass profile are appropriate markers for identifying and monitoring IR in a mixed-race population during regular screening and during diet-related interventions. Our work supports the need for race-specific tools in the evaluation of insulin-related outcomes.

Key words: insulin resistance, insulin sensitivity index, lipoprotein subclass, metabolic syndrome, racial difference, very low calorie diet

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## **INTRODUCTION**

### **Overview of Insulin Resistance**

Insulin resistance is central to the pathogenesis of metabolic syndrome, which is the cluster of metabolic abnormalities that include abdominal obesity, dyslipidemia, hypertension, and systemic insulin resistance. These metabolic disturbances confer an increased risk of type 2 diabetes and cardiovascular disease [1,2]. The prevalence of insulin resistance is increasing worldwide, further burdening healthcare systems. Earlier disease onset, longer disease duration, and accumulation of multiple adverse metabolic disturbances (i.e., components of the metabolic syndrome) have a profound influence on prognosis.

By definition, insulin resistance is characterized by the body's inability to mediate glucose disposal, mainly in skeletal muscle, resulting in high glucose and compensated higher insulin levels. Normally, insulin action is initiated when hormone binds to  $\alpha$ -subunits of the insulin receptor, leading to activation of tyrosine kinase domain of the  $\beta$ -subunit, followed by a series of transphosphorylation reactions, which causes tyrosine phosphorylation of several substrates, including insulin receptor substrate-1 (IRS-1). Phosphorylated tyrosine residues provide activation of phosphatidylinositol 3-kinase (PI 3-kinase), resulting in GLUT4 translocation and increased glucose transport [3]. In an insulin-resistant state, the insulin-stimulated muscle glucose disposal is decreased [4-6], which is primarily caused by impaired glucose transport [7]. Although GLUT4 protein content in muscle is not changed with

insulin resistance, defects in insulin signal transduction and GLUT4 translocation were reported [8].

Insulin signaling pathway abnormality is not only confined in skeletal muscle, but also in liver and adipose tissue. Therefore, beyond abnormal glucose metabolism, insulin resistance also is associated with a large number of metabolic disturbances, including atherogenic dyslipidemia [9,10], chronic low-grade inflammation [11], and obesity [12]. In the obese, insulin resistance often worsens, as do other metabolic derangements, due to dysregulated secretion of various adipose tissue factors. These factors are believed to involve pathogenesis of cardiovascular complications.

### **Identifying Insulin Resistance**

As the metabolic sequelae of perturbations in insulin dynamics expand across all age groups, early detection and appropriate interventions are paramount to reduce diabetes-related morbidity and mortality. Identifying insulin resistance usually is done by assessing complications, e.g., criteria of metabolic syndrome, but not by direct insulin resistance assessment. The euglycemic-hyperinsulinemic clamp is the direct, gold standard measurement of insulin dynamics [13], but it is infrequently used in clinical practice due to its costly, laborious, and invasive nature. This procedure requires at least 40 blood samples collected from each subject. Additionally, the process requires intravenous infusion of multiple substrates, including insulin, glucose, and electrolytes. Accordingly, numerous simple, inexpensive, and noninvasive surrogate measures of insulin dynamics have been devised.

### *Insulin Sensitivity Indices Based on Fasting Condition*

Several insulin sensitivity indices employing glucose and insulin levels at a fasting steady state have been developed, including fasting insulin level (FIL), homeostasis model assessment of insulin resistance (HOMA-IR), and quantitative insulin sensitivity check index (QUICKI). These indices primarily reflect hepatic insulin homeostasis. FIL is a simple and convenient marker for insulin resistance [14] when elevated in the presence of normo- or hyper-glycemia; however, an insulin assay has not been standardized for more universal applications. HOMA-IR is an interaction between fasting insulin and glucose levels [15]. It is demonstrated to have a linear correlation with the hyperinsulinemic-euglycemic clamp [16,17]. On the other hand, the coefficient of variation of HOMA-IR differs based on the type of insulin assay performed [17,18]. QUICKI also is calculated from fasting insulin and glucose levels with additional log transformation that is reported to provide a stronger linear correlation with the clamp than HOMA-IR [19]. A meta-analysis showed that QUICKI provides a high predictive power for predicting type 2 diabetes [20]. Interpretation of these three indices based on fasting insulin levels may be limited in type 2 diabetes, because insulin level becomes influenced by the  $\beta$ -cell defect and hyperglycemia [14].

### *Insulin Sensitivity Indices Based on Dynamic Tests*

Additionally, insensitivity indices derived from dynamic tests have been proposed. The Matsuda index, which is determined from oral glucose tolerance test (OGTT) results, indicates both hepatic and peripheral insulin sensitivity [21]. The simple index assessing insulin sensitivity using the oral glucose tolerance test ( $SI_{is}$ OGTT) is another index derived from OGTT with same principle as the Matsuda

index but a different equation [22]. Moreover, the Stumvoll index includes a demographic parameter, i.e., body mass index (BMI), with OGTT results [23]. The Avignon index also incorporates glucose distribution volume along with OGTT data in the equation [24]. Since the OGTT closely mimics the glucose and insulin dynamics of physiological conditions, these insulin sensitivity indices could potentially identify impaired insulin dynamics in individuals. However, glucose tolerance outcome is affected by various factors other than the metabolic actions of insulin, such as insulin secretion and incretin effects. Therefore, OGTT provides valuable information regarding glucose tolerance, but not insulin resistance. The frequently sampled intravenous glucose tolerance test (FSIVGTT) is another dynamic test that offers an indirect measurement of insulin dynamics [25]. This method provides the coefficient of variation for insulin resistance equivalent to the hyperinsulinemic-euglycemic clamp [26,27]. Although FSIVGTT is simple and more convenient for large-scale population studies than the hyperinsulinemic-euglycemic clamp, it still requires intravenous infusions and several blood samplings. Moreover, this approach may produce nonsystematic errors due to oversimplification of glucose homeostasis physiology [28]. Although these insulin sensitivity indices are widely used, there is no consensus as to which one provides convenience and sufficient information comparable to the hyperinsulinemic-euglycemic clamp.

#### *Other Approaches to Identify Insulin Resistance*

In addition to direct assessment of insulin dynamics, other metabolic parameters also may serve as surrogates of insulin resistance. A cluster of interrelated metabolic abnormalities may be gathered as a clinical construct to predict the development of type 2 diabetes and cardiovascular outcomes, i.e., insulin resistance



syndrome [29], metabolic syndrome [30-32], and diabetic risk score (DRS) [33].

Insulin also affects lipid metabolism. Dyslipidemia, i.e., high level of triglyceride and low level of high-density lipoprotein (HDL), contributes to two of five criteria of metabolic syndrome [31]. Both lipid abnormalities are derived from a conventional lipid panel, which evaluates only the total amount of cholesterol in each circulating lipid fraction; however, data regarding individual particles remain lacking.

Subfractions of lipoprotein, which are classified based on differences in particle size and density, may provide further information concerning insulin resistance and its related conditions. Currently, alterations in lipoprotein subclasses measured by nuclear magnetic resonance (NMR) spectroscopy also are considered as additional potential cardiometabolic risks [34,35]. Hence, applying lipoprotein subclass profile as a marker for insulin resistance is proposed. Some institutes also provide commercial products as an indicator of insulin resistance, e.g., the Lipoprotein Insulin Resistance (LP-IR) Score, which is calculated from lipoprotein subclass markers [36]. Furthermore, some adipose tissue factors, particularly inflammatory markers, may be used as alternative indicators of insulin resistance, e.g., interleukin 6 (IL-6), C-reactive protein (CRP) [37]. On the other hand, adiponectin level may indicate decreased risk of insulin resistance [38]. However, data-verified usefulness of these markers is still limited.

### **Gaps in the Literature**

Numerous studies have assessed the accuracy and precision of various indicators; however, these studies are often lacking in several respects. Some indicators, such as insulin level, lack standardization, while others are not completely validated against the hyperinsulinemic-euglycemic clamp as the gold standard of

measurement. In addition, data showing concomitant changes in insulin sensitivity and surrogate indicators after appropriate interventions are limited. Furthermore, several previous studies included both diabetic and non-diabetic participants, which might compromise interpretation of the results due to differential applications between the groups. Type 2 diabetes alters the correlation between glucose and insulin levels; consequently, surrogate markers may incorrectly indicate systemic insulin sensitivity.

Moreover, the impact of racial/ethnic differences is not well studied in terms of predictability of insulin resistance. The risk for insulin resistance as well as glucose and lipid perturbations differs among diverse racial/ethnic populations [39]. African Americans are known to hypersecrete insulin independent of systemic insulin sensitivity [40-46], and hepatic insulin clearance has been different between African Americans and European Americans [46-49]. However, African Americans exhibited lower triglyceride levels and higher HDL cholesterol than European Americans [50]. These discrepancies may influence the predictability of insulin sensitivity indicators in mixed racial/ethnic populations.

### **Experimental Aims**

In light of these gaps in the research literature, the following experimental aims were proposed to investigate 1) the predictability of insulin sensitivity indicators, both surrogates from glucose and insulin levels as well as lipoprotein subclasses, against the gold standard of measurement in European Americans and African Americans; and 2) the effect of weight loss on concomitant changes of these indicators after weight loss intervention.

### *Experimental Aim 1*

To determine the accuracy of the commonly used insulin sensitivity indices relative to the hyperinsulinemic-euglycemic clamp and evaluate whether there is a differential impact of race.

#### Hypothesis:

- 1) Commonly used insulin sensitivity indices are comparable with the hyperinsulinemic-euglycemic clamp in European Americans.
- 2) Predictability of these indices is different between European Americans and African Americans.

To investigate this aim, the hyperinsulinemic-euglycemic clamp and commonly used insulin sensitivity indices were cross-sectionally evaluated in non-diabetic European Americans and African Americans. Commonly used insulin sensitivity indices in our study include fasting insulin level, HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index. BMI-adjusted correlations between glucose disposal rate (GDR) obtained from the hyperinsulinemic-euglycemic clamp and insulin sensitivity indices were calculated for all patients and for groups stratified by race. Coefficient of determination to indicate predictability of each insulin sensitivity index was determined by multiple regression analysis, for which the model included surrogate indices of insulin sensitivity, BMI, race, gender, and interaction between insulin sensitivity indices and race as independent variables and the hyperglycemic-euglycemic clamp measure as the dependent variable.

### *Experimental Aim 2*

To investigate the relationship between insulin resistance and lipoprotein subclass profile and evaluate a differential impact of race.

Hypothesis:

- 1) Insulin resistance is associated with increased small LDL and large VLDL particles.
- 2) Relationship between small LDL and large VLDL particles with insulin resistance will differ according to race, such that African Americans will have fewer small LDL and VLDL particles than European Americans for any degree of insulin resistance.

For this aim, the hyperinsulinemic-euglycemic clamp and fasting lipoprotein subclass profile measured by NMR spectroscopy were cross-sectionally assessed in European Americans and African Americans. BMI-adjusted correlations between GDR and insulin lipoprotein subclass profile were analyzed for all patients and for groups stratified by race. Multiple regression analysis also was performed to determine the predictability of each lipoprotein subclass for insulin sensitivity according to models that included lipoprotein subclasses, race, and BMI as the independent variables and GDR as the dependent variable.

### *Experimental Aim 3*

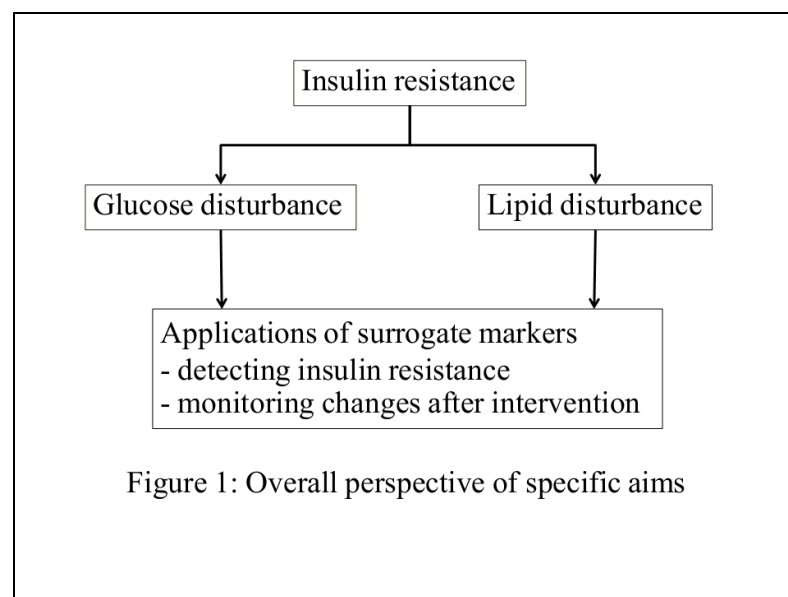
To determine changes of lipoprotein subclass profile and insulin sensitivity index after short-term very low calorie diet.

Hypothesis: Following a short-term very low calorie diet, the lipoprotein subclass profile will be beneficially altered, which is reflected by decreased large VLDL and small LDL particles. This improvement will be related to improved insulin sensitivity.

By study design, 26 obese participants were assigned to a 1-week isocaloric diet (containing 50% carbohydrate, 30% fat, and 20% protein) for weight maintenance, followed by 1 week of a very low calorie diet with the same macronutrient distribution. Fasting plasma samples for lipoprotein subclass profile, OGTT for insulin sensitivity assessment, and anthropometric measurement were compared between before and after very low calorie diet intervention.

### Overall Perspective

Figure 1 is a representation of the integration of the specific aims. Each aim is originated from the metabolic disturbances involved with insulin resistance and is investigated for further applications.



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GLUCOSE TOLERANCE TEST IN AFRICAN AMERICANS

by

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## ABSTRACT

**Objective:** To examine the utility of commonly used insulin sensitivity indices in non-diabetic European Americans (EAs) and African Americans (AAs).

**Methods:** 240 non-diabetic participants were studied. Euglycemic-hyperinsulinemic clamp was the gold-standard approach to assess glucose disposal rates normalized by lean body mass (GDR). The homeostatic model assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were calculated from fasting plasma glucose and insulin (FIL). Oral glucose tolerance test (OGTT) was performed to determine Matsuda index, the simple index assessing insulin sensitivity ( $SI_{isOGTT}$ ), Avignon index, and Stomvoll index. Relationships among these indices with GDR were analyzed by multiple regression.

**Results:** GDR values were similar in EA and AA subgroups; even so, AA exhibited higher FIL and were insulin resistant compared with EA as assessed by HOMA-IR, QUICKI, Matsuda index,  $SI_{isOGTT}$ , Avignon index, and Stumvoll index. In overall study population, GDR was significantly correlated with all studied insulin sensitivity indices ( $r = 0.381 - 0.513$ ); however, these indices were not superior to FIL in predicting GDR. Race and gender affected the strength of this relationship. In AA males, FIL and HOMA-IR were not correlated with GDR. In contrast, Matsuda index and  $SI_{isOGTT}$  were significantly correlated with GDR in AA males, and Matsuda index was superior to HOMA-IR and QUICKI in AAs overall.

**Conclusion:** Insulin sensitivity indices based on glucose and insulin levels should be used cautiously as measures of peripheral insulin sensitivity when comparing mixed-gender and mixed-race populations. Matsuda index and  $SI_{isOGTT}$  are reliable in studies that include AA males.

## INTRODUCTION

Insulin resistance is central to pathogenesis of cardiometabolic disease, and confers increased risk of type 2 diabetes and cardiovascular disease [1]. The gold-standard approach for measuring insulin resistance is euglycemic-hyperinsulinemic clamp [2]; however, it is rarely used in clinical practice and in epidemiological studies since it is laborious and requires intravenous infusions. Several surrogate indices employing glucose and insulin levels have been devised as alternative measures of insulin sensitivity, and are commonly used in cohort studies, including fasting insulin level (FIL), homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), Matsuda index, Avignon index, Stumvoll index, and the new simple index assessing insulin sensitivity using oral glucose tolerance test ( $SI_{is}OGTT$ ). FIL is a simple and practical surrogate marker for insulin resistance [3] when elevated in the presence of normo- or hyper-glycemia; however, insulin assay has not been standardized for more universal applications. HOMA-IR [4] and QUICKI [5] are models that incorporate both fasting insulin and glucose levels, although QUICKI employs a log-transformation that is reported to provide a stronger linear correlation with the clamp [5]. Matsuda index [6] and  $SI_{is}OGTT$  [7] are models that utilizes dynamic glucose and insulin values obtained during oral glucose tolerance tests (OGTT). Avignon index [8] and Stumvoll index [9] are also derived from OGTT with incorporation of glucose's volume of distribution or body mass index (BMI) in their equations. These indices are potentially of high value since they are facile and inexpensive in comparison with euglycemic-hyperinsulinemic clamp. Furthermore, it is difficult to clinically identify insulin resistance since individual variability in insulin sensitivity exists largely



independent of obesity in populations [10]. Clinical constructs such as Metabolic Syndrome and Prediabetes are used to assess risk for future diabetes and cardiometabolic disease; however, insulin sensitivity indices could potentially be used to more optimally identify insulin resistance in individuals as a central pathophysiological process responsible for cardiometabolic disease.

