Lipoprotein heterogeneity may help to detect individuals with insulin resistance

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Abstract

Aims/hypothesis The triacylglycerol (TG)-to-HDL-cholesterol ratio has been shown to detect insulin resistance. However, the added predictive value of a more comprehensive assessment of lipoprotein composition is unknown.

Methods We analysed cross-sectional data from 882 non-diabetic participants in the Insulin Resistance Atherosclerosis Study (IRAS). Lipoproteins were measured by nuclear magnetic resonance (NMR) spectroscopy. Determined by the frequently sampled intravenous glucose tolerance test, insulin resistance was defined as the lowest sex-specific quartile of insulin sensitivity.

Results The AUC of the receiver operating characteristic curve of HDL-cholesterol and TG levels for detecting insulin resistance was similar to that of the TG-to-HDL-cholesterol ratio (0.676 vs 0.673; $p=0.685$), but smaller than the AUC of NMR-detected lipoproteins (0.676 vs 0.745; $p<0.001$). NMR lipoproteins added discriminative value to HDL-cholesterol and TG levels (net reclassification improvement of 40.0%; $p<0.001$; and integrated discrimination improvement of 9.5%; $p<0.001$), with net benefit within predicted probabilities of between 10% and 50% by Vickers' decision-curve analysis. We also demonstrated additive value to demographic variables, BMI and levels of fasting glucose, TG, and HDL-cholesterol (net reclassification improvement of 14.0%; $p<0.001$; and integrated discrimination improvement of 4.5%; $p<0.001$).

Conclusions/interpretation NMR lipoproteins, which can be measured in the fasting state, add information to the TG and HDL-cholesterol ratio across a broad range on insulin resistance. Depending on the other risk factors of insulin resistance that are incorporated, NMR lipoproteins permit the correct reclassification of an additional 14–40% of individuals.

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Abbreviations

ApoA-I/B	Apolipoprotein A-I/B
CV	Coefficient of variation
FSIGTT	Frequently sampled intravenous glucose tolerance test
IDI	Integrated discrimination improvement
IRAS	Insulin Resistance Atherosclerosis Study
MINMOD	Mathematical modelling methods
NMR	Nuclear magnetic resonance
NRI	Net reclassification improvement

S_I Sensitivity index
TG   Triacylglycerol

Introduction

Dyslipidaemia is an important component of insulin resistance [1], with high triacylglycerol (TG) and low HDL-cholesterol levels as the most characteristic changes unveiled by conventional laboratory methods [1, 2]. HDL-cholesterol and/or TG levels have been shown to predict cardiovascular disease [3, 4] and type 2 diabetes [5] and have been used to estimate insulin resistance [6, 7]. However, the TG-to-HDL-cholesterol ratio has been shown to be a better marker than either component of the ratio (TG or HDL-cholesterol) alone for detecting insulin-resistant individuals and predicting future cardiovascular disease events [8–10]. Remnant cholesterol, another cholesterol fraction that can be estimated from the conventional lipid panel, may be of interest. Remnant cholesterol, which reflects the cholesterol content of TG-rich lipoproteins, has been associated with both chronic inflammation and cardiovascular disease [11].

Total- and LDL-cholesterol concentrations tend to be unaffected in insulin-resistant individuals. Therefore, conventional laboratory methods tend to miss many of the insulin resistance-related changes in LDL-cholesterol particles [12, 13]. These and other lipoprotein changes can be unveiled by a variety of methods, including gradient-gel electrophoresis, density gradient ultracentrifugation and nuclear magnetic resonance (NMR) spectroscopy [12, 14, 15]. With NMR spectroscopy, the following lipoprotein changes have been associated with insulin resistance: (1) larger VLDL particle size with greater concentration of large VLDL particles; (2) smaller LDL particle size with greater concentration of total- and small LDL particles and lower concentration of large LDL particles; and (3) smaller HDL particle size with lower concentration of large HDL particles and a modest increase of small HDL particles [12, 16].

