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The relationship between insulin resistance and ion mobility lipoprotein fractions

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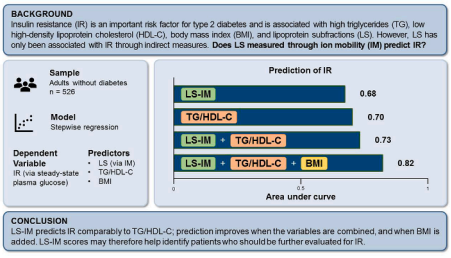
GRAPHICAL ABSTRACT



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ABSTRACT

Objective: Insulin resistance (IR) increases risk of type 2 diabetes and atherosclerotic cardiovascular disease and is associated with lipid and lipoprotein abnormalities including high triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C). Lipoprotein size and lipoprotein subfractions (LS) have also been used to assist in identifying persons with IR. Associations of LS and IR have not been validated using both direct measures of IR and direct measures of LS. We assessed the usefulness of fasting lipoprotein subfractions (LS) by ion mobility to identify individuals with IR.

Methods: Lipid panel, LS by ion mobility (LS-IM), and IR by steady-state plasma glucose (SSPG) concentration were assessed in 526 adult volunteers without diabetes. IR was defined as being in the highest tertile of SSPG concentration. LS-IM score was calculated by linear combination of regression coefficients from a stepwise regression analysis with SSPG concentration as the dependent variable. Improvement in prediction of IR was evaluated after combining LS-IM score with TG/HDL-C, TG/HDL-C and BMI as well as with TG/HDL-C, BMI, sex, race and ethnicity. IR prediction was evaluated by area under the receiver operating characteristic curve (AUC) and positive predictive value (PPV) considering the highest 5% of scores as positive test.

Results: Prediction of IR was similar by LS-IM score and TG/HDL-C (AUC=0.68; PPV=0.59 and AUC=0.70; PPV=0.59, respectively) and prediction was improved when LS-IM was combined with TG/HDL-C (AUC=0.73; PPV=0.70), TG/HDL-C and BMI (AUC=0.82; PPV=0.81) and with TG/HDL-C, BMI, sex, race and ethnicity (AUC=0.84; PPV=0.89).

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Conclusion: For identifying individuals with IR, LS-IM score and TG/HDL-C are comparable and their combination further improves IR prediction by TG/HDL-C alone. Among patients who have undergone IM testing, the LS-IM score may assist prioritization of subjects for further evaluation and interventions to reduce IR.

1. Introduction

Insulin resistance (IR) increases risk of type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (ASCVD) [1,2]. The role of IR in the pathogenesis of T2D and ASCVD and its association with metabolic abnormalities including elevated triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) concentrations was first formulated and presented by Reaven in 1988 [1]. Subsequent work by Reaven and colleagues and others demonstrated the association of IR with a myriad of metabolic abnormalities and various clinical syndromes including non-alcoholic fatty liver disease, obstructive sleep apnea, polycystic ovarian syndrome, and certain types of cancer that have deleterious health consequences [3]. Thus, identification of IR in individuals is of clinical importance because it could prompt changes in behavior and clinical management to reduce risk associated with IR.

IR can be measured directly by insulin suppression test or by a glucose clamp technique. However, because techniques for direct measurement of IR are labor intensive and expensive, indirect methods of IR assessment, such as fasting insulin, the oral glucose tolerance test, the homeostatic model assessment of insulin resistance (HOMA-IR) and others, are more commonly used in a clinical setting. These indirect methods have a variety of limitations but are generally well correlated with results from direct measurement [4]. Despite the availability of relatively simple indirect methods for IR assessment, patients lacking a clear indication for evaluation of T2D risk may not have IR assessed by their clinicians and will likely remain unaware of their elevated IR measure and the potentially increased risk of T2D and ASCVD.

A variety of other clinical measures have the potential to assist in the identification of individuals with IR. The association of these measures with IR may not be as strong as the indirect methods described above but, when readily available, provide additional diagnostic insights to identify and prioritize individuals for further IR assessment. Body mass index (BMI) is strongly associated with IR, but not all insulin resistant patients are obese [5]. IR is also associated with lipid and lipoprotein abnormalities that comprise high TG and low HDL-C concentrations and a preponderance of small dense low-density lipoprotein (LDL) particles [6–8]. TG to HDL-C concentration ratio (TG/HDL-C) can be used to identify insulin resistant individuals [9,10]. Lipoprotein size and LS concentrations have also been employed in the identification of persons with IR. In that context, an IR score based on nuclear magnetic resonance (NMR)-derived lipoprotein information was shown to have a strong association with multiple measures of IR [11–14].

LS can also be measured by ion mobility [15]. LS quantified by NMR and ion mobility are correlated, but not identical [16,17]. Ion mobility-based methods are a direct measure of lipoprotein particle counts according to their size, while NMR is an algorithmically derived measurement.

The association of directly measured LS by ion mobility (LS-IM) with a direct measure of IR has not been previously reported. Finding a strong association may provide patients who are already undergoing LS-IM testing and their clinicians with additional information about IR-driven risk of T2D and ASCVD. Therefore, we set out to describe the relationship between LS-IM and a direct measure of IR measured during the insulin suppression test and to determine the usefulness of LS to identify insulin resistant individuals.