Given the widespread use of insulin sensitivity indices in epidemiology and clinical trials, it is important to assess their predictive value for insulin resistance. Several studies have assessed correlations between various indices and clamp measures of insulin resistance [11-17]; however, these studies are often lacking in three aspects. First, the correlations often include non-diabetic subjects together with type 2 diabetic patients. Type 2 diabetes is a disease state with distortions in the relationship between circulating glucose and insulin values in a manner that does not reflect systemic insulin sensitivity. Hyperglycemia is the hallmark of type 2 diabetes and is accompanied by ‘glucose toxicity’ with respect to insulin secretion. Consequently, studies assessing the relationship between indices based on fasting glucose and insulin levels and clamp measures could reflect falsely inflated slopes and correlation coefficients in regression equations, when data from non-diabetic and diabetic subjects are included in the same regression analyses. Hence, rigorous analyses confined to non-diabetic subjects are needed to evaluate true value of insulin sensitivity indices. Secondly, even among non-diabetic subjects, there are factors influencing insulin secretion and circulating insulin concentrations independent of insulin sensitivity. Studies have shown that insulin secretory responses can primarily be impaired, independent of insulin resistance, and this trait is an independent risk factor for future diabetes [18]. Along these same lines, African-Americans are known

to hypersecrete insulin independent of systemic insulin sensitivity [19-25], and this could alter glucose-to-insulin ratios in a manner that distorts ability to use insulin sensitivity indices in studies involving multiple racial groups. Thus, careful analyses across racial and ethnic groups are warranted. Finally, few studies have addressed the relative values of multiple insulin sensitivity indices in the same population, with attention to the potential impact of race.

Our study attempted to address these short-comings in the literature. We performed euglycemic-hyperinsulinemic glucose clamp in a substantial number of non-diabetic European American (EA) and African American (AA) subjects, and compared the predictive value of FIL, HOMA-IR, log HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index as indices of peripheral insulin resistance.

## METHODS

### *Study Subjects*

Study subjects included 240 non-diabetic participants; 141 EA and 99 AA. Baseline characteristics are shown in Table 1. None of volunteers had cardiovascular, renal, or hepatic disease, and all were chemically euthyroid. Pregnant women were excluded and pre-menopausal females were studied between days 4-11 of the menstrual cycle by history. Race was determined by self-report. Informed consent was obtained from every participant, and the protocol was approved by University of Alabama at Birmingham Institutional Review Board.

## *Protocol*

Subjects were admitted to the Clinical Research Unit at the University of Alabama at Birmingham, where they received eucaloric diet consisting of 20% protein, 30% fat, and 50% carbohydrate of total calories during three-day stay. All procedures were conducted in the morning after 10-hour fast. Participants received standard 75-gram OGTT. Plasma glucose and insulin levels were obtained at 0, 30, 60, 90, 120, and 180 minutes. Insulin sensitivity indices were calculated by the following formulas [4-9]:

$$\text{HOMA-IR} = [\text{fasting plasma glucose level (FPG; mg/dl)} \times \text{fasting insulin level (FIL; } \mu\text{unit/ml)}] / 405$$

$$\text{QUICKI} = 1 / [\log \text{ FIL (} \mu\text{unit/ml)} + \log \text{ FPG (mg/dl)}]$$

$$\text{Matsuda index} = 10,000 / \text{square root of } [( \text{FPG} \times \text{FIL} ) \times ( \text{mean glucose} \times \text{mean insulin during OGTT} )]$$

$$\text{SI}_{\text{isOGTT}} = 1 / [\log (\text{sum glucose}_{t0+30+90+120} \text{ (mmol/l)}) + \log (\text{sum insulin}_{t0+30+90+120} \text{ (} \mu\text{unit/ml)})]$$

$$\text{Avignon index} = [(0.137 \times \text{Sib}) + \text{Si2h}] / 2$$

$$\text{Sib} = 10^8 / (\text{FIL (} \mu\text{unit/ml)} \times \text{FPG (mg/dl)} \times \text{volume distribution})$$

$$\text{Si2h} = 10^8 / (\text{plasma insulin at 2hr-OGTT (} \mu\text{unit/ml)} \times \text{plasma glucose at 2hr-OGTT (mg/dl)} \times \text{volume distribution})$$

$$\text{volume distribution} = 150 \text{ ml/kg of body weight.}$$

$$\text{Stumvoll index} = 0.226 - (0.0032 \times \text{BMI}) - (0.0000645 \times \text{insulin at 2hr-OGTT (pmol/l)}) - (0.0037 \times \text{plasma glucose at 1.5hr-OGTT (mmol/l)})$$

Lean body mass was determined by dual energy X-ray absorptiometry (DXA) with DPX-L, Version 1.33 (Lunar Radiation Corp., Madison, WI).

### *Assays*

Plasma glucose was measured by glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

### *Clamps*

Hyperinsulinemic-euglycemic clamps were performed at a maximally-effective insulin concentration as described [26]. In brief, a catheter was inserted into brachial vein to infuse insulin, glucose, and potassium phosphate. Insulin was administered at a rate of 200 mU/m<sup>2</sup>/minute for 4 hours, and this provided steady-state serum insulin levels that were maximally effective for promoting glucose uptake largely into skeletal muscle and which achieved full suppression of hepatic glucose output [27]. The mean of clamp-induced steady insulin level in EAs and AAs was 579 (SD 183) and 645 (SD 178) μU/ml (*P*=NS), respectively. A potassium phosphate solution was simultaneously infused to prevent hypokalemia. A variable-rate infusion of a 20% dextrose solution was used to maintain plasma glucose level. Plasma glucose was clamped at 90 mg/dl for at least 3 hours. Plasma glucose levels were evaluated every 5 minutes and plasma insulin every 30 minutes throughout the clamp.

Maximal glucose uptake for each individual was calculated from mean glucose infusion rate over the final three 20-minute intervals. Whole-body glucose uptake was calculated based on glucose infusion rate corrected for changes in the glucose pool size, assuming a distribution volume of 19% body weight and a pool fraction of 0.65. GDR was normalized per kilogram lean body mass, excluding bone mass determined by DXA.

### *Statistical Analysis*

Mean differences in patient characteristics were assessed by ANOVA. ANCOVA was used to detect mean differences in GDR and insulin sensitivity indices (FIL, HOMA-IR, log HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index), independent of BMI. BMI was rather higher in AA than in EA, therefore, BMI-adjusted correlations between GDR and insulin sensitivity indices were calculated for all patients and for groups stratified by race and gender. A Steiger's t-test was used to compare correlation coefficients among surrogate indices. Best fit analyses of the data correlating all indices with clamp measures of insulin sensitivity across gender and racial groups were performed. Coefficient of determination to indicate predictability of each insulin sensitivity index was determined by multiple regression analysis which the model included surrogate indices of insulin sensitivity, BMI, race, gender, and interaction between insulin sensitivity indices and race as independent variables and hyperglycemic-euglycemic clamp measure as dependent variable. P-values less than 0.05 were considered significant. Statistical analyses were performed using the SAS program version 9.2 (SAS Institute, Cary, NC).

## RESULTS

The analyses included 141 EAs (68 males and 73 females) and 99 AAs (43 males and 56 females). Hyperinsulinemic clamp measurements as well as HOMA-IR and QUICKI results were available in all 240 subjects, although Matsuda index,  $SI_{isOGTT}$ , Avignon index, and Stumvoll index were assessed only in 198 participants (119 EAs and 79 AAs) who were administered OGTTs. Descriptive characteristics of study participants stratified by race and gender are shown in Table 1. Mean age was similar in EAs and AAs, although BMI and waist circumference were somewhat higher in AAs than in EAs. Fasting glucose values were similar in all subgroups; however, FIL tended to be higher in females than in males, and were elevated in AA in comparison with their EA counterparts. Importantly, EAs and AAs were equally insulin sensitive with similar mean GDR values ( $P=NS$ ).

As shown in Figure 1, while both GDR and FPG were similar in EAs and AAs (panels A and B), FIL was higher in AAs (panel C), and AAs had lower QUICKI values (panel E), lower Matsuda index (panel F), lower  $SI_{isOGTT}$  (panel G), lower Avignon index (panel H), lower Stumvoll index (panel I), and higher HOMA-IR values (panel D) than EAs.

As delineated in Table 2, GDR was significantly negatively correlated with FIL, HOMA-IR, and log-transformed HOMA-IR, and positively correlated with QUICKI, Matsuda,  $SI_{isOGTT}$ , Avignon, and Stumvoll indices, with absolute  $r$  values ranging between 0.381 and 0.513 in overall cohort controlling for BMI. When stratified by race and gender, significant correlations persisted except that in AA males FIL and HOMA-IR failed to achieve a significant relationship with GDR. Log-transformation of HOMA-IR produced a significant correlation with GDR in AA

males but did not significantly strengthen this relationship in other groups stratified by race and gender. All relationships in Table 2 were also analyzed without adjustment for BMI which did not affect the results and conclusions (supplementary table A). Steiger's t-tests were performed to compare correlation coefficients among these insulin sensitivity indices with the GDR measure of insulin sensitivity. In entire cohort, neither HOMA-IR, log HOMA-IR, QUICKI, Matsuda index,  $SI_{is}OGTT$ , Avignon index, nor Stumvoll index were superior to FIL in predicting insulin sensitivity (i.e.,  $r$  values were similar;  $P=NS$ ). In EA subgroup, the strengths of the correlations with GDR were comparable, although HOMA-IR was marginally superior to FIL ( $P=0.04$ ) but similar to QUICKI and Matsuda index (supplementary table B); and Matsuda index was superior in a head-to-head comparison with QUICKI, but not with HOMA-IR. In AA females, all indices were similarly correlated with GDR, but it was in AA men that Matsuda index and  $SI_{is}OGTT$  emerged as stronger predictors of insulin sensitivity. The correlation between GDR and Matsuda in AA men was significantly stronger than with FIL, HOMA-IR, log HOMA-IR, and QUICKI. The correlation between GDR and  $SI_{is}OGTT$  in AA males was also significantly higher than FIL and HOMA-IR. When AA females and AA males were considered together, Matsuda was more strongly correlated with GDR than HOMA-IR or QUICKI but not different from FIL; while  $SI_{is}OGTT$  was only more strongly correlated with GDR than HOMA-IR. Further details are provided in supplementary tables.

Multiple linear, quadratic, and exponential models of fit were analyzed. Both linear and curvilinear models fit the data although differences in fit were not statistically significant. Scatter plots between GDR and insulin sensitivity indices are

shown in Figure 2 stratified by race and gender. These figures illustrate impact of race and gender on these relationships. For HOMA-IR (panel A), regression curves essentially overlapped in EA males and EA females; however, the slope was reduced in AA females and was completely flat in AA males. For QUICKI (panel B), regression lines overlapped for male subgroups (i.e., both EA and AA males), and for both female subgroups (EA and AA females), with the female regression lines having sharper slopes than that observed in males. For Matsuda index (panel C) and  $SI_{isOGTT}$  (panel D), all regression lines for EA and AA males and females exhibited a relatively greater degree of overlap and similarity of slope.

Table 3 shows results of multiple regression analyses assessing independent contributions of each index, race, gender, and BMI as determinants of GDR. FIL and HOMA-IR were similar in that the index, gender, BMI, and the interaction between index and race proved to be significant factors in the multiple regression equation, and together these factors explained 28-29% of variability in GDR. Log-transformation of HOMA-IR did not improve the  $R^2$  value in the multiple regression equation but eliminated the interaction between index and race. The interaction between QUICKI and gender also had a significant effect on predictability of GDR ( $\beta=-0.188$ ,  $P=0.03$ ), but this interaction was not operative in the models for other insulin sensitivity indices (data not shown). For Matsuda index,  $SI_{isOGTT}$ , and Strumvoll indices, the independent effects of the index, gender, and BMI could explain a greater degree of variability in GDR ranging between 33.3% and 36.5%.



## DISCUSSION

The purpose of this study was to examine the relative ability of key indices based on ambient glucose and insulin concentrations to predict insulin sensitivity, and to study the impact of gender and race on these relationships. We assessed insulin sensitivity via the gold-standard hyperinsulinemic-euglycemic clamp, which directly measures the ability of insulin to promote glucose uptake in peripheral tissues. Under the conditions of clamp studies, the degree of steady-state hyperinsulinemia is sufficient to completely suppress hepatic glucose output, and the observed GDRs reflect maximally stimulated glucose transport rates predominantly into skeletal muscle [2,27,28]. An important consideration is that EA and AA subgroups, and males and females within each racial group, are characterized by the same degree of insulin sensitivity measured by hyperinsulinemic clamp. Despite similarities in insulin sensitivity, AA display higher FIL, HOMA-IR and log HOMA-IR, and lower QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index values. Thus, the indices are indicative of greater insulin resistance in AA despite the subgroups are well matched to have similar mean clamp GDR measurements.

We [29] and others [30] have shown that sample populations of EAs and AAs have similar degrees of insulin sensitivity using hyperinsulinemic-euglycemic clamp. Furthermore, investigators using FIL, HOMA-IR, or frequently sampled IVGTTs that rely on interactions between ambient glucose and insulin values are likely to conclude that AA are more insulin resistant than EAs [19,21-25,31-39]. The current study provides a direct demonstration in the same subjects groups of the discrepancies between surrogate indices and clamp measures of insulin sensitivity in comparing EA and AA subgroups. There are several potential explanations. First, AAs have been

reported to hypersecrete insulin at any given level of insulin sensitivity [21-23]. Additionally, we have analyzed C-peptide to insulin molar ratios as an indicator of insulin clearance, and observed that AAs had a lower C-peptide to insulin ratio than EAs, as other authors have also reported [24,31,38,39]. Thus, both insulin hypersecretion and reduced clearance in AAs have the potential to impact relationships involving circulating insulin, glucose, and insulin sensitivity, and could confound the application of these indices to study racial differences in insulin sensitivity. Even so, the indices employing fasting levels of insulin and glucose assess systemic concentrations regardless of the impact of insulin secretion or clearance on fasting levels.

A more feasible explanation for these discrepancies relates to potential differences in relative insulin sensitivity affecting different organs, such as liver versus skeletal muscle. HOMA-IR and QUICKI are derived from fasting glucose and insulin levels [4,5] and are believed to primarily reflect hepatic insulin sensitivity [40]. Matsuda index,  $SI_{isOGTT}$ , Avignon index, and Stumvoll index are surrogate markers calculated from OGTT glucose and insulin values and are used as combined indicators of both hepatic and peripheral insulin sensitivity [6-9]. This is contrary to hyperinsulinemic clamps performed at maximally effective steady-state serum insulin levels that fully suppress hepatic glucose production and directly reflect glucose disposal predominantly into skeletal muscle. To explain why AAs were more insulin resistant than EAs when assessed by surrogate indices, while no difference in insulin sensitivity was observed by clamp, one could hypothesize that AAs are characterized by greater hepatic insulin resistance relative to insulin sensitivity in skeletal muscle and relative to hepatic insulin sensitivity in EAs. While relative hepatic insulin

resistance in AAs is an attractive hypothesis to explain the data, there has been little data published to directly support this idea, and this area is deserving of greater study.

Current data are consistent with previous literature in several aspects. Other authors have reported higher fasting insulin levels in lean AA adults [19] and in AA adolescents [31] when compared with their EA counterparts. Furthermore, the insulin area under the curve in response to OGTT was reported to be higher in both AA children and adults than in EAs [32,33]. Studies using hyperglycemic clamp to assess  $\beta$ -cell function have also found a higher insulin response and lower insulin sensitivity in AA adolescents and adults when compared to EAs [21,22,34,35]. Moreover, studies using the minimal model analysis of frequently sampled intravenous glucose tolerance test (FSIVGTT) have revealed lower insulin sensitivity, reduced hepatic insulin extraction and clearance, and increased acute insulin response in AA children and adults compared to EAs [23,24,36-39]. However, based on the current results, these previous reports using glucose and insulin levels to estimate insulin sensitivity, whether obtained under fasting conditions or after oral or IV glucose challenge, should not be interpreted to mean that AAs display greater peripheral insulin resistance. Race appears to alter insulin and glucose values in a manner that diminishes the ability of these indices to predict systemic or peripheral insulin resistance.