We hypothesised that lipoprotein composition as determined by NMR spectroscopy adds predictive value to conventional lipoproteins and apolipoproteins for the detection of insulin-resistant individuals. We tested this hypothesis using data from a large and ethnically diverse cohort of individuals, the Insulin Resistance Atherosclerosis Study (IRAS) [17]. In the IRAS, insulin sensitivity was measured by the frequently sampled intravenous glucose tolerance test (FSIGTT) [17]. We used the AUC of the receiver operating characteristic curve to evaluate the predictive discrimination of lipoproteins and apolipoproteins [18]. Calibration and reclassification tests and decision-curve analysis were used to overcome the limitations of the AUC, which include the lack of sensitivity to model improvement and absence of information on either absolute predicted risk or risk reclassification [19–24].

Methods

Study participants The design and methods of the IRAS have previously been described in detail [17]. Briefly, the study was conducted at four clinical centres in the USA. At centres in Oakland and Los Angeles, California, non-Hispanic whites and African-Americans were recruited from Kaiser Permanente, a nonprofit health maintenance organisation. Centres in San Antonio, Texas and San Luis Valley, Colorado recruited non-Hispanic whites and Hispanics from two ongoing population-based studies (the San Antonio Heart Study and the San Luis Valley Diabetes Study). A total of 1,625 individuals aged 40–69 years were enrolled in the IRAS (56% women), which occurred between October 1992 and April 1994. The IRAS protocol was approved by local institutional review committees and all participants provided written informed consent.

Among the 1,065 non-diabetic participants, 183 were excluded because of excessive alcohol intake (≥28 and ≥14 g/day in men and women, respectively) or treatment with lipid-lowering agents. Therefore, the present report analysed data on 882 participants (349 non-Hispanic whites, 218 African-Americans and 315 Hispanics). Information on demographics, glucose tolerance status, conventional lipoproteins, NMR lipoproteins and insulin sensitivity was available in all 882 individuals. Apolipoproteins A-I (apoA-I) and B (apoB) were measured in 870 and 879 participants, respectively.

Acquisition of data and definition of variables Age, sex, race/ethnicity, family history of diabetes and treatment with glucose- and lipid-lowering medications were obtained by self-report. Anthropometric measurements were obtained using standardised protocols. The IRAS protocol required two visits, 1 week apart, of approximately 4 h each. Participants were asked prior to each visit to fast for 12 h, to abstain from heavy exercise and alcohol for 24 h and to refrain from smoking on the morning of the examination. During the first visit, a 75 g OGTT was administered to assess glucose tolerance status. During the second baseline visit, insulin sensitivity and insulin secretion were measured using the FSIGTT. An injection of regular insulin was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance. Glucose in the form of 50% [wt/vol.] solution (0.3 g/kg) and regular human insulin (0.03 U/kg) were injected through an intravenous line at 0 and 20 min, respectively. Blood was collected at −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100 and 180 min for measurement of plasma glucose and insulin. Insulin sensitivity, expressed as the insulin sensitivity index (S_I), was calculated using mathematical modelling methods (MINMOD version 3.0 [1994], Los Angeles, CA, USA) [25].

Laboratory analyses of plasma glucose and insulin took place at the University of Southern California (Los Angeles,

CA, USA). Plasma insulin concentration was measured by the dextran-charcoal radioimmunoassay (coefficient of variation [CV] of 19%) [26]. Plasma lipids and lipoproteins were obtained from fasting single fresh plasma samples using Lipid Research Clinic methods and were measured at the central IRAS laboratory at the Medlantic Research Institute, Washington, DC, USA [27]. We estimated non-HDL-cholesterol as the difference between total and HDL-cholesterol, and remnant cholesterol as the difference between non-HDL-cholesterol and LDL-cholesterol.

Plasma total apoA-I and apoB concentrations were assayed by immunoprecipitation (SPQ kit from Incstar, Stillwater, MN, USA) and ELISA techniques at MedStar Laboratory (Washington, DC, USA) (CV of 4.1%) [28]. LDL size was determined by gradient-gel electrophoresis (CV of 2%) [29]. Lipoprotein subclass particle concentrations and average VLDL, LDL and HDL particle diameters were measured by NMR spectroscopy (LipoScience Inc, Raleigh, NC, USA) [15, 16, 30]. Particle concentrations were given by the measured amplitudes of the characteristic lipid methyl group NMR signals they emit. Nine subclasses were investigated: large VLDL (including chylomicrons if present; >60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large LDL (21.2–23 nm), 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). VLDL and LDL subclass particle concentrations are given in units of nanomoles per litre and HDL in micromoles per litre. The sum of each particle subclasses provides total VLDL, LDL and HDL particle concentrations. Weighted-average VLDL, LDL and HDL particle sizes (in nanometers) were calculated as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. NMR spectroscopy and conventional laboratory methods have a high degree of agreement in quantifying lipoprotein subclass concentrations.