2. Methods

2.1. Study population

This cross-sectional analysis includes 526 participants derived from 1072 apparently healthy individuals who had volunteered to participate in studies of IR between 1999 and 2011. Participants were recruited from the San Francisco Bay Area through advertisements in the local newspapers. The studies excluded pregnant women, individuals older than 79 or younger than 18 years, persons with history of cardiovascular disease, and patients with diabetes requiring insulin treatment. For this analysis, we excluded 149 participants who had fasting glucose $\geq$ 126 mg/dL and 397 participants with missing data for at least one of the following measures: race, ethnicity, body mass index (BMI), TG, HDL-C, LDL cholesterol, systolic blood pressure, diastolic blood pressure, alanine transaminase, or any of the ion mobility LS (Supplemental Figure S1).

The Institutional Review Board at Stanford approved all studies, and all participants gave written informed consent.

2.2. Clinical and measurements

The study visits were conducted at Stanford Clinical and Translational Research Unit. Race and ethnicity were self-reported. Height and weight were measured without shoes and in light clothing; and BMI was calculated by dividing weight in kilograms by height in meters squared. Blood pressure was measured by an automatic blood pressure recorder after participants were seated quietly in a chair for 5 minutes with their feet on the floor and their arm supported at heart level. Three blood pressure measurements were obtained at 1-minute intervals using an appropriately sized cuff and were averaged.

2.3. Insulin suppression test

The degree of IR was directly measured by the modified and validated version of the Insulin Suppression Test (IST), which quantifies the ability of a steady-state of physiological hyperinsulinemia to stimulate glucose uptake [18,19].

After an overnight fast, an intravenous catheter was placed in each arm. One arm was used for drawing blood samples and the other for giving a continuous infusion of octreotide acetate (0.27 $\mu g/m^2$/min), insulin (32 mU/m^2/min), and glucose (267 mg/m^2/min) for 180 minutes. Blood was sampled every 30 minutes for 150 minutes and then every 10 minutes to measure steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations.

During the IST, octreotide acetate inhibits endogenous insulin secretion and the infusion of insulin results in similar SSPI concentration (physiological hyperinsulinemia) among all individuals. The ability of physiological hyperinsulinemia to stimulate uptake of infused glucose is indicated by the SSPG concentration. The higher the SSPG concentration, the lower the insulin-stimulated glucose uptake, and the more insulin resistant a person. IR measured during the IST highly correlates with that measured during the euglycemic, hyperinsulinemic clamp test [20,21]. Individuals in the top tertile of SSPG concentration were defined as being insulin resistant. This decision was based on the results of a prospective study where subjects in the tertile with the highest SSPG concentration developed more ASCVD than those in the tertile with the lowest SSPG concentration [2].

2.4. Lipid and lipoprotein measurements

Lipid panel were measured after overnight fasting at Stanford Health Care Clinical Laboratory and the Friedewald equation was used to calculate LDL cholesterol [22].

2.5. Ion mobility measurements

LS levels were assessed by ion mobility, as previously described [23, 24], at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA). The LS and their definitions are provided in **Supplemental Table S1**.

2.6. Statistical methods

Pearson's correlation coefficient (r) was used as a measure of pairwise correlation.

The associations of the TG/HDL-C and each LS-IM measure with SSPG concentration were assessed in separate linear regression models adjusting for age, sex, race, ethnicity and BMI. To incorporate multiple ion mobility variables and covariates in a single model, a backward stepwise regression model was performed using the Aikaike Information Criterion (AIC) as the metric to compare models. Using the regression coefficients for the LS-IM measures in the final stepwise model, an ion mobility score (LS-IM score) was calculated. The score for each subject was a linear combination of the LS-IM variables from the stepwise model calculated as B1*Var1 + B2*Var2 + … + Bp*Varp where B1 to Bp are the regression coefficients and Var1 to Varp are the subject specific values for p LS-IM variables in the final model. In a similar fashion, scores were calculated for other combinations of variables from the model: 1) LS-IM score + TG/HDL-C; 2) BMI + TG/HDL-C; 3) BMI + TG/HDL-C + LS-IM score; 4) BMI + TG/HDL-C + Sex + Race + Ethnicity; 5) BMI + TG/HDL-C + Sex + Race + Ethnicity + LS-IM score.; Since the standard deviations of the individual LS-IM measures vary and to allow comparison of the relative effect sizes, all continuous variables were standardized by transform to standard deviation (SD) units when included in the regression models. Tertiles of the LS-IM score, calculated using the ion mobility coefficients of the stepwise model, were plotted against SSPG concentration in tertiles of BMI for women and men. Similarly, tertiles of TG/HDL-C and tertiles of the score combining LS-IM score + TG/HDL-C were plotted against SSPG concentration in tertiles of BMI for women and men.