While several reports have found correlations between surrogate indices and insulin sensitivity [11-17], there has been no definite conclusion regarding which surrogate marker is the most predictive of insulin sensitivity. Our study examined differences in the relationships among GDR and surrogate markers of insulin sensitivity in non-diabetic population. These results revealed that HOMA-IR, log

HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index are not superior to FIL in predicting GDR in overall non-diabetic population. Furthermore, while all indices were significantly correlated with GDR, the correlation coefficients were rather modest ranging from 0.381 to 0.513. This finding is consistent with previous study which demonstrated equivalent usefulness of FIL, HOMA-IR, and QUICKI in non-diabetic subjects [14]. Conversely, another report including both nondiabetic and diabetic subjects found the superiority of QUICKI and log-transformation of HOMA-IR to FIL [15]. In general, the correlations between measures of insulin sensitivity and various indices are stronger when patients with type 2 diabetes are included in the analyses, with representative values ranging from 0.51 to 0.88 [4-6,11-13,15-17], when compared with the current data in non-diabetic subjects. Factors other than insulin resistance contribute to the rise in glucose in type 2 diabetic patients, and glucose toxicity affects circulating insulin levels independently from insulin resistance. Therefore, indices based on fasting glucose and insulin levels may not accurately track with changes in insulin sensitivity. The higher correlation coefficients when diabetic patients are included in these regressions may partially represent an artifact created by the extremely high glucose values in patients who are predictably insulin resistant by virtue of having diabetes. Consequently, indices based on glucose and insulin values exaggerate the relative degree of insulin resistance, resulting in stronger correlation coefficients in studies including both type 2 diabetic and non-diabetic participants than is evident in studies restricted to non-diabetic subjects. Since insulin resistance is an integral feature of type 2 diabetes, these indices are most valuable to the extent they can identify insulin resistance in non-diabetic individuals. Based on our current data, results using these indices should be interpreted cautiously when used as an estimate of peripheral insulin sensitivity.

Our correlation analyses also found that race and gender significantly impacted the relationship between each index and GDR, and affected the utility of the different indices in specific ethnic and gender subgroups. When stratified by race and gender, significant correlations persisted between GDR and all studied indices with the notable exception that, in AA males, FIL and HOMA-IR were no longer related to GDR and the strength of the relationship between QUICKI and GDR was weakened. Log-transformation of HOMA-IR was required to achieve a statistically significant relationship with GDR in AA males. Importantly, however, Matsuda index and  $SI_{isOGTT}$  emerged as better indices of insulin sensitivity in AA males without diminution in the strength of the correlation with GDR. These findings may or may not relate to differences in hepatic insulin sensitivity as discussed above; however, it appears that indices which include both fasting and post-challenge glucose and insulin concentrations, i.e., Matsuda index and  $SI_{isOGTT}$ , are better predictors of peripheral insulin sensitivity in AA men than indices that rely only on fasting glucose and insulin levels. In contrast, indices derived from OGTT which incorporate measures of glucose's volume of distribution (Avignon index) and BMI (Stumvoll index) provide no additional predictive value. Moreover, in males-only analyses consisting of both EAs and AAs, Matsuda index and  $SI_{isOGTT}$  proved to be the best predictors of GDR. The data indicate that indices derived from OGTT, i.e. Matsuda index and  $SI_{isOGTT}$ , are preferred surrogate indices of insulin sensitivity in any study of AAs, and in mixed-race studies that include AAs, particularly AA males.

The multiple regression models for prediction of GDR included as independent variables each surrogate index of insulin sensitivity, BMI, race, gender, and interaction between index and race. These analyses highlighted the differential

impact of race and gender. Models for FIL, HOMA-IR, and Matsuda index demonstrated independent effects of the index, gender, BMI, and the interaction between index and race in predicting GDR. The model for QUICKI, SI<sub>is</sub>OGTT, and Avignon index established the independent significance of the index, BMI, and gender, and with QUICKI there was an interaction between QUICKI and gender (data not shown). The model for Stumvoll index indicated independent effects of only the index and gender. The overall predictive value ( $R^2$ ) in these models was generally higher for indices derived from OGTT, i.e. SI<sub>is</sub>OGTT (0.365), Matsuda index (0.333), and Stumvoll index (0.336), than that observed for FIL (0.280), HOMA-IR (0.290), log HOMA-IR (0.280), or QUICKI (0.265).

One strength of our study is that we enrolled a relatively large non-diabetic cohort with a significant population of both AAs and EAs, providing adequate power to analyze ethnic and gender differences. In comparing our results with other publications, it is important to consider that our study enrolled mixed racial groups, including EAs and AAs of both genders, while other publications involved more homogenous ethnic populations predominated by Caucasians [4-6,11-13]. Furthermore, our study included both obese and nonobese participants; some studies found that predictability of insulin sensitivity indices is lower in lean individuals than in obese counterparts [16,17]. One weakness of our study is that race was determined by-self report which may not accurately reflect ancestral genetic admixture.

## CONCLUSIONS

1) In AAs and EAs with similar peripheral insulin sensitivity measured by hyperinsulinemic clamp, AAs exhibited higher FIL and exhibited more insulin resistance than EAs, as assessed by HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index. 2) In a mixed-race and gender sample population, HOMA-IR, QUICKI, Matsuda index,  $SI_{is}$ OGTT, Avignon index, and Stumvoll index were not superior to FIL alone in predicting GDR. 3) Racial and gender differences were detected in the ability of the indices to predict insulin sensitivity. Most remarkably, FIL and HOMA-IR were not correlated with GDR in AA males. In contrast, Matsuda index and  $SI_{is}$ OGTT were significantly correlated with GDR in AA males, and Matsuda index was superior to HOMA-IR and QUICKI in overall AA subgroup consisting of males and females. These data indicate that commonly used indices based on glucose and insulin levels should be used cautiously as measures of peripheral insulin sensitivity when comparing mixed-gender and mixed-race populations. Matsuda index and  $SI_{is}$ OGTT appear to be most reliable in studies of AAs.

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V.P. performed statistical analyses and wrote the manuscript. K.H.I., and M.F.L. performed statistical analyses, critically reviewed the manuscript, and provided editorial recommendations. A.J.M. critically reviewed the manuscript, and provided editorial recommendations. W.T.G. conceived and designed the study, critically reviewed the manuscript, and provided editorial recommendations. W.T.G. is also the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Table 1:** Descriptive characteristics of study subjects.

	European Americans (EAs)			African Americans (AAs)			<i>P</i> -value		
	total	male	female	total	male	female	EA VS AA total	EA VS AA male	EA VS AA female
Number	141	68	73	99	43	56			
Prediabetes subjects* (%)	23	23	23	35	33	38			
Age (years)	37 ± 11	34 ± 9	40 ± 11	37 ± 9	38 ± 10	37 ± 9	0.76	0.03	0.09
Waist circumference (cm)	90 ± 13	91 ± 11	90 ± 14	96 ± 13	97 ± 13	96 ± 13	<0.001	0.01	<0.001
Body mass index (kg/m <sup>2</sup> )	28 ± 5	26 ± 4	29 ± 6	31 ± 5	29 ± 4	33 ± 5	<0.001	0.001	<0.001
Fasting plasma glucose (mg/dl)	90 ± 8	90 ± 8	90 ± 9	91 ± 10	91 ± 10	90 ± 9	0.59	0.52	0.86
Fasting serum insulin (μU/ml)	9 ± 7	8 ± 6	11 ± 9	15 ± 11	13 ± 11	17 ± 11	<0.001	0.005	0.001
Fasting C-peptide (ng/ml)	2.2 ± 1.0	2.3 ± 1.1	2.2 ± 1.0	2.3 ± 1.4	2.4 ± 1.6	2.2 ± 1.1	0.67	0.64	0.96
Fasting C-peptide to insulin molar ratio	17 ± 11	20 ± 14	15 ± 8	10 ± 9	13 ± 12	8 ± 5	<0.001	0.02	<0.001
2 hr OGTT plasma glucose(mg/dl)	117 ± 26	109 ± 25	124 ± 25	119 ± 26	116 ± 26	121 ± 27	0.52	0.18	0.57
2 hr OGTT serum insulin (μU/ml)	53 ± 43	41 ± 26	61 ± 50	72 ± 57	62 ± 68	80 ± 47	0.004	0.07	0.03
Glucose Disposal Rate (mg/min/kg lean body mass)	14 ± 4	14 ± 3	15 ± 4	14 ± 4	13 ± 3	15 ± 4	0.58	0.66	0.59

\* includes subjects with impaired fasting plasma glucose and/or impaired glucose tolerance test

**Table 2:** Correlation coefficients between common indices of insulin sensitivity and glucose disposal rate (GDR) per lean body mass with BMI adjustment; and comparison of correlation coefficients between common indices of insulin sensitivity and GDR.

	Correlation coefficients between common indices of insulin sensitivity and GDR										<i>P</i> -value compare correlation coefficients between insulin sensitivity indices and GDR†										
	Indices employed fasting condition					Indices derived from oral glucose tolerance test															
	N	FIL	HOMA	log HOMA	QUICKI	N	Matsuda index	Simple index	Avignon index	Stumvoll index	HOMA VS FIL	log HOMA VS FIL	QUICKI VS FIL	Matsuda VS FIL	Avignon VS FIL	Stumvoll VS FIL	Simple index VS FIL	Matsuda VS HOMA	Matsuda VS log HOMA	Simple index VS HOMA	Simple index VS log HOMA
		<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>		<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>										
total subjects	240	-0.423*	-0.430*	-0.444*	0.421*	198	0.486*	0.513*	0.381*	0.464*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
all EA	141	-0.492*	-0.525*	-0.465*	0.424*	119	0.470*	0.500*	0.432*	0.484*	0.04	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
EA male	68	-0.540*	-0.564*	-0.557*	0.504*	51	0.599*	0.620*	0.507*	0.609*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
EA female	73	-0.511*	-0.544*	-0.471*	0.461*	68	0.459*	0.533*	0.497*	0.564*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
all AA	99	-0.389*	-0.373*	-0.418*	0.419*	79	0.540*	0.539*	0.304*	0.451*	NS	NS	NS	NS	NS	NS	NS	0.04	NS	0.04	NS
AA male	43	-0.128	-0.085	-0.335#	0.328#	34	0.512§	0.523*	0.357#	0.474§	NS	0.008	0.006	0.002	NS	0.02	0.005	0.002	0.02	0.004	NS
AA female	56	-0.558*	-0.553*	-0.538*	0.547*	45	0.600*	0.607*	0.457*	0.546*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
all male	111	-0.340*	-0.330*	-0.472*	0.442*	85	0.542*	0.567*	0.444*	0.520*	NS	0.003	0.05	0.007	NS	0.03	0.005	0.009	NS	0.004	NS
all female	129	-0.522*	-0.534*	-0.506*	0.503*	113	0.518*	0.570*	0.471*	0.556*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

N is the number in each group, R is the correlation coefficient. NS = not significant *p*-value. All bold values are statistically significant. # *P* < 0.05, \$ *P* < 0.01, \* *P* < 0.001; † Significance indicates superiority of the top index VS the bottom index.

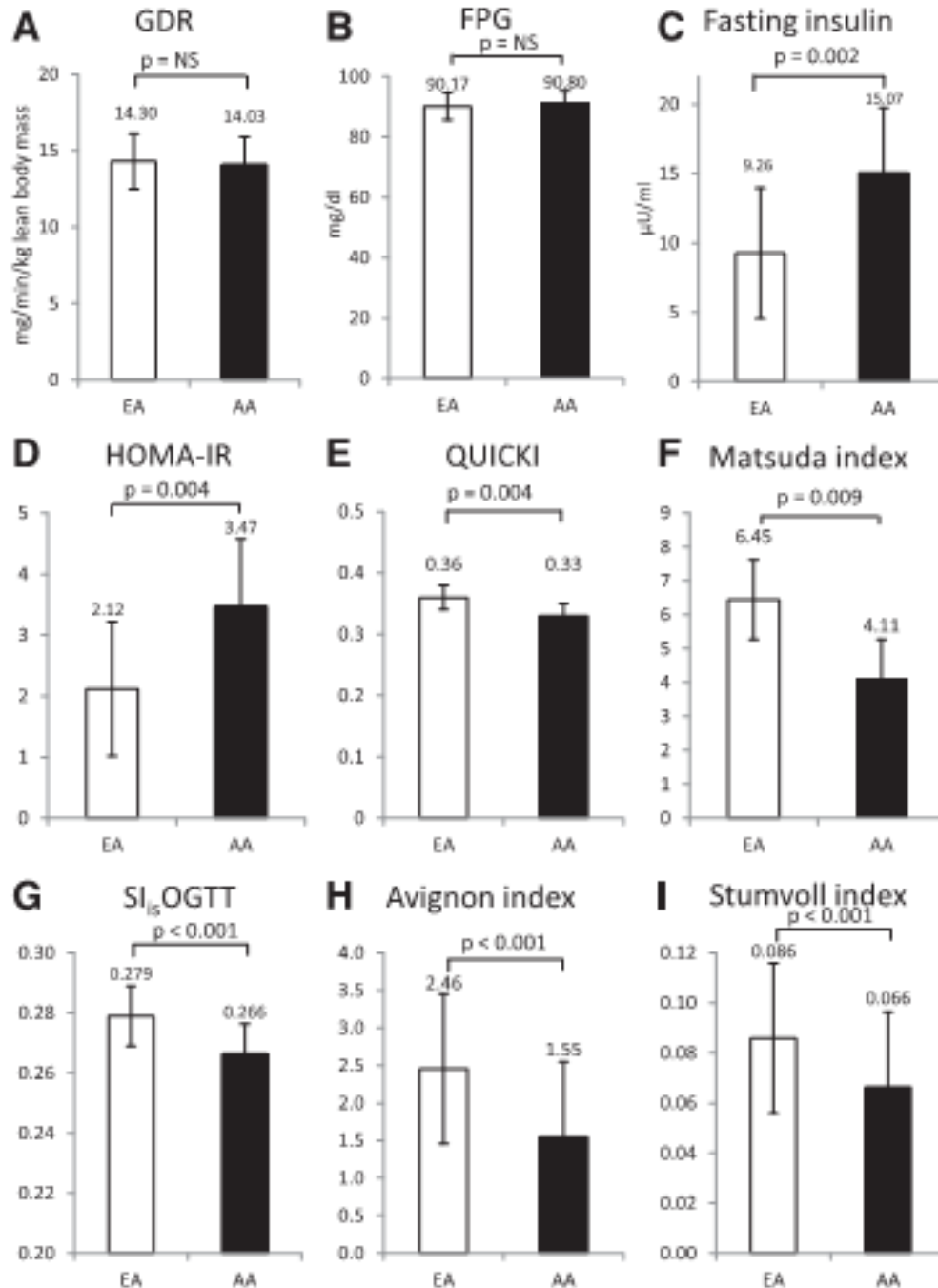
**Table 3:** Coefficient of determinations using a model which includes surrogate indices of insulin sensitivity as independent variables and the hyperglycemic-euglycemic clamp measure as the dependent variable in multiple regression analyses.