Diabetes was defined as fasting plasma glucose ≥7.0 mmol/l, 2 h plasma glucose ≥11.1 mmol/l and/or treatment with glucose-lowering medications [31]. Insulin resistance was defined as the lowest sex-specific S_I quartile [32].

Statistical analyses Statistical analyses were performed using SAS statistical software (version 9.2, SAS Institute, Cary, NC, USA) and R Project statistical software packages (version 2.9.2, the R Foundation for Statistical Computing, Vienna, Austria). The relation of lipoproteins and apolipoproteins to S_I was examined by Pearson's correlation coefficients (r). The proportion of the variance (R^2) of S_I explained by lipoproteins and apolipoproteins was determined by linear regression analysis. The predictive discrimination of lipoproteins and apolipoproteins for the detection of insulin-resistant individuals was assessed by the AUC of the receiver operating characteristic curve [18]. AUCs were compared by the De Long

method [33]. The Youden's J statistic was used to determine the cut-off point with the best performance for identifying persons with insulin resistance. Model calibration was evaluated by the Hosmer-Lemeshow χ^2 statistic. Models that had a χ^2 value >20 ($p<0.01$) were considered not well calibrated [34]. The incremental value for risk prediction was determined by reclassification metrics (net reclassification improvement [NRI], category-free NRI and integrated discrimination improvement [IDI]) with the %add_predictive macro (http://analytics.ncsu.edu/sesug/2010/SDA07.Kennedy.pdf, accessed 28 May 2015) and %ROCPLUS macro (www.mayo.edu/research/departments-divisions/department-health-sciences-research/division-biomedical-statistics-informatics/software/locally-written-sas-macros, accessed 28 May 2015) [20–23, 35, 36]. We used the 0.12, 0.27 and 0.50 probabilities of having insulin resistance to generate four strata. These cut-off points (low, intermediate and high) matched probabilities of having insulin resistance at BMI of 25, 30 and 35 kg/m^2 (overweight, obesity and morbidly obesity cut-off points), respectively. Since NRI requires predefined clinically meaningful strata, these cut-off points remain arbitrary. To overcome this limitation, we produced Vickers' decision curves to identify both the range of threshold probabilities in which NMR lipoproteins had added value to TG and HDL-cholesterol concentrations for detecting insulin resistance and the magnitude of benefit [24]. This analysis was carried out using a statistical code available in R (https://www.mskcc.org/departments/epidemiology-biostatistics/health-outcomes/decision-curve-analysis-01, accessed 13 August 2015) [24]. Log-transformed TG and TG-to-HDL-cholesterol ratio were used in all analyses to meet the assumptions of the tests. We also used the natural log transformation of (S_I+1) given that some participants had S_I=0. We considered a p value <0.050 (two-sided) to be significant.

Results

All participants were free of diabetes and were not taking lipid-lowering medications. Participant characteristics are shown in Table 1.

Measures of obesity, TG and HDL-cholesterol levels, TG-to-HDL-cholesterol ratio and apoB concentration had relatively strong correlations with S_I (Table 2). Non-HDL-cholesterol and estimated remnant cholesterol in the fasting state also had a significant relationship with S_I, but neither relationship was as strong as that of TG levels. Large VLDL particles, total and small LDL particles, large HDL particles, and VLDL, LDL and HDL particle sizes also had significant relationships with S_I. All these relationships were quite similar in the three ethnic groups. Conventional lipoproteins and apolipoproteins accounted for 14.8% (95% CI 10.5, 19.1) of the variance of S_I, whereas NMR