Receiver operating characteristic (ROC) curves were plotted and the area under the curve (AUC) and 95% confidence intervals were calculated using Delong's method [25] for each of the scores discussed above when predicting IR defined as the top tertile of SSPG. Statistical comparisons among the scores discussed above were performed by likelihood ratio test in nested logistic regression models [26]. The positive predictive value (PPV) of identifying individuals in the top tertile of SSPG concentration was determined for each of the scores when considering the highest 5% of values for a score as a positive test. Wilson's method [27] was used to calculate confidence intervals for the PPV.

The Bonferroni method [28] was used to determine significance levels adjusting for multiple comparisons.

All analyses were performed using the R programming language [29].

3. Results

The median age of study participants was 50 years and about two-thirds (65%) were women (**Supplemental Table S2**). The majority of subjects were non-Hispanic (92%) and 70% were white. Nearly half (48%) of participants were obese (BMI $\geq$ 30.0 kg/m^2) and 38% were overweight (BMI 25.0 to 29.9 kg/m^2).

As expected, there was a significant positive correlation between SSPG concentration and both BMI and TG/HDL-C (r=0.54 and 0.32 respectively) (**Supplemental Table S3**). Pairwise correlations (r) between BMI and the various LS-IM measures were less than 0.2 in absolute magnitude for all LS-IM measures and the only measure to reach statistical significance (p-value < 0.0008) was HDL large which was negatively correlated with BMI (r=-0.17). Pairwise correlations between SSPG concentration and the individual LS-IM measures reaching statistical significance (p-value < 0.0008) included: negative correlations of SSPG concentration with LDL peak particle size (r=-0.26), IDL small (r=-0.21), HDL large (r=-0.18), and LDL large a (r=-0.17); and positive correlations of SSPG concentration with LDL small (r=0.27), LDL very small a (r=0.23), LDL total (r=0.19), LDL medium (r=0.19) and LDL very small b (r=0.17). Stronger correlations were found between TG/HDL-C and the LS-IM measures: significant correlations (p-value < 0.0008) were found for the following measures: negative correlations of TG/HDL-C with LDL peak particle size (r=-0.72), LDL Large a (r=-0.50), IDL small (r=-0.38), LDL large b (r=-0.37), HDL large (r=-0.33), HDL total (r=-0.18); and positive correlations of TG/HDL-C with LDL very small b (r=0.64), LDL very small a (r=0.62), LDL very small c (r=0.60), LDL Small (r=0.50), VLDL large (r=0.46), LDL very small d (r=0.38), VLDL medium (r=0.35), LDL total (r=0.32), and Non HDL total (r=0.23).

Table 1
Results of stepwise linear regression analysis

Variable	Beta	95% CI
BMI	33.5	28.4 to 38.6
Male (reference=Female)	-12.0	-23.1 to -1
Hispanic (reference=Non-Hispanic)	15.7	-2.2 to 33.7
Race (reference=White)		
Native American	5.3	-39.8 to 50.4
East Asian	21.5	3.9 to 39.2
Black	-6.0	-24.9 to 12.8
South Asian	21.0	6.2 to 35.7
TG/HDL-C	17.1	9.8 to 24.4
VLDL Medium	-8.8	-15.6 to -2.1
IDL Small	-19.0	-30 to -8.1
LDL Large a	11.0	-0.7 to 22.6
LDL Medium	10.2	4.5 to 15.9
LDL Very Small b	14.8	5.1 to 24.6
LDL Very Small c	-16.8	-28.4 to -5.1
LDL Very Small d	8.6	0.4 to 16.8
HDL Small	7.9	2.2 to 13.6

Beta represents the change in SSPG concentration (mg/dL) per each one SD change (continuous variables) or from the reference category (categorical variables).
SD for continuous variables are: BMI=5.4, TG/HDL-C =2.45, VLDL Medium=15.8, IDL Small=50.3, LDL Large a=92.6, LDL Medium=8.1, LDL Very Small b=62.6, LDL Very Small c=34.6, LDL Very Small d=19.5 and HDL Small=3267 with all lipoprotein measures in nmol/L.
R-squared of model=0.43
HDL: High-density lipoprotein
IDL: Intermediate-density lipoprotein
LDL: Low-density lipoprotein
VLDL: Very low-density lipoprotein

The majority of LS-IM measures were associated with a significant change in SSPG concentration as demonstrated by confidence intervals that did not span zero (**Supplemental Table S4**). The largest changes per SD in LS-IM measures were for LDL peak particle size (SSPG concentration decreased by 16.7 mg/dL per 1 SD increase in peak particle size) and LDL Small (SSPG concentration increased by 15.8 mg/dL per 1 SD increase in LDL small particle number). Large effect sizes were also found for the non LS-IM measures of TG/HDL-C and BMI. SSPG concentration increased by 17 mg/dL per 1SD increase in TG/HDL-C. Since BMI was included as a covariate in each of the models for the individual LS-IM measures and TG/HDL-C, there were a total of 20 estimates for the effect size of BMI. Among the 20 models, SSPG concentration increased by an average of 40 mg/dL per 1 SD increase in BMI.