	FIL		HOMA		Log HOMA		QUICKI		Matsuda		SI <sub>is</sub> OGTT		Avignon		Stumvoll	
	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value	$\beta$	<i>P</i> -value
insulin sensitivity index	<b>-0.567</b>	<0.001	<b>-0.599</b>	<0.001	<b>-0.430</b>	<0.001	<b>0.369</b>	<0.001	<b>0.407</b>	<0.001	<b>0.471</b>	<0.001	<b>0.367</b>	<0.001	<b>0.681</b>	<0.001
race	0.086	0.14	0.083	0.158	0.056	0.47	0.074	0.21	0.106	0.09	-0.75	0.28	0.048	0.608	0.164	0.23
Gender	<b>-0.287</b>	<0.001	<b>-0.281</b>	<0.001	<b>-0.295</b>	<0.001	<b>-0.301</b>	<0.001	<b>-0.292</b>	<0.001	<b>-0.317</b>	<0.001	<b>-0.312</b>	<0.001	<b>-0.332</b>	<0.001
BMI	<b>-0.178</b>	0.009	<b>-0.184</b>	0.006	<b>-0.168</b>	0.01	<b>-0.18</b>	0.009	-0.128	0.07	<b>-0.143</b>	0.03	<b>-0.164</b>	0.03	0.104	0.21
interaction between index and race	<b>0.195</b>	0.05	<b>-0.233</b>	0.017	0.013	0.89	0.05	0.689	<b>0.151</b>	0.03	0.839	0.23	0.135	0.89	-0.123	0.38
$R^2$	<b>0.280*</b>		<b>0.290*</b>		<b>0.280*</b>		<b>0.265*</b>		<b>0.333*</b>		<b>0.365*</b>		<b>0.239*</b>		<b>0.336*</b>	

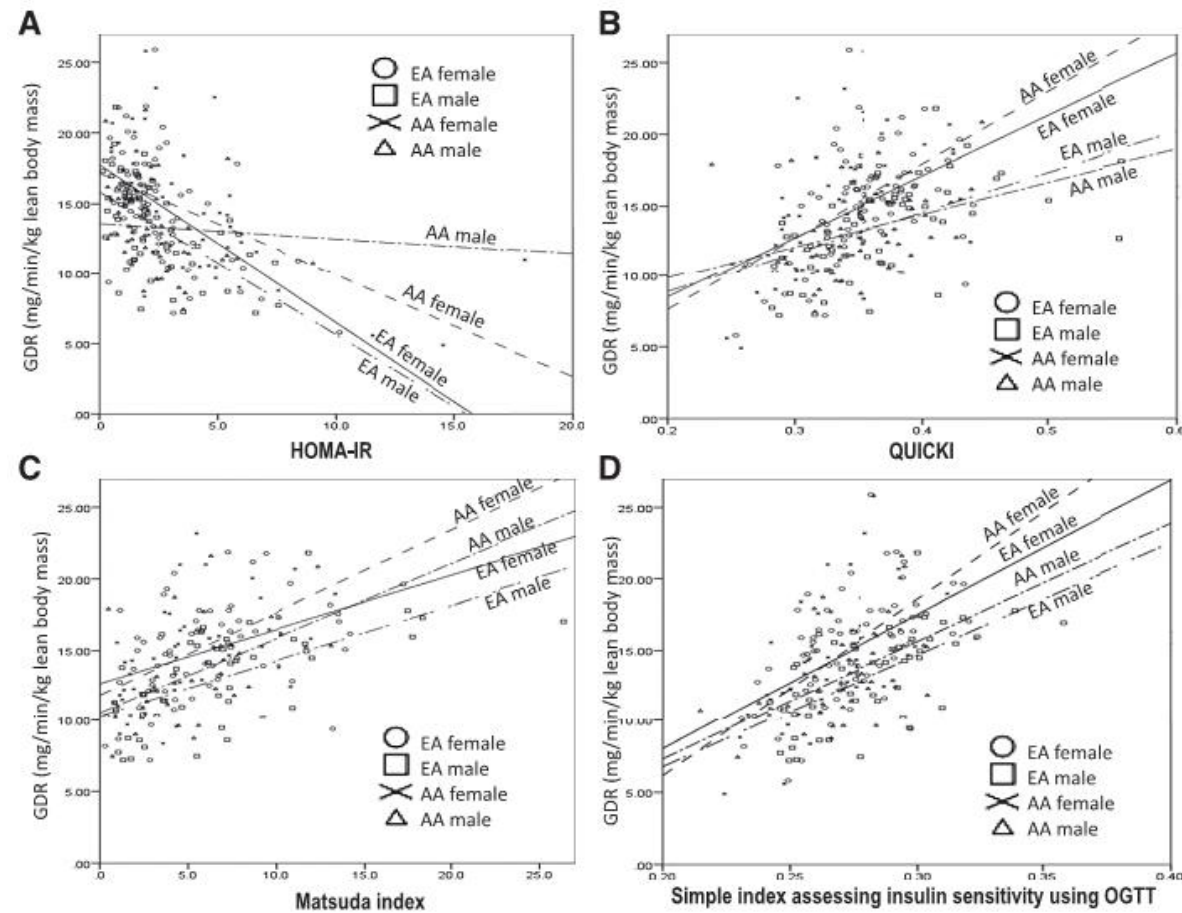
$\beta$  is the standard coefficient of each independent variable and  $R^2$  the coefficient of determination of each model. Race (African American as 1, European American as 0), gender (male as 1, female as 0) and BMI are entered as common independent variables in all models and FIL, or HOMA, or log HOMA, or QUICKI, or Matsuda index as an additional independent variable in individual models. All bold values are statistically significant. \* $P < 0.00$



**Figure 1.** Mean differences in insulin sensitivity indices between European Americans (EAs) and African Americans (AAs) as assessed by ANCOVA adjusted for BMI. GDR, glucose disposal rate measured by hyperinsulinemic clamp; FPG, fasting plasma glucose; FIL, fasting insulin level; HOMA-IR, the homostatic model assessment for insulin resistance; QUICKI, the quantitative insulin sensitivity check index;  $SI_{isOGTT}$ , the simple index assessing insulin sensitivity using oral glucose tolerance test.



**Figure 2.** Correlation between the glucose disposal rate measured by hyperinsulinemic clamp (GDR) and HOMA-IR, QUICKI, Matsuda index, Simple index assessing insulin sensitivity using oral glucose tolerance test in nondiabetic subjects comparing race and gender. AA indicates African Americans and EA European Americans. Circle = EA female; square = EA male; cross = AA female; triangle = AA male.



RACIAL DIFFERENCES IN LIPOPROTEIN SUBCLASSES AND RELATIONSHIP  
TO INSULIN RESISTANCE

by

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## ABSTRACT

**Objective:** To evaluate impact of race on lipoprotein subclasses determined by nuclear magnetic resonance (NMR), and differential relationships with progressive insulin resistance.

**Methods:** 273 participants, 161 European Americans (EAs) and 112 African Americans (AAs), were studied using euglycemic-hyperinsulinemic clamp to assess glucose disposal rate (GDR), and fasting lipoproteins were assessed by conventional lipid panel and NMR lipoprotein subclass profile.

**Results:** In subgroups of AAs and EAs matched for insulin sensitivity, AAs had higher triglycerides and lower HDL; lower VLDL particle concentrations and mean VLDL size; lower IDL levels; lower LDL particle concentrations due to decreased small LDL particles accompanied by increased LDL size; and higher levels of large and small HDL and lower medium HDL. In both AAs and EAs, progressive insulin resistance was associated with larger VLDL due to increased large VLDL particles, and decreased LDL size due to increased small LDL particles. IDL was negatively correlated with GDR only in AAs and emerged as the strongest independent predictor of insulin sensitivity.

**Conclusions:** These findings demonstrate differences in lipoprotein subclasses in AAs and EAs, and in dyslipidemia associated with insulin resistance. The data support alternative approaches in evaluation of lipids/lipoproteins as an indicator of cardiometabolic disease risk in AAs.

## INTRODUCTION

Insulin resistance is characterized by an atherogenic dyslipidemia that features a decrease in HDL-c and an increase in triglyceride levels [1]. Accordingly, the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP ATP III) include these two abnormalities in the criteria for metabolic syndrome [2]. In addition to these traditional atherosclerosis risk factors, alterations in lipoprotein subclasses may also confer additional risk [3-12]. The conventional clinical lipid panel assessing total cholesterol, HDL-c, triglycerides, and calculated LDL-c does not provide information regarding subclasses of particles with variable size and density within the major lipoprotein classes of VLDL, LDL, and HDL. However, nuclear magnetic resonance (NMR) spectroscopy is being increasingly used to measure the size and concentration of lipoprotein subclass particles [13]. Unlike conventional methodology that measure lipoproteins based on their lipid or apolipoprotein content, NMR spectroscopy determines spectral signals emitted from lipoprotein particles which vary on the basis of particle diameter [14].

Prior studies in this field indicated that increments in small LDL and large VLDL particles, and possibly decrease in large HDL particles, were associated with insulin resistance [3,15-18]. While these studies have better defined the dyslipidemia associated with insulin resistance, the generalized application of this knowledge is still limited because the majority of participants in these studies were Caucasian. There is no systematic research determine whether the relationship between lipoprotein subclass and

insulin resistance differs among racial groups, particularly in African Americans (AAs) who tend to exhibit higher HDL-c and lower triglyceride levels with higher degree of insulin resistance when compared with Caucasians [19]. The current study evaluated racial differences in lipoprotein subclasses determined by NMR spectroscopy and differences in the relationship between alterations in lipoprotein subclasses and insulin sensitivity assessed by the hyperinsulinemic euglycemic clamp technique.

## METHODS

### *Study Subjects*

Study subjects included 273 participants with and without Type 2 Diabetes Mellitus (T2DM); 161 European Americans (EAs) and 112 AAs. All participants were equilibrated on a weight-maintenance diet (28-32 kcal/kg/day) consisting of 50% carbohydrate, 30% fat, and 20% protein for 3 days prior to study. None of participants were actively engaged in regular exercise, and weight was stable ( $\pm 3\%$ ) for at least 3 months before study. None of participants had cardiovascular, renal, or hepatic disease, and all were clinically and chemically euthyroid. No subjects were taking any medications known to affect carbohydrate and lipoprotein metabolism. Pregnant women were excluded and premenopausal females were studied between days 4 and 11 of the menstrual cycle by history. Race was determined by self-report. 17% of subjects had T2DM treated with diet or sulfonylurea and/or metformin oral hypoglycemic agents; however, the medications were withdrawn for at least 3 weeks prior to study as the patients were monitored on an outpatient basis. Baseline characteristics are shown in

Table 1. Informed consent was obtained from every participant, and the protocol was approved by University of Alabama at Birmingham Institutional Review Board.

### *Insulin Sensitivity Measurement*

Hyperinsulinemic-euglycemic clamps were employed as the gold standard measure of insulin sensitivity, as we have previously described [20]. In brief, a catheter was inserted into brachial vein to infuse, glucose, and potassium phosphate. Insulin was administered at a rate of 200 mU/m<sup>2</sup>/min for 4 h, and this provided steady-state serum insulin levels that were maximally effective for promoting glucose uptake largely into skeletal muscle and that achieved full suppression of hepatic glucose output [21]. A variable-rate infusion of a 20% dextrose solution was used to maintain plasma glucose level. Plasma glucose was clamped at 90 mg/dl for at least 3 h. Plasma glucose levels were evaluated every 5 min and plasma insulin was measured every 30 min throughout the clamp. Maximal glucose uptake for each participant was calculated from mean glucose infusion rate over the final three 20-min intervals. Whole-body glucose uptake was calculated based on glucose infusion rate corrected for changes in the glucose pool size, assuming a distribution volume of 19% body weight and a pool fraction of 0.65. Glucose disposal rate (GDR) was normalized per kilogram of lean body mass, excluding bone mass determined by dual-energy X-ray absorptiometry.

We also assessed commonly used insulin sensitivity indices that derive from mathematical manipulations of ambient glucose and insulin concentrations, including the homeostatic model assessment for insulin resistance (HOMA-IR) based on measurements in fasting blood samples and the Matsuda index based on oral glucose tolerance tests

(OGTT). A standard 75-g OGTT was performed in 204 participants (120 EAs and 84 AAs) in the morning after a 10-h fast. Plasma glucose and insulin levels were obtained at 0, 30, 60, 90, 120, and 180 min. Insulin sensitivity indices were calculated by the following equations [22,23].

$$\text{HOMA-IR} = [\text{fasting plasma glucose level (FPG; mg/dl)} \times \text{fasting insulin level (FIL; } \mu\text{unit/ml)}] / 405$$

$$\text{Matsuda index} = 10,000 / \text{square root of } [(FPG \times FIL) \times (\text{mean glucose} \times \text{mean insulin during OGTT})]$$

Lean body mass was determined by dual-energy X-ray absorptiometry with DPX-L version 1.33 (Lunar Radiation, Madison, WI).

#### *Lipoprotein Subclass Profile*

Blood for the lipoprotein subclass profile was obtained at 8 AM after an overnight fast at the same time as the sample for the conventional lipid panel. The NMR lipoprotein subclass profile was measured using a 400-mHz proton NMR analyzer at LipoScience (Raleigh, NC). The two principles of this method are: 1) each lipoprotein subclass based on size simultaneously produces a unique NMR signal; and 2) the measured subclass signal amplitudes are directly relative to the numbers of subclass particles producing the signal, irrespective of difference in particle lipid component [24]. The data identify 9 lipoprotein subclasses: large VLDL (60-200nm), medium VLDL (35-60 nm), small VLDL (27-35 nm), IDL (23-27 nm), large LDL (21.2-23nm), small LDL (18-21.2 nm), large HDL (8.8-13 nm), medium HDL (8.2-8.8 nm), and small HDL (7.3-8.2 nm). Weighted average size of VLDL, LDL, and HDL particles was analyzed from the



difference subclass concentrations by summation of the diameter of each lipoprotein subclass multiplied by mass percentage as estimated from the intensity of NMR signal [24].

### *Assays*

Plasma glucose was measured by glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using a Millipore Specific Insulin RIA kit (Millipore Corp., Billerica, MA). Conventional lipid panel, including total cholesterol, HDL cholesterol, and triglycerides was determined by colorimetric method on Sirrus analyzer (Stanbio Laboratory, Boerne, TX). LDL cholesterol was calculated using the Freidewald equation.

### *Statistical Analysis*

Mean differences in patient characteristics were assessed by ANOVA. Body mass index (BMI) was somewhat higher in AAs than in EAs; thus, BMI-adjusted partial correlations between GDR and lipoprotein subclasses were analyzed for all patients and for groups stratified by race and gender. Best-fit analyses of the data were performed correlating all lipoprotein subclasses with clamp measures of insulin sensitivity across racial and gender groups. Multiple regression analysis was performed to determine the predictability of each lipoprotein subclass for insulin sensitivity according to models that included lipoprotein subclasses, BMI, race, and gender as the independent variables, and GDR from hyperglycemic-euglycemic clamp measurement as the dependent variable. *P*

< 0.05 was considered significant. Statistical analyses were analyzed using the SPSS program version 20.0 (IBM, Armonk, NY)

## RESULTS

The analyses included 161 EAs (67 males and 94 females) and 112 AAs (44 males and 68 females). Descriptive characteristics of study participants stratified by race are shown in Table 1. Mean age was similar in EAs and AAs, although BMI and waist circumference were modestly higher in AAs than EAs. Importantly, both fasting plasma glucose and peripheral insulin sensitivity indicated by GDR were similar in EAs and AAs; even so, fasting insulin level was increased and HOMA-IR and Matsuda index reflected more insulin resistance in AAs than in EAs, as we have previously reported [25]. Regarding the conventional lipid panel, in EAs and AAs matched for the degree of insulin sensitivity, mean total cholesterol and LDL-c were similar, while AAs had significantly higher HDL-c and lower triglyceride than EAs. Table 2 demonstrates racial differences in prevalence rates of metabolic syndrome risk factors with higher rates of elevated blood pressure and reduced rates of dyslipidemia (i.e., abnormal HDL-c and triglycerides) in AAs compared with EAs.

Figure 1 shows lipoprotein subclass profile parameters stratified by race after controlling for gender, age, and BMI. AAs had lower total VLDL particles compared with EAs, and this was explained by reduced concentrations of large and medium sized VLDL particles without significant effects on small VLDL particles (Fig 1, panel A). While LDL-c levels were similar in AAs and EAs, AAs exhibited lower concentrations of LDL particles due entirely to diminished levels of small LDL without any significant

change in large LDL particles (Fig 1, panel B). Finally, while HDL-c was increased in AAs, HDL particle concentration was similar in AAs and EAs; however, AAs had higher concentrations of small and large HDL particles and decreased levels of medium HDL particles when compared with EAs (Fig 1, panel C). Regarding overall mean size of lipoproteins, AAs were observed to have smaller VLDL, larger LDL, and similar sized HDL compared with EAs (Fig 1, panel D).

We then examined the quantitative relationship between lipoprotein subclasses and insulin sensitivity assessed by clamp measures of GDR in AAs and EAs. These data, controlled for age and BMI, are delineated in Table 3. In both AAs and EAs, GDR was negatively correlated with triglyceride levels, and this was entirely due to a progressive rise in large VLDL particles with worsening insulin resistance accompanied by an increase in mean VLDL particle size. Other than a negative correlation between medium VLDL and GDR in AA males, there were no significant relationships between GDR and medium VLDL or small VLDL particles. GDR was negatively correlated with LDL-c in AA males but not in AA females or in EAs. However, there was a strong negative correlation between GDR and small LDL particle concentrations, and a positive correlation with LDL particle size, across all gender and racial subgroups. Insulin resistance was not associated with any changes in large LDL concentrations. In both genders and races, GDR was positively correlated with HDL-c, and this was primarily due to an association with large HDL particle concentrations. A final interesting observation was the significant correlation between IDL particle concentration and GDR only in AA males and females but not in EAs. All relationships in Table 3 also were analyzed by multiple linear, logarithmic, quadratic, and exponential models of fit. Both

linear and curvilinear models fit the data, although differences in fit were not statistically significant.

To more rigorously examine these relationships, scatter plots correlating GDR and key lipoprotein subclasses in both AAs and EAs are shown in Figure 2. For small LDL particles (Fig. 2A) and large VLDL particles (Fig. 2B), regression curves essentially overlap in both racial groups. In contrast, regression lines for IDL particles (Fig. 2C) demonstrate the significant relationship with GDR in AAs whereas IDL and GDR are clearly not related in EAs.