Eight of the LS-IM measures, as well as BMI, TG/HDL-C, sex,

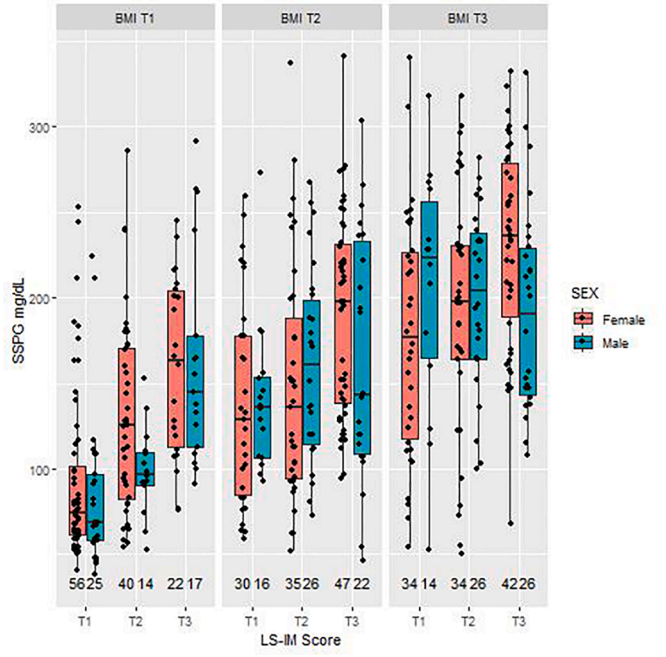

Fig. 1A. Relationship among insulin resistance, BMI, and LS-IM score
SSPG concentration by tertiles (T) of LS-IM Score on x-axis (T1: < -5.7, T2: >= -5.7 to < 8.04, T3: >= 8.04) and BMI in panels (T1: < 27.7 kg/m^2, T2: > 27.7 to <=32.1 kg/m^2, T3: >32.1 kg/m^2).
Number of subjects printed below each box plot.
SSPG concentration is the direct measure of insulin resistance where a higher SSPG concentration indicates greater degree of insulin resistance than a lower SSPG concentration.
LS-IM Score = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small with all LS-IM values in standard deviation units and standard deviations of 15.8, 50.3, 92.6, 8.1, 62.6, 34.6, 19.5 and 3267 nmol/L, respectively.
SSPG: Steady-state plasma glucose
LS-IM: Ion mobility based subfractionation
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
IDL: Intermediate-density lipoprotein
VLDL: Very low-density lipoprotein

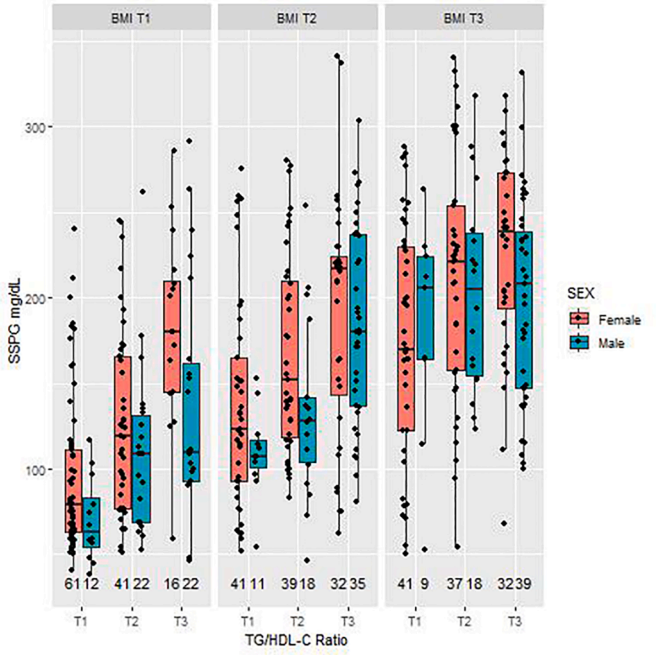

Fig. 1B. Relationship among insulin resistance, BMI, and TG/HDL-C
SSPG concentration by tertiles (T) of LS-IM Score on x-axis (T1: < -5.7, T2: >= -5.7 to < 8.04, T3: >= 8.04) and BMI in panels (T1: < 27.7 kg/m^2, T2: > 27.7 to <=32.1 kg/m^2, T3: >32.1 kg/m^2).
Number of subjects printed below each box plot.
SSPG concentration is the direct measure of insulin resistance where a higher SSPG concentration indicates greater degree of insulin resistance than a lower SSPG concentration.
SSPG: Steady-state plasma glucose
TG/HDL-C: Ratio of triglycerides to high density lipoprotein cholesterol concentration

ethnicity, and race were the final variables remaining in a backwards stepwise regression analysis with SSPG concentration as a dependent variable (Table 1). Tertiles of the LS-IM score showed a strong positive relationship across all tertiles of BMI in women and in the lower two tertiles of BMI in men (Figure 1A) and the relationship appears similar when tertiles of TG/HDL-C or tertiles of the score representing the combination of LS-IM + TG/HDL-C are plotted (**Figures 1B and 1C**).

Receiver operating characteristic (ROC) curves for predicting IR (SSPG concentration in the top tertile) were plotted for the LS-IM score, TG/HDL-C, and LS-IM score + TG/HDL-C (Figure 2A); BMI, BMI+TG/HDL-C and LS-IM score+BMI+TG/HDL-C (Figure 2B); BMI+TG/HDL-C+Sex+Race+Ethnicity and LS-IM score+BMI+TG/HDL-C+Sex+Race+Ethnicity (Figure 2C); . The combination of LS-IM score + TG/HDL-C improved prediction of IR compared with TG/HDL-C alone (AUC=0.73 vs 0.70 respectively; p-value < 0.0001) (Table 2). Similarly LS-IM score + BMI + TG/HDL-C improved prediction compared with BMI+TG/HDL-C alone (AUC= 0.82 vs 0.79 respectively; p-value< 0.0001) and LS-IM score also resulted in improved prediction when added to BMI + TG/HDL-C + Sex + Race + Ethnicity (AUC=0.84 vs 0.81 respectively; p-value < 0.0001). In the subset of non-obese individuals, the additions of LS-IM score to TG/HDL-C, BMI+TG/HDL-C, and BMI+TG/HDL-C+Sex+Race+Ethnicity also resulted in improved prediction (p-value < 0.0001) (Table 2).

The positive predictive values (PPV) were calculated for identifying subjects in the top tertile of SSPG concentration when considering the highest five percent of values for each of the same groups of variables. The PPVs ranged from 59% when considering TG/HDL-C or the IM score alone to 89% when considering the full model of LS-IM score + BMI + TG/HDL-C + Sex + Race + Ethnicity (Table 2).

4. Conclusions

This study of LS and IR has three main findings. First, we found that multiple LS are associated with IR when using both a direct measure of LS (LS-IM) and a direct measure of IR (SSPG concentration). Second, we found that prediction of IR by LS-IM score was similar to that by TG/HDL-C. Third, we found that LS-IM score contributes information that is independent of TG/HDL-C, BMI, sex, race and ethnicity as evidenced by improvement in prediction of IR when LS-IM is used in combination with these factors. Specifically, when predicting individuals in the top tertile of SSPG concentration, the AUC and PPV for the LS-IM score alone and TG/HDL-C alone were similar, but when used together they significantly improved the AUC and PPV (Table 2). Furthermore, the AUC and PPV improved when LS-IM was added to the combination of TG/HDL-C and BMI and also when it was added to the combination of sex, race, ethnicity, BMI and TG/HDL-C.

Our results also demonstrate the potential utility of the LS-IM score, TG/HDL-C or their combination to identify individuals who are most insulin resistant and have greater degrees of dyslipidemia in either obese or non-obese groups. As shown in **Figure 1A, 1B and 1C**, within each tertile of BMI, individuals with higher scores (LS-IM, TG/HDL-C and LS-IM+TG/HDL-C) were generally more insulin resistant (higher SSPG concentration) than those with the lower LS-IM scores. As shown in

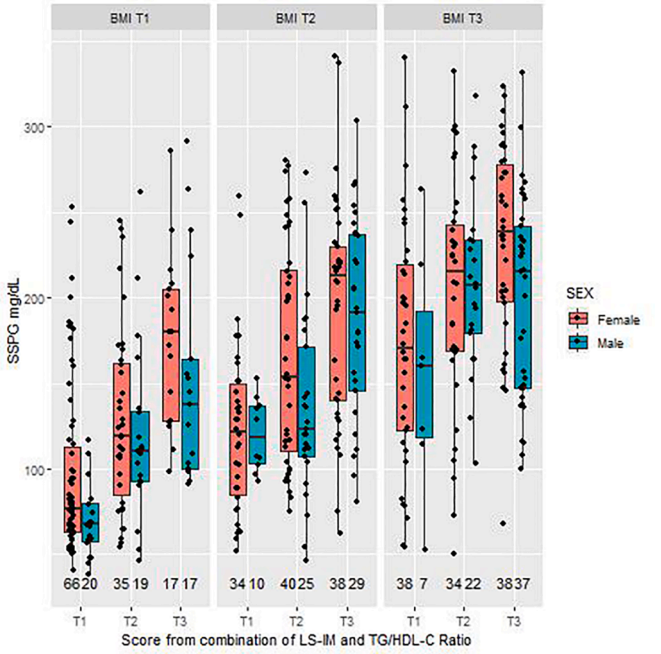

Fig. 1C. Relationship among insulin resistance, BMI, and LS-IM score+TG/HDL-C

SSPG concentration by tertiles (T) of LS-IM Score on x-axis (T1: < -5.7, T2: >= -5.7 to < 8.04, T3: >= 8.04) and BMI in panels (T1: < 27.7 kg/m^2, T2: > 27.7 to <=32.1 kg/m^2, T3: >32.1 kg/m^2).

Number of subjects printed below each box plot.

SSPG concentration is the direct measure of insulin resistance where a higher SSPG concentration indicates greater degree of insulin resistance than a lower SSPG concentration.

LS-IM Score + TG/HDL-C = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small + 17.1*TG/HDL-C with all LS-IM values in standard deviation units and standard deviations of 15.8, 50.3, 92.6, 8.1, 62.6, 34.6, 19.5, 3267 nmol/L, respectively and TG/HDL-C in standard deviation units with standard deviation of 17.1.