Table 4 shows the results of multiple regression analyses assessing independent contributions of each lipoprotein subclass, race, gender, and BMI as determinants of insulin sensitivity assessed by GDR. Small LDL particles, large VLDL particles, gender, and BMI proved to be significant factors in the multiple regression equation, and together these factors explained 40.5% of the variability in GDR in EAs and a higher proportion of 48.2% in AAs. Furthermore, coefficients of determination pertaining to the individual independent factors varied as a function of race. Both large VLDL and small LDL were important determinants of GDR in EA and AA; however, only in AA did IDL emerge as a strong independent predictor of insulin resistance.

## DISCUSSION

We have studied subgroups of AAs and EAs matched for the degree of insulin sensitivity as assessed by hyperinsulinemic-euglycemic clamp, and illustrated racial differences in the conventional lipid panel, the NMR lipoprotein subclass profile, and, for

the first time, racial differences in the impact of insulin resistance on lipoprotein subclasses. As previously reported [19,26-28], AAs had significant higher HDL-c and lower triglycerides with the same level of total cholesterol and LDL-c when compared with EAs. The current study, however, compares AAs and EAs with similar degree of insulin sensitivity, thus controlling for any effects of any racial differences in insulin sensitivity. Since triglycerides and HDL-c constitute 2 out of the 5 risk factor categories involved in the diagnosis of metabolic syndrome (i.e., according to NCEP ATP III), clearly the application of the same criteria across racial groups has the potential to underestimate cardiometabolic disease risk in AAs. While EAs and AAs in our cohort had a comparable prevalence of metabolic syndrome, there were racial differences in the frequency of risk factors qualifying individuals for the diagnosis. Compared with EAs, AAs had significantly more abdominal obesity and high blood pressure, but more favorable lipid levels with a fewer percentage of individuals exhibiting high triglycerides and low HDL-c. This finding is in agreement with observations reported in the Insulin Resistance Atherosclerosis Study [19], and is consistent with our previous demonstration that the ATPIII criteria for the metabolic syndrome are characterized by high specificity and low sensitivity in identifying insulin resistant individuals with dyslipidemia [29].

We have further delineated racial differences in lipoprotein subclasses. Again, at the same level of peripheral insulin resistance and after adjustment for age, gender, and BMI, AAs were observed to have a decrease in the mean concentration of VLDL particles as a result of reductions in large and medium sized VLDL, and a decrease in mean VLDL particle size, compared with EAs. The higher levels of HDL-c in AAs were due to more large and small HDL particles, but fewer medium HDL particles, resulting in

similar overall HDL size compared with EAs. While LDL-c levels were similar, the AAs had fewer total LDL particles and significantly reduced small LDL particles, resulting in larger mean LDL size than in EAs. Since VLDL, HDL, and LDL subclasses can be variably associated with cardiovascular disease risk [13,14] and insulin resistance, these findings underscore the ability of lipoprotein subclass evaluation to provide information relevant to cardiometabolic disease risk, over and above the conventional lipid panel, in different racial groups.

Our final objective was to assess relationships between insulin sensitivity and lipoprotein subclasses, and determine whether these relationships were different in comparing AAs and EAs. Our findings showed that insulin sensitivity influenced subclasses within all three lipoprotein classes. In the combined cohort, we confirmed our previous study in EAs [3] showing that progressive insulin resistance (i.e., decreasing GDR) was associated with: i) an increase in VLDL size and an increase in large VLDL particle concentrations; ii) a decrease in LDL size as a result of a marked increase in small LDL particles without any change in large LDL together with an overall increase in the number of LDL particles; and iii) a decrease in HDL size as a result of depletion of large HDL particles. These data are also consistent with a study using the frequently sampled intravenous glucose tolerance test (IVGTT) to measure insulin sensitivity [15] and recapitulates the lipoprotein subclass profile observed in patients with Metabolic Syndrome, Prediabetes, and T2DM [16-18,29,30].

While these correlations between GDR and lipoprotein subclasses were generally observed in both AAs and EAs, there were key racial differences in 3 aspects. First,

insulin resistance in AA males was associated with more marked alterations in LDL. GDR was inversely correlated with total cholesterol and LDL-c only in AA males, and the relationship with total LDL and small LDL particle concentrations were stronger than in EA males. Secondly, GDR was more highly correlated with HDL-c, mean HDL particle size, and total HDL and large HDL particle concentrations in AAs. Finally, an unanticipated distinguishing feature was the observation that insulin resistance was singularly associated with an increase in IDL only in AAs. This latter observation was reinforced in multiple regression models for prediction of GDR. In both AAs and EAs, large VLDL and small LDL particle concentrations were independent determinants of GDR; however, in AAs, IDL also entered the equations as the factor with the highest coefficient of determination. These models were able to predict 40.5% of the variability of insulin sensitivity in EAs and 48.2% of variation in AAs. Interestingly, the ability of lipoprotein subclasses to predict insulin sensitivity was generally stronger than widely-used indices of glucose homeostasis that employ mathematical manipulations of circulating glucose and insulin concentrations [25].

The racial differences in lipoprotein subclasses point to variations in enzyme activities responsible for hydrolyzing lipoprotein lipid components. Lipoprotein lipase removes triglycerides from VLDL, resulting in IDL, which is further converted to LDL by both lipoprotein lipase and hepatic lipase [31]. Hepatic lipase hydrolyzes phospholipids and triglycerides; and promotes uptake of lipoproteins into liver cells [32,33]. Hepatic lipase also plays a role in HDL remodeling via hydrolysis of triglycerides and phospholipids to yield smaller HDL particles. In insulin resistance and T2DM, hepatic lipase activity is increased [34,35] and lipoprotein lipase activity is

diminished [36-38]. Furthermore, AAs have been observed to have lower serum hepatic lipase activity [39,40] and higher lipoprotein lipase activity [41,42] than in other ethnic groups. These racial differences in hepatic lipase and lipoprotein lipase activities could feasibly explain differential relationships between GDR and lipoprotein subclasses with worsening insulin resistance.

## CONCLUSIONS

In subgroups of AAs and EAs matched for insulin sensitivity, 1) AAs exhibited higher triglycerides and lower HDL without a difference in LDL-c. 2) In the NMR lipoprotein subclass profile, AAs were found to have lower VLDL particle concentrations and mean VLDL size due to reductions in large and medium VLDL particles; lower IDL levels; lower LDL particle concentrations due to marked reductions in small LDL particles accompanied by an increase in LDL size; and higher levels of large and small HDL and lower medium HDL resulting in no change in HDL particle concentration or mean HDL size. 3) In both AAs and EAs, progressive insulin resistance (i.e., decreasing GDR) was associated with larger VLDL due to an increase in large VLDL and a decrease in LDL size due to an increase in small LDL. 4) In AA males, insulin resistance was associated with increments in total and LDL cholesterol and a stronger correlation with increments in total and small LDL particle concentrations. 5) IDL was inversely correlated with GDR only in AA and emerged as the strongest independent predictor of insulin sensitivity

These data define differences in circulating lipoprotein subclasses between AAs and EAs, and in the dyslipidemia associated with insulin resistance. In AAs but not EAs,



insulin resistance is associated with increased IDL concentrations, and a more adverse effect on total and small LDL particle concentrations in AA males. The data support alternative approaches in assessment of lipids/lipoproteins as an indicator of cardiometabolic disease risk in AAs.

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None authors have conflict of interests to declare.

V.P. performed statistical analyses and wrote the manuscript. W.T.G. conceived and designed the study, critically reviewed the manuscript, and provided editorial recommendations. W.T.G. is also the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Table 1:** Descriptive characteristics of study subjects

	EA	AA	p-value
N	161	112	
male/female (%)	41.6/58.4	39.3/60.7	
Type 2 Diabetes (%)	17.4	16.1	
Prediabetes, including IFG and IGT (%)	20.5	32.1	
age (years)	39 $\pm$ 11	39 $\pm$ 9	0.88
body mass index (kg/m <sup>2</sup> )	28.4 $\pm$ 5.5	31.0 $\pm$ 5.2	< 0.001
waist circumference (cm)	93 $\pm$ 14	96 $\pm$ 12	0.04
glucose disposal rate (mg/min/kg lean body mass)	13.0 $\pm$ 4.3	13.0 $\pm$ 4.3	0.97
fasting plasma glucose (mg/dl)	106 $\pm$ 43	109 $\pm$ 50	0.57
fasting insulin level ( $\mu$ U/ml)	10.2 $\pm$ 8.1	15.2 $\pm$ 11.0	< 0.001
HOMA-IR	2.69 $\pm$ 2.45	3.97 $\pm$ 3.13	< 0.001
Matsuda index	6.66 $\pm$ 4.72	4.19 $\pm$ 2.56	< 0.001
total cholesterol (mg/dl)	185 $\pm$ 55	185 $\pm$ 46	0.99
triglyceride (mg/dl)	136 $\pm$ 100	102 $\pm$ 50	< 0.001
LDL cholesterol (mg/dl)	111 $\pm$ 34	113 $\pm$ 38	0.88
HDL cholesterol (mg/dl)	42 $\pm$ 15	49 $\pm$ 17	0.002

**Table 2:** Metabolic syndrome risk factors regarding NCEP ATP III stratified by race

	EA	AA	<i>P</i> -value
FPG $\geq$ 100 mg/dl	26.71%	28.57%	0.76
BP $\geq$ 130/85 mg/dl	12.42%	30.36%	< 0.001
triglyceride $\geq$ 150 mg/dl	27.95%	12.50%	0.002
HDL < 40 mg/dl in men, < 50 mg/dl in women	66.46%	49.11%	0.004
abdominal obesity*	41.61%	58.04%	0.009
number risks of metabolic syndrome			0.89
1	32.92%	27.68%	
2	25.47%	25.00%	
3	16.77%	21.43%	
4	8.70%	8.04%	
5	1.24%	0.89%	

\* waist circumference  $\geq$  40 in. in men,  $\geq$  35 in. in women

**Table 3:** Correlation coefficients between lipid subclasses and glucose disposal rate (GDR) per lean body mass controlled by age and BMI

	N	triglyceride	HDL	LDL	total cholesterol	total VLDL particles	large VLDL	medium VLDL	small VLDL	total LDL particles	IDL	large LDL	small LDL	total HDL particles	large HDL	medium HDL	small HDL	VLDL size	LDL size	HDL size
total subjects	273	-0.373*	0.286*	-0.189\$	-0.134#	-0.121	-0.427*	-0.127#	0.027	-0.461*	-0.164\$	0.088	-0.494*	0.156#	0.255*	-0.010	0.077	-0.385*	0.349*	0.158#
all EA	161	-0.411*	0.223\$	-0.080	-0.095	-0.014	-0.422*	-0.053	0.156	-0.400*	-0.051	0.064	-0.466*	0.098	0.216\$	-0.006	0.027	-0.415*	0.334*	0.131
EA male	67	-0.505*	0.187	-0.078	-0.228	-0.083	-0.490*	-0.194	0.240	-0.519*	-0.190	0.071	-0.554*	-0.081	0.216	-0.278#	0.072	-0.499*	0.374\$	0.101
EA female	94	-0.366*	0.207	-0.079	-0.081	0.074	-0.377*	0.134	0.120	-0.331\$	0.014	0.016	-0.408*	0.116	0.177	0.034	0.030	-0.373*	0.276#	0.114
all AA	112	-0.350*	0.366*	-0.305\$	-0.189	-0.245#	-0.529*	-0.261#	-0.119	-0.570*	-0.311\$	0.101	-0.540*	0.202#	0.309\$	-0.040	0.149	-0.354*	0.359*	0.215#
AA male	44	-0.373#	0.261	-0.421\$	-0.324#	-0.249	-0.589*	-0.458\$	0.007	-0.657*	-0.329#	-0.021	-0.607*	0.189	0.260	0.019	0.213	-0.442\$	0.254	0.198
AA female	68	-0.341#	0.400\$	-0.236	-0.105	-0.246	-0.519*	-0.127	-0.220	-0.528*	-0.303#	0.174	-0.505*	0.207	0.316#	-0.096	0.119	-0.323#	0.397\$	0.206
all male	111	-0.464*	0.239#	-0.256\$	-0.299\$	-0.178	-0.489*	-0.293\$	0.123	-0.576*	-0.254\$	0.048	-0.574*	0.087	0.232#	-0.114	0.131	-0.463*	0.339*	0.128
all female	162	-0.333*	0.298*	-0.159	-0.083	-0.064	-0.391*	0.035	-0.028	-0.404*	-0.119	0.081	-0.454*	0.158	0.263\$	0.0003	0.049	-0.350*	0.335*	0.170#

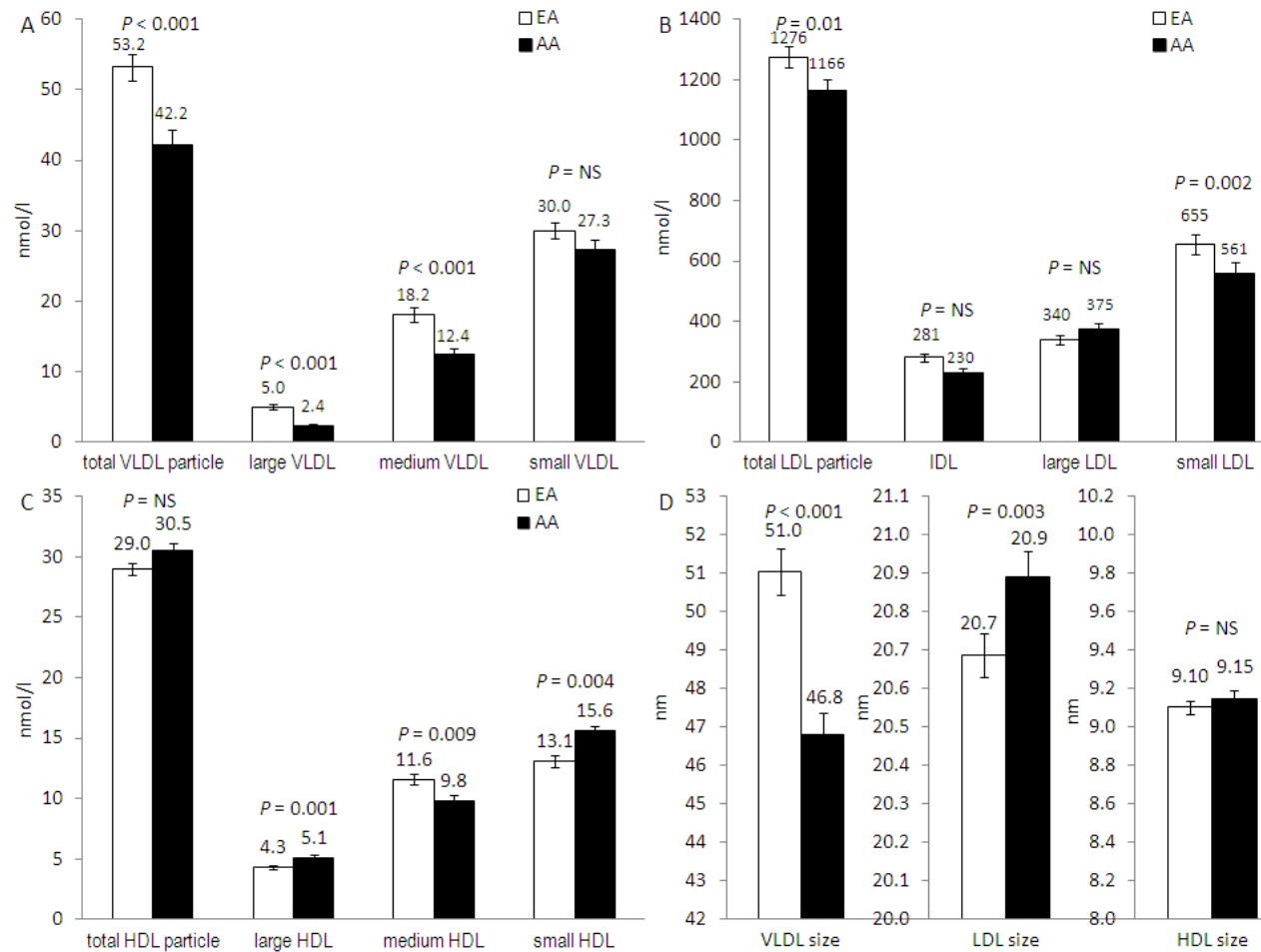
N is the number in each group. All bold values are statistically significant. #  $P < 0.05$ , \$  $P < 0.01$ , \*  $P < 0.001$

**Table 4:** Coefficient of determinations using a model which includes lipid subclass profiles as independent variables and the hyperglycemic-euglycemic clamp measure as the dependent variable in multiple regression analyses.