SSPG: Steady-state plasma glucose

LS-IM: Ion mobility based subfractionation

HDL: High-density lipoprotein

LDL: Low-density lipoprotein

IDL: Intermediate-density lipoprotein

VLDL: Very low-density lipoprotein

TG/HDL-C: Ratio of triglycerides to high density lipoprotein cholesterol concentration

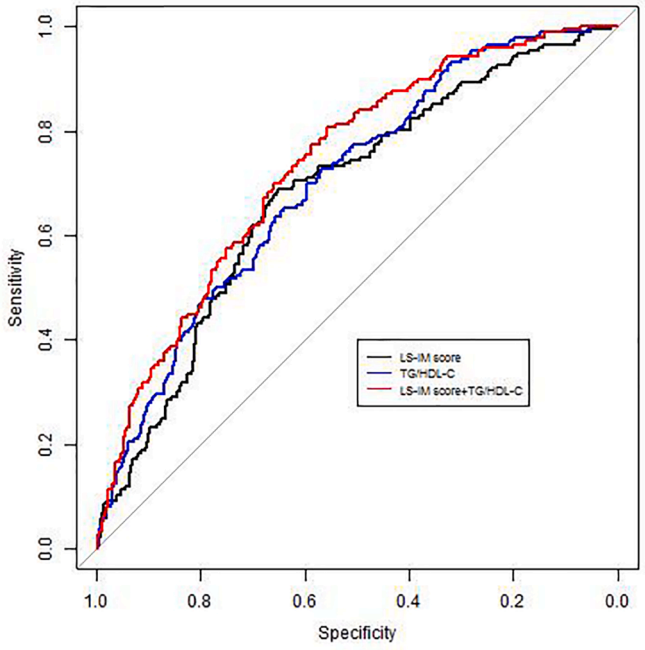

Fig. 2A. Receiver operating characteristic curves to predict SSPG concentration in top tertile (>196 mg/dL): TG/HDL-C, LS-IM Score and TG/HDL-C+LS-IM Score

LS-IM Score = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small

TG/HDL-C +LS-IM Score = 17.1*TG/HDL-C + LS-IM Score

SSPG: Steady-state plasma glucose

LS-IM: Ion mobility based lipoprotein subfractionation

HDL: High-density lipoprotein

LDL: Low-density lipoprotein

IDL: Intermediate-density lipoprotein

VLDL: Very low-density lipoprotein

TG/HDL-C: Ratio of triglycerides to high density lipoprotein cholesterol concentration

Table 2, the AUCs for predicting IR among non-obese individuals were not diminished for LS-IM score, TG/HDL-C or their combination. These observations are consistent with our previous finding that, at a given BMI, insulin resistant individuals have higher TG and lower HDL-C concentrations than insulin sensitive persons [5].

Dyslipidemia of IR is characterized by elevated TG and low HDL-C concentrations as well as by a preponderance of small dense LDL [7], postprandial lipemia [30], and increased concentration of partially oxidized LDL [31]. Several lipid and LS abnormalities measured by NMR are also seen in persons with IR [11]. Consistent with these previously reported findings, we show that several of the LS-IM measures were associated with IR (SSPG concentration). Specifically, a combination of eight LS were combined to form the LS-IM score; and of those, increased VLDL Medium, IDL Small, and LDL Very small c result in lower LS-IM scores and lower risk of IR while increased LDL Large a, LDL Medium, LDL Very small b, LDL Very small d and HDL Small result in higher LS-IM scores and higher risk of IR. Due to correlations among each of these measures, the strength and direction of the regression coefficients can be difficult to interpret such as that for LDL Very small c which is in the opposite direction of the other very small LDL regions. Nevertheless, the improvement in prediction of IR when LS-IM score is added to TG/HDL-C or to TG/HDL-C+BMI demonstrates the predictive value of the LS-IM score as a whole. From a pathophysiological perspective, these associations are thought to arise in part from increased hepatic production and reduced clearance of VLDL from plasma as well as from increased hepatic lipase activity and subsequent hydrolysis of phospholipids in LDL and HDL particles leading to smaller and denser LDL particles and a decrease in large HDL particles and an increase in small HDL particles [8].

It is difficult to compare the LS-IM score described here with the previously described LP-IR score derived from NMR [14]. The LP-IR score was based on HOMA-IR as a measure of IR while the current score was based on SSPG concentration, a direct measure of IR. In addition, the size ranges of the defined regions vary between the two scores. However, both scores demonstrate particles from a wide span of size ranges that independently contribute to the association with IR.