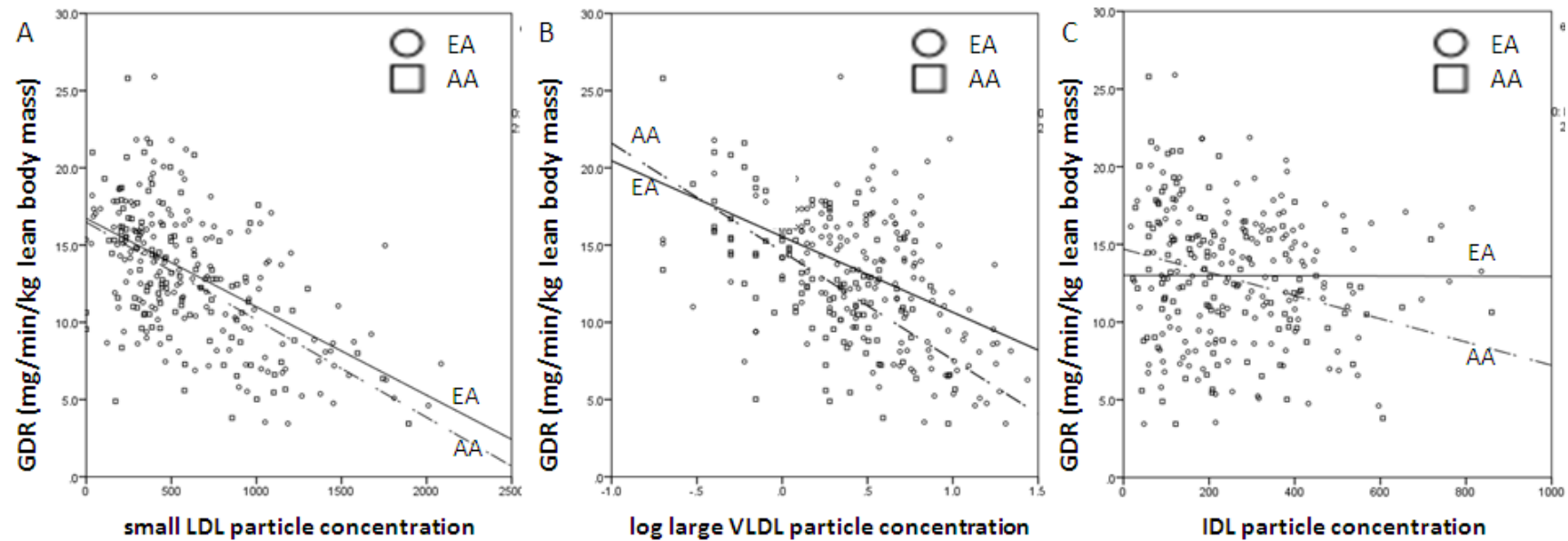
	All participants		EA		AA	
	$\beta$	P-value	$\beta$	P-value	$\beta$	P-value
ethnics	-0.097	0.07				
gender	<b>-0.110</b>	0.03	<b>-0.176</b>	0.008	-0.010	0.89
BMI	<b>-0.178</b>	0.001	<b>-0.176</b>	0.01	<b>-0.214</b>	0.005
IDL	<b>-0.204</b>	< 0.001	-0.089	0.17	<b>-0.332</b>	< 0.001
small LDL	<b>-0.410</b>	< 0.001	<b>-0.376</b>	< 0.001	<b>-0.382</b>	< 0.001
large VLDL	<b>-0.177</b>	0.01	<b>-0.173</b>	0.07	<b>-0.287</b>	0.002
$R^2$	<b>0.396*</b>		<b>0.405*</b>		<b>0.482*</b>	

$\beta$  is the standard coefficient of each independent variable and  $R^2$  the coefficient of determination of each model. Race (African American as 1, European American as 0), gender (male as 1, female as 0) and BMI are entered as common independent variables in all models and IDL, small LDL, large VLDL, or HOMA, or Matsuda index as an additional independent variable in individual models. All bold values are statistically significant. \* $P < 0.001$ .

**Figure 1.** Mean differences in lipoprotein subclass between European Americans (EA) and African Americans (AA) as assessed by ANCOVA, controlling for gender, age, and BMI. [A] VLDL particle concentration; [B] LDL particle concentration; [C] HDL particle concentration; [D] mean size of each lipoprotein class. White bar, EA; black bar, AA.



**Figure 2.** Correlation between the glucose disposal rate measured by hyperinsulinemic clamp (GDR) and particular lipoprotein subclasses. [A] small LDL particle; [B] large VLDL particle; [C] IDL particle. Note: Large VLDL particle was presented with log-transformation due to non-normally distributed data, however, both linear and curvilinear models fit the data in which the differences in fit did not change the significant finding. Circle, EA; square, AA.



CHANGES IN LIPOPROTEIN SUBCLASSES FOLLOWING INTERVENTIONS  
THAT INCREASE INSULIN SENSITIVITY: EFFECTS OF VERY LOW CALORIE  
DIET (VLCD) AND THIAZOLIDINEDIONE

by

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## ABSTRACT

**Background:** Insulin resistance is related to several cardiovascular disease risk factors. Lipoprotein subclasses may be used to detect changes in insulin sensitivity after interventions. Our study aimed to determine changes in insulin sensitivity and lipoprotein subclass profiles in two different interventions: short-term very low calorie diet (VLCD) and thiazolidinedione (TZD).

**Methods:** Two separate cohorts were tested: 26 participants with a 1-week VLCD of 800–1,000 kcal/day and nine participants with a 12-week course of rosiglitazone. Body composition, insulin sensitivity indicated by Matsuda index, serum for conventional lipid profiles, and lipoprotein subclasses were evaluated before and after interventions.

**Results:** After 1-week VLCD, participants had significant weight loss, decreased waist circumference, decreased total fat mass, and improved insulin sensitivity indicated by increased Matsuda index. Following 12-week TZD intervention, participants had no changes in weight or total fat mass but improved insulin sensitivity. Both interventions also reduced triglyceride levels, with different changes in VLDL subclasses. The 1-week VLCD produced a decrease in large VLDL particles, while an increase in small VLDL particles was observed after 12-week TZD. Although both interventions were accompanied by no changes in total and LDL cholesterol levels, a decrease in small LDL particles was observed. Moreover, small LDL particles were negatively associated with Matsuda index in both VLCD and TZD interventions.

**Conclusions:** Concomitant changes in improved insulin sensitivity and lipoprotein subclass profile were observed after VLCD and TZD interventions. The findings



emphasized the utility of lipoprotein subclass, particularly small LDL particles, as an indicator in monitoring insulin sensitivity changes after intervention.

## INTRODUCTION

Insulin resistance is closely associated with numerous metabolic derangements and multiple cardiovascular risk factors that have an impact on morbidity and mortality. Abdominal obesity, hypertension, hypertriglyceridemia, and low level of high-density lipoprotein (HDL) cholesterol are metabolic risk factors predisposed to type 2 diabetes mellitus and cardiovascular disease [1,2]. The dyslipidemia that accompanies insulin resistance is characterized by increased triglycerides and decreased HDL cholesterol levels and is ascertained by its conventional lipid panel (triglycerides, total cholesterol, HDL, and calculated low-density lipoprotein [LDL] levels). However, the conventional lipid panel does not provide all risk information due to inadequate data regarding subclasses of particles within the major lipoprotein classes of HDL, LDL, and very low-density lipoprotein (VLDL). We [3] and others [4-6] have reported that insulin resistance and cardiometabolic disease are associated with changes in lipoprotein particles, and that lipoprotein subclasses, particularly small LDL particles, can independently confer increased cardiovascular disease risk.

Lipoprotein subclasses can be altered by various conditions, e.g., dietary components, body size, long-term weight loss, lipid-lowering agents, and insulin resistance state [7,8]. We previously have shown that increments in large VLDL and small LDL particles were associated with worsening insulin resistance [9]. However, less is known about whether perturbations that improve insulin sensitivity are associated with a commensurate change in lipoprotein subclasses. Therefore, proving this relationship would emphasize the role of lipoprotein subclass profile as a useful predictor of insulin

resistance as a function of alterations in insulin sensitivity that could occur as a function of diet, medications, or other environmental influences.

Our study employed two completely different interventions to improve insulin sensitivity: very low calorie diet (VLCD) and thiazolidinedione administration. VLCD, a dietary intervention that contains energy less than 800 kcal/day, was demonstrated to rapidly induce significant weight loss due to the caloric restriction, ketosis-induced appetite suppression, and diuresis [10]. Moreover, short-term VLCD, i.e., 1 week, leads to an improvement in insulin sensitivity together with an increase in free fatty acid levels [11]. On the other hand, thiazolidinediones (TZD), or peroxisome proliferator-activated receptor (PPAR) $\gamma$  agonists, were shown to improve insulin sensitivity commensurate with a decrease in free fatty acids and an increase in weight due to increased fluid retention and alteration of fat distribution after several months of the intervention [12,13].

## METHODS

### *Study Subjects*

This study included 26 participants with VLCD intervention and 9 participants with TZD administration. Participants were weight stable (weight loss or gain less than 3 percent over past 6 months) overweight or obese adults aged 21–60 years. None of the participants had cardiovascular, renal, or hepatic disease, and all were clinically and chemically euthyroid. Prior to the study, all participants with type 2 diabetes were being treated with diet or sulfonylurea and/or metformin oral hypoglycemic drugs but were withdrawn from therapy for at least 3 weeks and monitored on an outpatient basis. No

subjects were taking any other medications known to affect carbohydrate and lipoprotein metabolism. None of the participants engaged in regular exercise. Pregnant women were excluded, and premenopausal females were studied between days 4 and 11 of the menstrual cycle by history. Race was determined by self-report. Informed consent was obtained from every participant, and the protocol was approved by the University of Alabama at Birmingham Institutional Review Board.

### *Study protocol*

Two non-overlapping groups of participants were used. Participants with VLCD intervention received 1-week period isocaloric diet (containing 50% carbohydrate, 30% fat, and 20% protein) for weight maintenance, followed by 1-week period VLCD (800 kcal/day for female, 1,000 kcal/day for male) with the same macronutrient distribution as the initial diet. All meals were provided by the metabolic kitchen at the UAB clinical research unit. Participants with TZD intervention were treated as outpatients for 12 weeks with 8 mg/day rosiglitazone. No dietary changes were recommended in this group. Anthropometric and body composition measurements, plasma samples, and oral glucose tolerance test (OGTT) results were obtained before and after both interventions.

### *Anthropometric and body composition measurements*

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meter ( $\text{kg}/\text{m}^2$ ). Waist circumference (cm) was assessed by a tension-controlled tape measure by Novel Products (Rockton, IL). Body composition measures, including total fat mass, percent of body fat, and lean body mass, were determined by

dual-energy X-ray absorptiometry with DPX-L version 1.33 (Lunar Radiation, Madison, WI).

#### *Insulin sensitivity measurement*

Insulin sensitivity was assessed by the oral glucose tolerance test (OGTT), in which plasma glucose and insulin levels were obtained at 0, 30, 60, 90, 120, and 180 min. The Matsuda index, one of the commonly used insulin sensitivity indices, was calculated by the following equations [14]:

$$\text{Matsuda index} = 10,000 / \text{square root of } [(FPG \times FIL) \times (\text{mean glucose} \times \text{mean insulin during OGTT})]$$

#### *Lipoprotein subclass profile*

Blood for the lipoprotein subclass profile was obtained at 8 a.m. after an overnight fast, at the same time as the sample for the conventional lipid panel. The NMR lipoprotein subclass profile was measured using a 400-mHz proton NMR analyzer at LipoScience (Raleigh, NC). The two principles of this method are: 1) each different lipoprotein subclass based on size simultaneously produces a unique NMR signal; and 2) the measured subclass signal amplitudes are directly relative to the numbers of subclass particles producing the signal, irrespective of difference in particle lipid component [15]. The data identify 9 lipoprotein subclasses: large VLDL (60–200nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large LDL (21.2–23nm), small LDL (18–21.2 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Weighted average size of VLDL, LDL, and HDL particles was analyzed from the difference subclass concentrations by summation of the diameter of each

lipoprotein subclass multiplied by mass percentage as estimated from the intensity of NMR signal [15].

### *Assays*

Plasma glucose was measured by glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using a Millipore specific insulin RIA kit (Millipore Corp., Billerica, MA). Conventional lipid panel, including total cholesterol, HDL cholesterol, and triglycerides, was determined by colorimetric method on Sirrus analyzer (Stanbio Laboratory, Boerne, TX). LDL cholesterol was calculated using the Freidewald equation.

### *Statistical analysis*

All data were given as means  $\pm$  standard deviation unless otherwise indicated. Mean differences in metabolic and anthropometric variables before and after interventions were analyzed by 1-tailed pair t-test.  $P < 0.05$  was considered significant. Statistical analyses were performed using the SPSS program version 20.0 (IBM, Armonk, NY).

## RESULTS

The analyses included 26 participants who completed 1 week of VLCD intervention and 9 participants who completed TZD intervention. Descriptive characteristics of study participants before and after interventions are shown in Table 1. In the group studied before and after VLCD, 81% were female. Two thirds had type 2 diabetes mellitus or prediabetes. The mean age was 44 years, and the mean BMI was 36.0

kg/m<sup>2</sup>. After 1 week of VLCD, participants had significant weight loss, decreased waist circumference, and decreased total fat mass but no change in percent of body fat. They also had improved insulin sensitivity as indicated by Matsuda index, and, in the conventional lipid panel, triglycerides and HDL cholesterol were decreased.

In the group studied before and after TZD, 44% were female. One third had type 2 diabetes mellitus or prediabetes. The mean age was 44 years, and the mean BMI was 31.8 kg/m<sup>2</sup>. After TZD intervention, participants had no changes in weight, BMI, total fat mass, or percent of body fat. However, Matsuda index indicated that insulin sensitivity was significantly improved. In the conventional lipid panel, triglycerides levels were significantly decreased, while HDL cholesterol levels were increased in this group.

The studies allowed us to assess effects on two very different perturbations, both of which enhanced insulin sensitivity, on lipoprotein subclasses. Changes in lipoprotein subclass profile after VLCD and TZD intervention were shown in Figure 1 and Figure 2, respectively. After VLCD intervention, subclass analyses revealed that a decrease in triglycerides from the conventional lipid panel was due to a lower total VLDL particle concentration, which was caused by a decreased large VLDL particle concentration. These changes were accompanied by a decrement in VLDL size (Fig. 1, panel A). While LDL cholesterol did not change after VLCD, lipoprotein subclass analyses showed a lower small LDL particle concentration (Fig. 1, panel B). The short period of VLCD resulted in decreased HDL cholesterol level due to lower medium HDL particle concentration without change in HDL size (Fig. 1, panel C).

Following TZD intervention, the decrease in triglycerides was explained by a decrement in VLDL size due to an increased small VLDL particle concentration (Fig. 2, panel A). Although LDL cholesterol did not change after TZD, a lower small LDL particle concentration with an increment in LDL size was observed in lipoprotein subclass analyses (Fig. 2, panel B). Increased HDL cholesterol from the conventional lipid profile was due to increased large and medium HDL particles, along with decreased small HDL particles (Fig. 2, panel C). However, HDL size did not change after TZD.

To exam whether these changes in lipoprotein subclasses reflected the improvement in insulin sensitivity, we constructed scatter plots correlating Matsuda index and various lipoprotein subclass concentrations before and after each intervention. Large VLDL (Fig. 3, panel A) and small LDL (Fig. 3, panel C) particles exhibited significantly negative correlation with Matsuda index in participants with VLCD, while participants with TZD exhibited a strong trend with decrements in small LDL particle concentrations as insulin sensitivity increased as reflected by an increasing Matsuda index (Fig. 3, panel D). However, a large VLDL particle concentration was not correlated with insulin sensitivity in this group (Fig. 3, panel B).

## DISCUSSION

To explore the impact of alterations in insulin sensitivity on lipoprotein subclasses, we studied patients before and after two markedly different perturbations. Short-term VLCD produced significant weight loss, while no weight change was observed after long-term TZD intervention. Both interventions resulted in increased insulin sensitivity as indicated by the Matsuda index. Moreover, triglycerides levels



decreased after both interventions. However, HDL cholesterol decreased after short-term VLCD, while it increased after long-term TZD administration. Free fatty acid levels were reported to increase with VLCD [11]; however, free fatty acid levels are known to decrease with TZD [12,16].

Despite the marked differences in interventions, the increase in insulin sensitivity was accompanied by similar effects on small LDL particles. After both VLCD and TZD interventions, a decrease in small LDL particles was observed. In TZD administration, total LDL particle concentrations also decreased, consequently leading to an increment in LDL size since large LDL particles were unaffected. However, no changes in total LDL particles or LDL size were observed after short-term VLCD.

While triglycerides were reduced by both interventions, the underlying mechanisms were different, as reflected by the differential effects on VLDL subclasses. Decreased large and medium LDL particle concentrations were accompanied by a decrement in VLDL size after VLCD. For TZD intervention, redistribution to small VLDL particles without changes in total VLDL particles resulted in a decrement in VLDL size.

Although HDL size was not changed following both interventions, effects on HDL cholesterol and HDL subclasses were in opposite directions. Short-term VLCD was related to reduced HDL levels due to decreased medium HDL particles without changes in large or small HDL particles, while long-term TZD was associated with increased HDL levels due to increased large and medium HDL particles with decreased small HDL particles.

These discrepancies in lipid/lipoprotein changes could be explained by several reasons. The first factor is a dissimilar mechanism of action between VLCD and TZD interventions. VLCD was demonstrated to increase lipolysis and elevate free fatty acid levels, followed by a decrease in intramyocellular lipids, which resulted in an improved insulin signaling pathway [11]. On the other hand, TZD improved both hepatic and peripheral insulin sensitivity via activation of PPAR- $\gamma$  and increasing expression of the glucose transporter-4-receptor. In addition, it affected adipocyte differentiation, resulting in decreased circulating free fatty acids [12,16]. Another explanation is difference in duration of management. Some parameters, particularly HDL, may variably respond based on interval of measurement. Several studies reported that transient reduction in HDL cholesterol level could occur during acute caloric restriction; however, it tended to increase with long-term weight loss [17,18].

One of similarities between VLCD and TZD interventions is concomitant changes in insulin sensitivity and particular lipoprotein subclasses. Pooled data from before and after interventions showed a significant correlation between small LDL particles and insulin sensitivity in the short-term VLCD group ( $r = -0.274$ ,  $P = 0.05$ ) with the same trend in long-term TZD intervention ( $r = -0.435$ ,  $P = 0.07$ ). In addition to the result from our previous reports that small LDL particles could predict insulin sensitivity [3,9], this finding extended the application of small LDL particles as a tool in monitoring changes after interventions. Although large VLDL particles also were proposed to be another predictor of insulin sensitivity [3,9], our study found that large VLDL particles were associated with insulin sensitivity in only VLCD intervention ( $r = -0.316$ ,  $P = 0.02$ ), but not with TZD treatment ( $r = 0.180$ ,  $P = 0.48$ ). This could be explained by no significant

change in large VLDL particles after long-term TZD administration. However, decreased triglycerides levels and smaller VLDL size with this intervention were caused by increased small VLDL particles – thus, the change in lipoprotein subclass that was consistent was decreased small LDL particles. We found that particular subclass to be integrally associated with insulin resistance in larger cross-sectional studies [3,9]. Therefore, this subclass seems highly related to change in insulin sensitivity, both at baseline and after intervention.

Although some TZD have the further potential to react with the PPAR- $\alpha$  receptor, which responds for lipid metabolism [12,19], we believed that the lipid/lipoprotein changes in our study were a primary consequence of improved insulin sensitivity rather than PPAR- $\alpha$  pathway because rosiglitazone was reported to have very modest effect on this mechanism [20,21]. Furthermore, our results opposed the hypothesis that adverse effects on lipids of rosiglitazone might be a potential factor of increased risk of myocardial infarction shown by prior meta-analyses [22,23]. On the other hand, current findings pointed out that rosiglitazone had beneficial lipids outcomes.

The limitation of this study is the small number of participants who completed rosiglitazone administration. However, significant changes in several parameters were still observed after the intervention.

## CONCLUSIONS

Concomitant changes in improved insulin sensitivity and lipoprotein subclass profile, particularly decreased small LDL particles, were observed after both short-term

VLCD and long-term rosiglitazone interventions. This finding highlights the usefulness of lipoprotein subclass profile as a marker in detecting insulin sensitivity change after intervention.

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Neither of the authors has any conflict of interest to declare.

V.P. performed statistical analyses and wrote the manuscript. W.T.G. conceived and designed the study, critically reviewed the manuscript, and provided editorial recommendations. W.T.G. is also the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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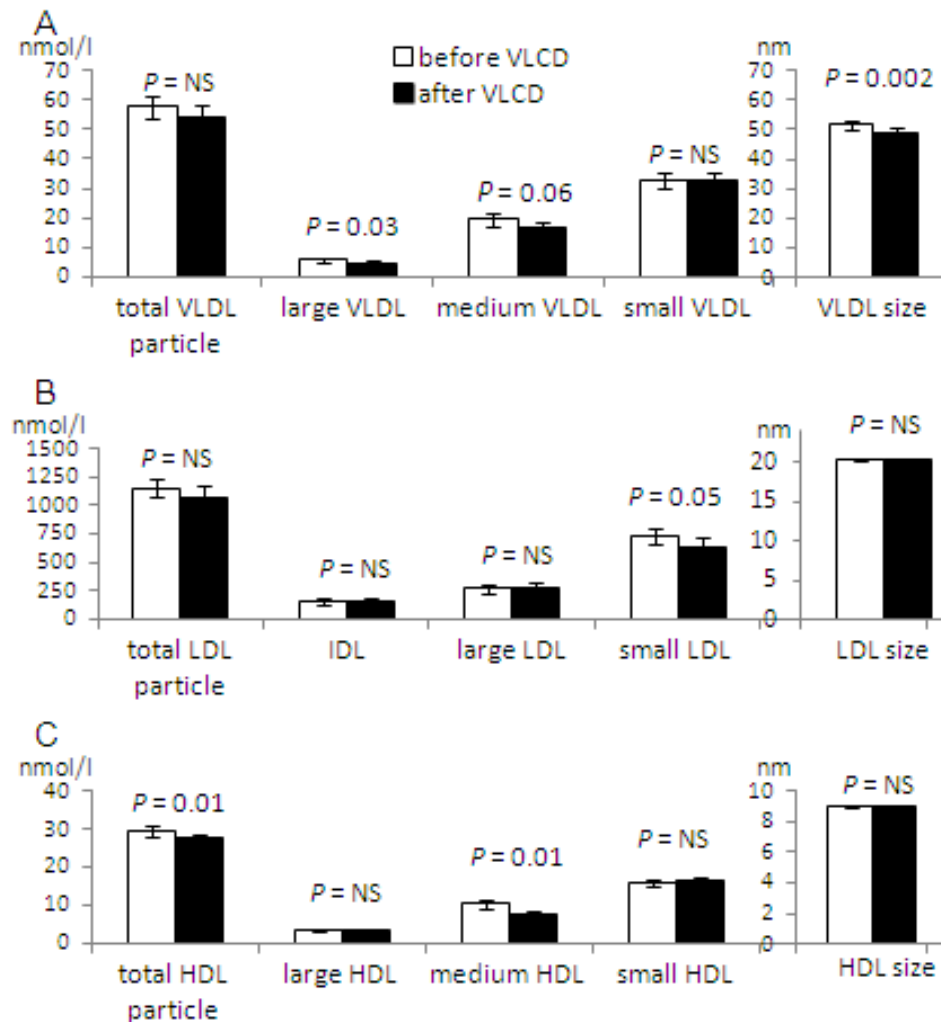


**Table 1:** Descriptive characteristics of study subjects.

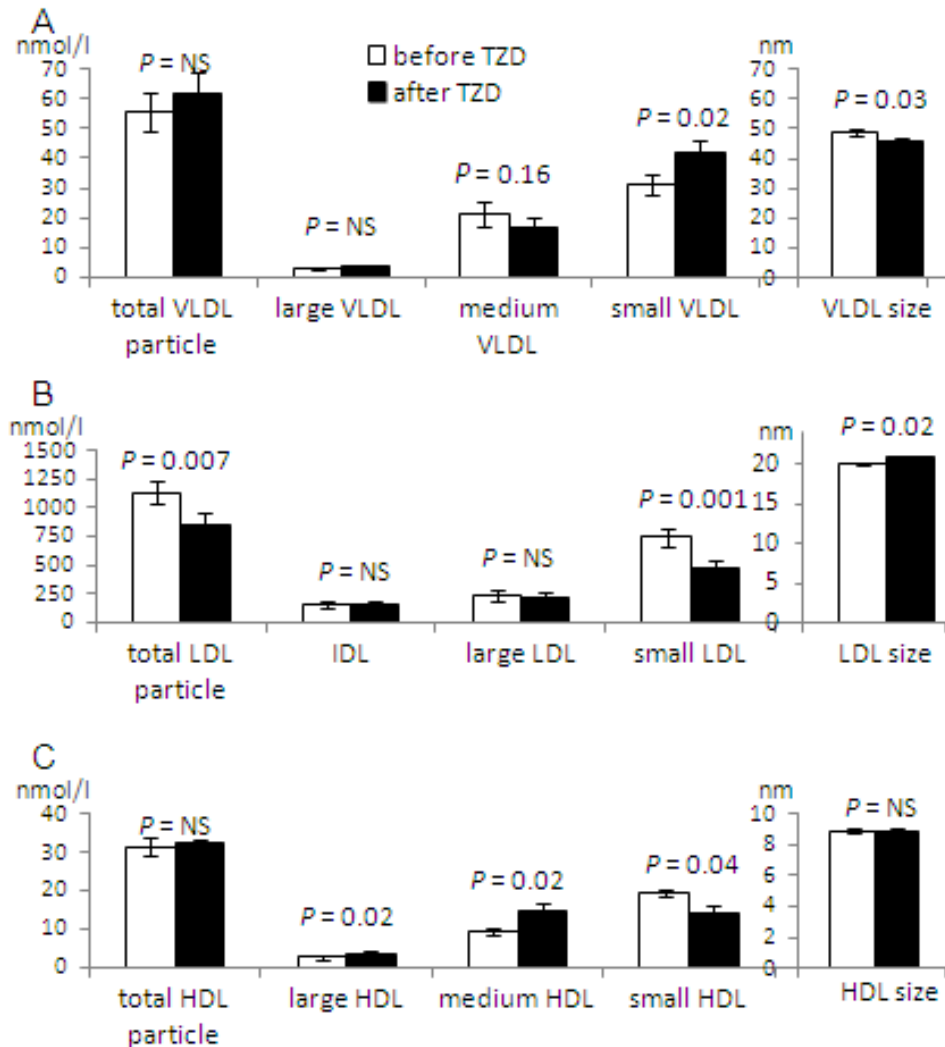
	before VLCD	after VLCD	p-value	before TZD	after TZD	p-value
N	26			9		
Gender	5M/21F			5M/4F		
European American:African American	61.5%:38.5%			44%:56%		
type 2 diabetes (%)	31			11		
prediabetes (IFG or IGT, %)	38			22		
age (year)	44 ± 8			44 ± 10		
weight (kg)	100.5 ± 17.3	97.7 ± 16.9	< 0.001	94.6 ± 18.3	95.0 ± 19.2	NS
body mass index (kg/m <sup>2</sup> )	36.0 ± 5.4	34.9 ± 5.4	< 0.001	31.8 ± 4.3	32.1 ± 4.5	NS
waist circumference (cm)	110.4 ± 15.2	108.3 ± 14.5	< 0.001	104.6 ± 12.2	103.8 ± 12.9	NS
percent of body fat (%)	44.7 ± 6.9	45.1 ± 7.1	NS	39.8 ± 7.2	40.2 ± 7.7	NS
total fat mass (kg)	42.0 ± 8.8	41.4 ± 8.7	0.01	36.2 ± 10.0	36.9 ± 10.1	NS
lean body mass (kg)	52.1 ± 9.8	50.4 ± 9.8	< 0.001	54.3 ± 12.1	54.4 ± 13.1	NS
fasting plasma glucose (mg/dl)	137 ± 77	122 ± 60	0.01	110 ± 33	96 ± 16	0.06
fasting insulin level (μU/ml)	19.2 ± 10.7	17.7 ± 11.9	NS	21.0 ± 7.2	14.0 ± 4.2	0.002
Matsuda index	2.54 ± 1.68	3.08 ± 2.17	0.02	2.51 ± 1.30	3.93 ± 1.44	0.003
total cholesterol (mg/dl)	186 ± 36	182 ± 40	NS	179 ± 45	185 ± 33	NS
triglyceride (mg/dl)	148 ± 58	127 ± 62	0.005	136 ± 43	106 ± 53	0.02
LDL cholesterol (mg/dl)	113 ± 33	117 ± 38	NS	116 ± 38	121 ± 35	NS
HDL cholesterol (mg/dl)	43 ± 14	39 ± 13	< 0.001	37 ± 15	43 ± 18	0.01

VLCD: very low calorie diet; TZD: thiazolidinedione; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; NS: not significant.

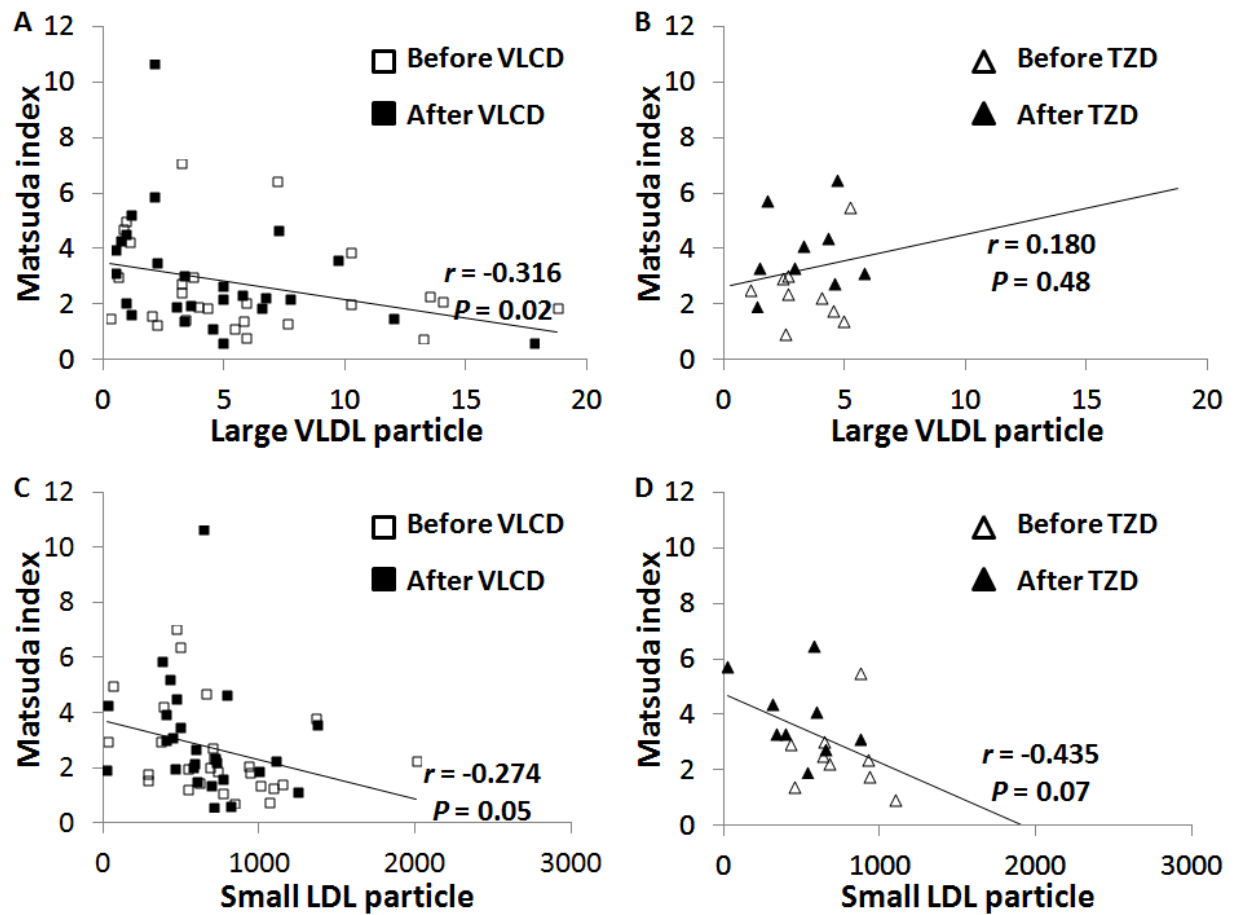
**Figure 1.** Mean differences in lipoprotein subclass between before and after very low calorie diet (VLCD) as assessed by pair t-test. [A] VLDL particle concentration and VLDL size; [B] LDL particle concentration and LDL size; [C] HDL particle concentration and HDL size. NS indicates  $P$ -value  $> 0.2$ . White bar, before VLCD; black bar, after VLCD.



**Figure 2.** Mean differences in lipoprotein subclass between before and after thiazolidinedione administration (TZD) as assessed by pair t-test. [A] VLDL particle concentration and VLDL size; [B] LDL particle concentration and LDL size; [C] HDL particle concentration and HDL size. NS indicates  $P$ -value  $> 0.2$ . White bar, before TZD; black bar, after TZD.