Identification of IR in individuals is of clinical importance because it could prompt changes in behavior and clinical management to reduce risk associated with IR. We have previously shown that TG/HDL-C can be used to identify insulin resistant individuals [9,10]. TG/HDL-C ratio and BMI are simple measures that can be used to identify individuals with increased cardiometabolic risk and would be preferable in clinical or research settings where the LS-IM measurements cannot be performed. The LS-IM score could potentially be used alone or in combination with TG/HDL-C and/or BMI among patients who are already

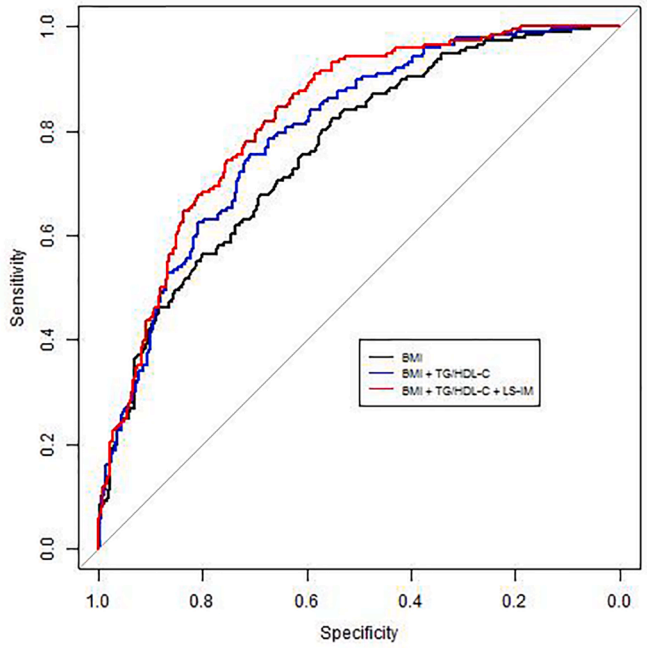

Fig. 2B. Receiver operating characteristic curves to predict SSPG concentration in top tertile (>196 mg/dL): BMI, BMI+TG/HDL-C and BMI+TG/HDL-C+LS-IM Score
LS-IM Score = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small
BMI+TG/HDL-C = 33.5*BMI + 17.1*TG/HDL-C
BMI+TG/HDL-C = 33.5*BMI + 17.1*TG/HDL-C + LS-IM Score
SSPG: Steady-state plasma glucose
LS-IM: Ion mobility based lipoprotein subfractionation
HDL: High-density lipoprotein
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IDL: Intermediate-density lipoprotein
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TG/HDL-C: Ratio of triglycerides to high density lipoprotein cholesterol concentration

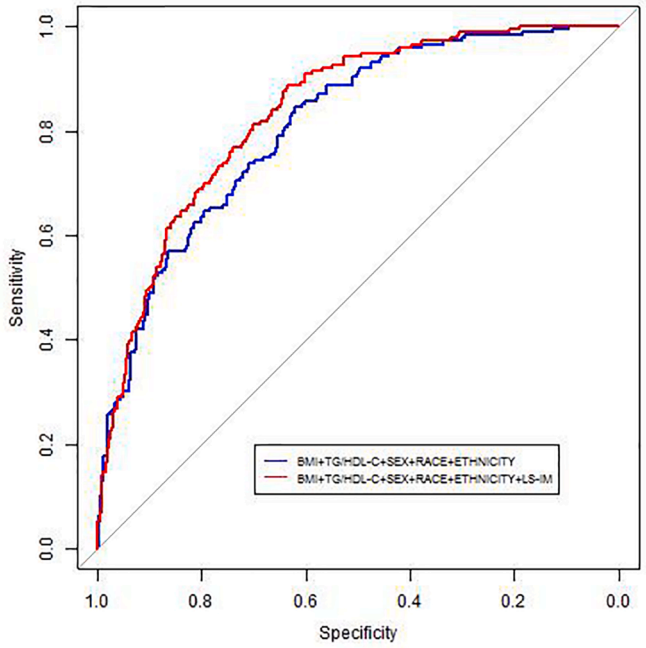

Fig. 2C. Receiver operating characteristic curves to predict SSPG concentration in top tertile (>196 mg/dL): BMI+TG/HDL-C+Sex+Race+Ethnicity and BMI+TG/HDL-C+Sex+Race+Ethnicity+LS-IM Score
LS-IM Score = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small
LS-IM Score + TG/HDL-C = IM Score + 17.1*TG/HDL-C
BMI+TG/HDL+Sex+Race+Ethnicity = 33.5*BMI + 17.1*TG/HDL-C – 12*Male + 15.7*Hispanic + 5.3*Native American + 21.5*East Asian -6*Black + 21*South Asian
BMI+TG/HDL+Sex+Race+Ethnicity+LS-IM Score = 33.5*BMI + 17.1*TG/HDL-C – 12*Male + 15.7*Hispanic + 5.3*Native American + 21.5*East Asian -6*Black + 21*South Asian + LS-IM Score
SSPG: Steady-state plasma glucose
LS-IM: Ion mobility based lipoprotein subfractionation
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
IDL: Intermediate-density lipoprotein
VLDL: Very low-density lipoprotein
TG/HDL-C: Ratio of triglycerides to high density lipoprotein cholesterol concentration

undergoing LS-IM testing. These insights can be used to provide additional information to alert the patient and clinician to the probable existence of significant IR at no additional cost.

Strengths of our study include the fact that we validated the usefulness of the LS-IM score for prediction of IR using a gold-standard measure of IR. In addition, we improved risk prediction using already available data from LS-IM clinical testing where those measurements are available (no additional cost). Limitations of our study include that the individuals in our study are not typical of the population undergoing LS-IM testing. The individuals studied were apparently healthy volunteers while those undergoing LS-IM testing are predominantly referred for testing by their clinicians for evaluation of risk of ASCVD. Future studies will be needed to assess the prediction of IR by the LS-IM score in the population of patients undergoing LS-IM testing.

In conclusion, LS-IM measurements, in addition to TG/HDL-C and/or the combination of TG/HDL-C and BMI, improve prediction of IR. Among individuals who have undergone LS-IM testing, this information could be used to prioritize lifestyle interventions to improve IR and the associated risk of T2D and ASCVD. Targeted interventions including increased exercise and weight loss have been shown to be particularly helpful in improving IR and decreasing the progression of individuals to T2D. This information can also be used to identify individuals who may be candidates for further testing by other validated measures such as fasting insulin or the IR score [32] and ultimately identify individuals who otherwise may be unaware of their IR and corresponding higher risk of T2D and ASCVD.

Author contributions

CR wrote the manuscript and performed the statistical analysis; FA wrote sections of, reviewed and edited the manuscript. DS, JWK and MM reviewed and edited the manuscript. CR is the guarantor of this work and, as such, had full access to all of 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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CR, DS, MM are employees of and own stock in Quest Diagnostics. JWK and FA have no relevant conflicts of interest.

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Table 2
Area under ROC curve (AUC) and positive predictive values (PPV) for predicting individuals in the top tertile of SSPG concentration (>196 mg/dL)

Strata	Model	AUC (95% CI)	P-value*	PPV† (95% CI)	R-squared‡
All	LS-IM score	0.68 (0.63 to 0.73)		0.59 (0.41 to 0.75)	0.12
	TG/HDL-C	0.70 (0.65 to 0.75)		0.59 (0.41 to 0.75)	0.10
	LS-IM score+TG/HDL-C	0.73 (0.69 to 0.78)	<0.0001[a]	0.70 (0.52 to 0.84)	0.21
	BMI	0.76 (0.72 to 0.80)		0.74 (0.55 to 0.87)	0.29
	BMI+TG/HDL-C	0.79 (0.76 to 0.83)		0.81 (0.63 to 91.8)	0.35
	LS-IM score+ BMI+TG/HDL-C	0.82 (0.79 to 0.86)	<0.0001[a]	0.81 (0.63 to 91.8)	0.41
	BMI+TG/HDL-C+Sex+Race+Ethnicity	0.81 (0.77 to 0.85)		0.85 (0.68 to 0.94)	0.38
	LS-IM score+BMI+TG/HDL-C+Sex+Race+Ethnicity	0.84 (0.80 to 0.87)	<0.0001[a]	0.89 (0.72 to 0.96)	0.43
BMI<30	LS-IM score	0.77 (0.70 to 0.84)		NA[b]	0.18
	TG/HDL-C	0.72 (0.65 to 0.79)		NA[b]	0.07
	LS-IM score+TG/HDL-C	0.79 (0.72 to 0.85)	<0.0001[a]	NA[b]	0.24
	BMI	0.71 (0.64 to 0.79)		NA[b]	0.17
	BMI+TG/HDL-C	0.78 (0.71 to 0.85)		NA[b]	0.21
	LS-IM score+ BMI+TG/HDL-C	0.82 (0.76 to 0.88)	<0.0001[a]	NA[b]	0.32
	BMI+TG/HDL-C+Sex+Race+Ethnicity	0.78 (0.72 to 0.85)		NA[b]	0.24
	LS-IM score+BMI+TG/HDL-C+Sex+Race+Ethnicity	0.83 (0.77 to 0.89)	<0.0001[a]	NA[b]	0.35

* P-value comparing AUC of current row with row immediately above. a) indicates p-value is significant after adjusting for 6 statistical tests in table (< 0.008).

† PPV when considering highest 5% of values as a positive test; b) sample size of N=13 among top 5% of non-obese subjects too small to obtain reliable PPV

‡ R-squared of model with continuous SSPG concentration (mg/dL)

LS-IM Score = -8.8*VLDL Medium – 19*IDL Small + 11*LDL Large a + 10.2*LDL Medium + 14.8*LDL very small b – 16.8*LDL very small c + 8.6*LDL very small d + 7.9*HDL small

LS-IM Score + TG/HDL-C = LS-IM Score + 17.1*TG/HDL-C

BMI + TG/HDL-C = 33.5*BMI + 17.1*TG/HDL-C

BMI + TG/HDL-C + Sex + Race + Ethnicity = 33.5*BMI + 17.1*TG/HDL-C – 12*Male + 15.7*Hispanic + 5.3*Native American + 21.5*East Asian -6*Black + 21*South Asian

HDL: High-density lipoprotein

IDL: Intermediate-density lipoprotein

LDL: Low-density lipoprotein

LS-IM: Ion mobility based lipoprotein subfractionation

SSPG: Steady-state plasma glucose

TG/HDL-C: Triglyceride to high-density lipoprotein cholesterol ratio

VLDL: Very low-density lipoprotein

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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