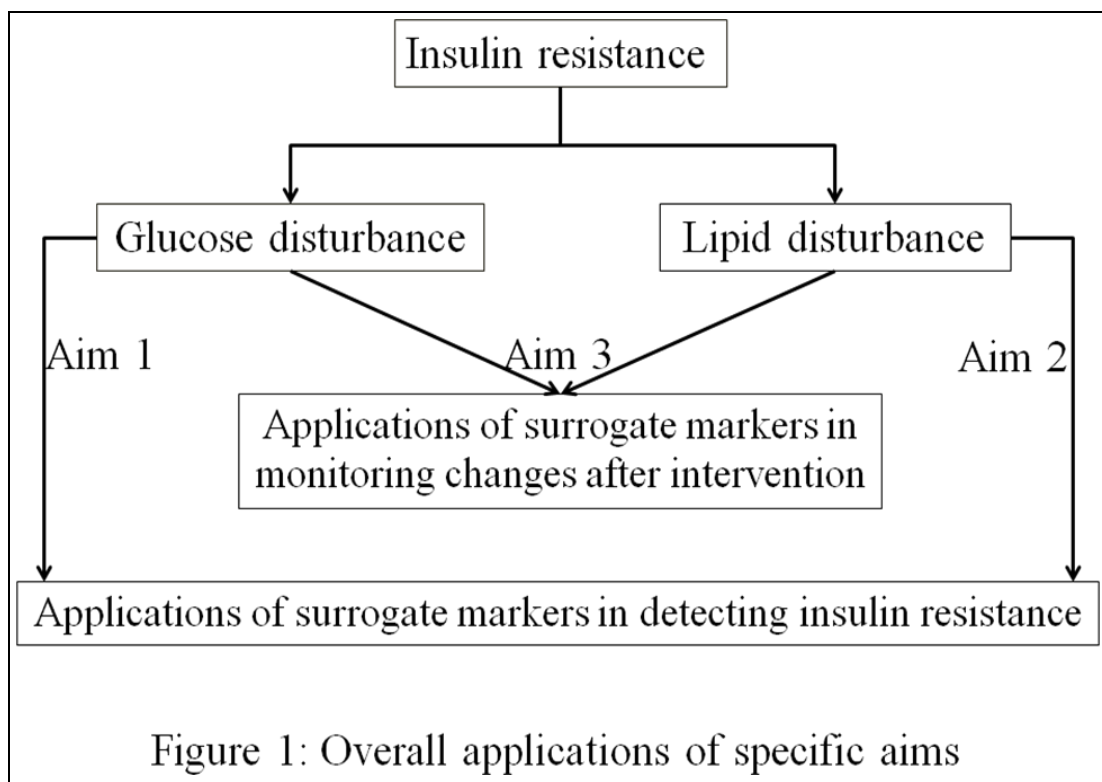


**Figure 3.** Scatter plots show correlation between the Matsuda index and particular lipoprotein subclasses before and after each intervention. [A] Correlation between the Matsuda index and large VLDL particle in participants with very low calorie diet (VLCD) intervention, [B] correlation between the Matsuda index and large VLDL particle in participants thiazolidinedione (TZD) intervention, [C] correlation between the Matsuda index and small LDL particle in participants with VLCD intervention, [D] correlation between the Matsuda index and small LDL particle in participants TZD intervention. White square, before VLCD; black square, after VLCD; white triangle, before TZD; black triangle, after TZD.



## GENERAL DISCUSSION

Insulin resistance is a combination of complex abnormalities involving multiple pathways resulting in a large number of related disorders that affect cardiovascular outcomes. Identifying insulin resistance is important, but difficult. The primary aims of these studies were 1) to determine the accuracy of the commonly used insulin sensitivity indices relative to the hyperinsulinemic-euglycemic clamp and evaluate whether there is a differential impact of race, 2) to investigate the relationship between insulin resistance and lipoprotein subclass profile and evaluate a differential impact of race, and 3) to determine changes of lipoprotein subclass profile and insulin sensitivity index after short-term very low calorie diet.



The overall perspective is presented in a modified version of Figure 1, which includes the applications of specific aims. The major finding from this study was that lipoprotein subclass may reflect a better marker for insulin resistance than glucose measures when using a diverse sample, suggesting the need to collectively evaluate both lipid and glucose disturbance.

In specific aim 1, we observed that European Americans (EAs) and African Americans (AAs) had similar peripheral insulin resistance as measured by the hyperinsulinemic-euglycemic clamp. In contrast, AAs were more insulin resistant when assessed by insulin sensitivity indices based on fasting condition and dynamic tests. The discrepancy in predictability of commonly used insulin sensitivity indices between EAs and AAs could be explained by several hypotheses. First, EAs and AAs have different insulin metabolism, particularly at the level of the hepatocytes. We and other investigators [46-49] have demonstrated a significantly lower C-peptide to insulin molar ratio as an indicator of hepatic insulin clearance in AAs. Moreover, insulin hypersecretion was also reported in AAs [40-42]. These two factors could influence relationships involving circulating insulin, glucose, and insulin sensitivity and could confound the interpretation of these insulin sensitivity indices in different racial groups.

Another possible explanation is the difference in metabolism among various organs between EAs and AAs. The hyperinsulinemic-euglycemic clamp as the gold standard measurement of insulin sensitivity directly determines the ability of insulin to promote glucose uptake in skeletal muscle under the condition of completely suppressed hepatic glucose output [13,51], while all commonly used insulin sensitivity indices depend on plasma glucose and insulin levels. Fasting insulin level,

HOMA-IR, and QUICKI are performed in a fasting steady state. Therefore, they mainly reflect hepatic insulin sensitivity. The Matsuda index,  $SI_{isOGTT}$ , Avignon index, and Stumvoll index include OGTT data in their formulas, which indicate both hepatic and peripheral insulin sensitivity [21]. The hypothesis that AAs have greater insulin resistance relative to peripheral insulin resistance may explain the discrepancy in predictability of commonly used insulin sensitivity indices between EAs and AAs. However, this theory requires further research to be proven.

Regarding racial differences in the predictability of commonly used insulin sensitivity indices, it appears that indices that incorporate glucose and insulin concentrations from both fasting and dynamic states, i.e., the Matsuda index and  $SI_{isOGTT}$ , are superior predictors of peripheral insulin sensitivity in AAs than indices that rely only on fasting glucose and insulin levels. On the other hand, indices derived from OGTT that include additional factors such as glucose's volume of distribution in the Avignon index and BMI in the Stumvoll index, provide no further predictive value. Although informative, assessing insulin resistance using glucose and insulin levels as predictors continues to limit our capacity to identify disease severity. However, lipid metabolism also substantially altered in the insulin resistance state provides an additional pathway to explore.

In specific aim 2, we observed a similar prevalence of metabolic syndrome in EAs and AAs; however, the components reflected by metabolic syndrome differ according to race. AAs had more abdominal obesity and hypertension, while EAs had more atherogenic dyslipidemia. In addition, similar to previous reports, EAs had significant lower HDL-c and higher triglycerides with the same level of total cholesterol and LDL-c when compared with AAs [52-55]. To more comprehensively

assess the relationship between lipid disturbance and insulin resistance, we evaluated lipoprotein subclasses. We observed racial differences in lipoprotein subclasses with the matched degree of insulin resistance. AAs were found to have lower VLDL particle concentrations due to reductions in large and medium VLDL particles accompanied by smaller VLDL size; lower IDL levels; lower LDL particle concentrations due to marked reductions in small LDL particles and, as a result, increased LDL size; and higher levels of large and small HDL and lower medium HDL, resulting in no change in HDL particle concentration or mean HDL size.

Regarding racial difference in lipoprotein subclass profile, there were discrepancies in correlations between GDR and lipoprotein subclasses. Although both large VLDL and small LDL particle concentrations produced strongly negative correlations with GDR in both EAs and AAs, IDL had a remarkably negative association with GDR only in AAs. This finding might be influenced by variations in enzyme activities responsible for hydrolyzing lipoprotein lipid components.

Lipoprotein lipase removes triglycerides from VLDL, resulting in IDL, which is further converted to LDL by both lipoprotein lipase and hepatic lipase [56]. In insulin resistance, hepatic lipase activity is increased [57,58], and lipoprotein lipase activity is reduced [59-61]. Moreover, AAs have been observed to have lower serum hepatic lipase activity [62,63] and higher lipoprotein lipase activity [64,65] than in other racial groups. These racial differences in hepatic lipase and lipoprotein lipase activities could possibly explain differential relationships between GDR and lipoprotein subclasses with worsening insulin resistance. As VLDL, HDL, and LDL subclasses can be related to insulin resistance and cardiovascular disease risk [66,67], these findings emphasized the ability of lipoprotein subclass assessment as an



alternative indicator of insulin resistance and its related disease risk in diverse racial populations. A main finding of this study was that lipoprotein subclass profile serves as a better indicator of insulin resistance than proxy measures. Perhaps this can be used to further understand racial difference in insulin resistance.

Beyond racial differences, we wanted to determine whether lipoprotein subclass profile can be used to identify insulin resistance under the auspice of intervention. In specific aim 3, concomitant favorable changes in insulin sensitivity and certain lipoprotein subclasses, including decreased large VLDL and small LDL particles, were demonstrated after short-term very low calorie diet (VLCD). This finding extended the beneficial effects of VLCD in addition to improving insulin sensitivity, lowering blood pressure, and decreasing triglyceride levels [68]. The proposed underlying mechanism is due to a decrease in intramyocellular lipid content, which was accompanied by an improved insulin signal pathway and further enhanced various substrate metabolism, particularly lipoprotein [69,70]. Besides advantages of short-term VLCD, the findings also highlighted the application of the Matsuda index and lipoprotein subclass profile as indicators for monitoring changes after interventions. These markers are sensitive enough to reflect short-term change. Additionally, they may provide predictive values for insulin resistance and related co-morbidities.

### **Summary**

The gold standard measurement is excessively complicated for realistic practice. Since insulin resistance involves numerous disturbances, several approaches are proposed as surrogate methods to indicate insulin dynamics. Most commonly used insulin sensitivity indices depend on plasma glucose and insulin values, either in a

fasting steady-state condition or during dynamic tests. Lipoprotein subclass profile measured by NMR spectroscopy also is another promising indicator. However, applications of these options are limited in certain situations, particularly when applied to a diverse racial/ethnic population. The racial difference in underlying metabolism, involving various enzymes, accounts for the explanation. Any commonly used insulin sensitivity indices can be applied to EA subgroup, while the Matsuda index and  $SI_{OGTT}$  appear to be more appropriate than other indices for AAs. Despite large VLDL and small LDL particles from lipoprotein subclass profile also being good indicators of insulin sensitivity for EAs and AAs, IDL particles are emerging as additional strong predictors for the AA subgroup. The value of these markers is not only confined to detecting insulin resistance; appropriate indicators for monitoring changes after interventions also are warranted. The current studies proved that both the Matsuda index and lipoprotein subclass profile, particularly small LDL particles, are appropriate markers for detecting as well as monitoring insulin dynamics in a mixed-race population.

### **Strengths and limitations**

Strengths of these studies included a large cohort with a significant population of both AAs and EAs, providing sufficient power to analyze racial/ethnic differences. Moreover, lipoprotein subclass profile was assessed by nuclear magnetic resonance (NMR) spectroscopy, which directly measures lipoprotein particles without influence by variation of composition [67]. However, our studies are not without limitations. Race was determined by self-report, which may not take into account biological correlates and only included EAs and AAs. The sample size was modest, particularly

for specific aim 3. Further evaluation of a larger, more diverse sample including other racial groups is warranted.

### **Future Direction**

This project expands current understanding of racial differences in indicators of insulin resistance. However, some findings may need further explanation. Difference in degree of hepatic and peripheral insulin resistance in AAs is an attractive hypothesis to clarify the discrepancy among insulin sensitivity indices in this racial group; however, there has been little data published to directly support this idea.

In addition to abnormal insulin signaling pathways resulting in glucose and lipid/lipoprotein disturbance, insulin resistance also involves other various derangements, including dysregulated adipokines secretion and inflammatory process. Hence, some adipokines, e.g., plasminogen activator inhibitor 1 (PAI-1) [71], and certain inflammatory markers, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) [72], may be served as markers for insulin resistance. However, additional applications need to be further investigated.

Recently, metabolomics or small-molecule metabolites, e.g., amino acids, acylcarnitines, which potentially represented metabolic dysregulation, were demonstrated to have a relationship with insulin sensitivity and to predict development of type 2 diabetes as well as cardiovascular risks [73-76]; however, this area requires further exploration.

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APPENDIX  
IRB APPROVAL FORM

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for investigators for additional information.
- Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.

<b>1. Today's Date</b>		3/4/2011	
<b>2. Principal Investigator (PI)</b>			
Name (with degree)	W. Timothy Garvey, M.D.	Blazer ID	Garveyt
Department	Nutrition Sciences	Division (if applicable)	SHP
Office Address	Webb Bldg Room 232	Office Phone	934-6103
E-mail	garveyt@uab.edu	Fax Number	996-4082
Contact person who should receive copies of IRB correspondence (Optional)			
Name	Dana Y. Rigsby, RN	E-Mail	ccnrm@uab.edu
Phone	996-4015	Fax Number	996-4082
Office Address (if different from PI)			
<b>3. UAB IRB Protocol Identification</b>			
3.a. Protocol Number		F030603009	
3.b. Protocol Title		Mechanisms of Human Insulin Resistance	
3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable			
<input type="checkbox"/>	Study has not yet begun	No participants, data, or specimens have been entered.	
<input checked="" type="checkbox"/>	In progress, open to accrual	Number of participants, data, or specimens entered: 150	
<input type="checkbox"/>	Enrollment temporarily suspended by sponsor		
<input type="checkbox"/>	Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)		
	Date closed:	Number of participants receiving interventions:	
		Number of participants in long-term follow-up only:	
<input type="checkbox"/>	Closed to accrual, and only data analysis continues		
	Date closed:	Total number of participants entered:	
<b>4. Types of Change</b>			
Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.			
<input type="checkbox"/>	Protocol revision (change in the IRB-approved protocol) In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.		
<input type="checkbox"/>	Protocol amendment (addition to the IRB-approved protocol) In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.		
<input checked="" type="checkbox"/>	Add or remove personnel In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.		
<input type="checkbox"/>	Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP).		
<input type="checkbox"/>	Change in source of funding; change or add funding In Item 5.c., describe the change or addition in detail, include the applicable OGCA tracking number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.		
<input type="checkbox"/>	Add or remove performance sites In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.		



<input type="checkbox"/>	<b>Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)</b> To assist you in revising or preparing your submission, please see the <a href="#">IRB Guidebook for Investigators</a> or call the IRB office at 934-3789.
<input type="checkbox"/>	<b>Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)</b> In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.
<input type="checkbox"/>	<b>Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)</b> In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.
<input type="checkbox"/>	<b>Revise or amend consent, assent form(s)</b> Complete Item 5.d.
<input type="checkbox"/>	<b>Addendum (new) consent form</b> Complete Item 5.d.
<input type="checkbox"/>	<b>Add or revise recruitment materials</b> Complete Item 5.d.
<input type="checkbox"/>	<b>Other (e.g., investigator brochure)</b> Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable. Include a copy of all affected documents, with revisions highlighted as applicable.

<b>5. Description and Rationale</b> In Item 5.a. and 5.b, check Yes or No and see instructions for Yes responses. In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.	
<input type="checkbox"/> Yes <input type="checkbox"/> No	<b>5.a. Are any of the participants enrolled as normal, healthy controls?</b> If yes, describe in detail in Item 5.c. how this change will affect those participants.
<input type="checkbox"/> Yes <input type="checkbox"/> No	<b>5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?</b> If yes, FAP-designated units complete a FAP submission and send to <a href="mailto:fap@uab.edu">fap@uab.edu</a> . Identify the FAP-designated unit in Item 5.c. For more details on the UAB FAP, see <a href="http://www.uab.edu/cto">www.uab.edu/cto</a> .
<b>5.c. Protocol Changes:</b> In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.	
▶ Please add Veeradej Pisprasert, PhD student, Department of Nutrition Sciences, as a Research Associate to this protocol. <span style="float: right;"><i>Updated SIRB</i></span>	
<b>5.d. Consent and Recruitment Changes:</b> In the space below, (a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them; (b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and (c) indicate either how and when you will re-consent enrolled participants or why re-consenting is not necessary (not applicable for recruitment materials).	
Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies: • a copy of the currently approved document (showing the IRB approval stamp, if applicable) • a revised copy highlighting all proposed changes with "tracked" changes • a revised copy for the IRB approval stamp.	

Signature of Principal Investigator

*Matthew Garvey*

Date 3/4/11

**FOR IRB USE ONLY**

☐ Received & Noted ☒ Approved Expedited\* ☐ To Convened IRB

Reslie Cooper, CIP  
Signature (Chair, Vice-Chair, Designee)

3-11-11  
Date

DOLA 1-26-11

Change to Expedited Category Y / N / NA NA

\*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111