Table 1 Participant characteristics

Characteristic	
n	882
Age, years	54.4 (8.5)
Female, %	56.5
African-American, %	24.7
Hispanic, %	35.7
BMI, kg/m^2	28.3 (5.7)
Fasting glucose, mmol/l	5.43 (0.57)
S_I, $\times 10^{-5}$ min^{-1} pmol^{-1} ml^{-1}	3.69 (3.41)
Lipoproteins, mmol/l	
Total cholesterol	5.45 (1.10)
LDL-cholesterol	3.63 (0.89)
HDL-cholesterol	1.21 (0.39)
Non-HDL-cholesterol	4.23 (1.14)
Estimated remnant cholesterol	0.61 (0.73)
TG	1.50 (0.97)
TG-to-HDL-cholesterol ratio	3.45 (3.42)
LDL size by gradient-gel electrophoresis, nm	26.10 (0.97)
Apolipoproteins, g/l	
ApoA-I	1.307 (0.283)
ApoB	1.039 (0.246)
ApoB-to-apoA-I ratio	0.83 (0.29)
Lipoprotein heterogeneity (NMR)	
Total VLDL particles, nmol/l	64.3 (30.7)
Large	2.88 (2.96)
Medium	17.7 (13.1)
Small	43.7 (20.0)
Total LDL particles, nmol/l	1,166 (384)
IDL	43.1 (25.7)
Large	498.9 (211.5)
Small	624.5 (459.7)
Total HDL particles, μmol/l	31.5 (5.4)
Large	5.00 (2.77)
Medium	2.74 (3.65)
Small	23.7 (5.2)
Particle size, nm	
VLDL	49.1 (10.6)
LDL	21.34 (0.79)
HDL	8.96 (0.47)

Data are n, percentage or mean (SD)

lipoproteins explained 18.7% (95% CI 14.1, 23.2). The proportion of the S_I variance explained by TG and HDL-cholesterol levels (12.3% [95% CI 8.3, 16.3]) was comparable with that explained by the TG-to-HDL-cholesterol ratio (11.8% [95% CI 7.8, 15.7]).

Table 3 presents the ability of conventionally measured lipoproteins and apolipoproteins to detect insulin resistance as defined by the lowest quartile of S_I. The AUC of HDL-cholesterol and TG levels was greater than that either of TG concentration (0.676 vs 0.641; $p=0.020$) or of HDL-cholesterol concentration (0.651; $p=0.012$), but was similar to the AUC of TG-to-HDL-cholesterol ratio (0.673; $p=0.685$). Non-HDL-cholesterol, estimated remnant cholesterol, and apolipoprotein levels and LDL size by gradient-gel electrophoresis did not significantly increase the AUC of HDL-cholesterol and TG levels (0.691 vs 0.676; $p=0.063$). NMR lipoproteins had a higher AUC than HDL-cholesterol and TG levels (0.745 vs 0.676; $p<0.001$; Table 3). This was demonstrated in men (0.753 vs 0.641; $p<0.001$) and women (0.775 vs 0.722; $p=0.021$) and in Hispanics (0.738 vs 0.632; $p=0.002$) and non-Hispanic whites (0.809 vs 0.697; $p<0.001$). In African-Americans, however, this finding did not reach statistical significance (0.753 vs 0.720; $p=0.350$). In addition, the predictive discrimination of a clinical model of readily available variables (age, sex, ethnicity, clinic, BMI and fasting glucose) was increased by NMR lipoproteins (0.857 vs 0.828; $p<0.001$), but not by HDL-cholesterol and TG levels (0.840 vs 0.828; $p=0.073$).

Non-HDL-cholesterol, estimated remnant cholesterol, and apolipoproteins levels and LDL size by gradient-gel electrophoresis were not used in further testing, because none of them increased the predictive discrimination of TG and HDL-cholesterol levels. Figure 1a shows the incremental value of NMR lipoproteins to TG and HDL-cholesterol (AUC 0.764 vs 0.676; $p<0.001$). Both models were well calibrated (Electronic Supplemental Material [ESM] Table 1). Besides having higher predictive discrimination (greater AUC), NMR lipoproteins added discriminative value (as measured by NRI, category-free NRI and IDI) to HDL-cholesterol plus TG levels. NMR lipoproteins reclassified a net of 40% of individuals more appropriately than HDL-cholesterol and TG levels. NMR lipoproteins added predictive discrimination to TG and HDL-cholesterol in obese and non-obese individuals and in those with normal and impaired glucose tolerance (ESM Table 2). Figure 1b shows the incremental value of NMR lipoproteins within the context of a clinic model that included age, sex, ethnicity, clinic, BMI, fasting glucose, TG and HDL-cholesterol (AUC 0.840 vs 0.860; $p=0.002$). NMR lipoproteins reclassified a net of 14% of individuals more appropriately than the clinical model (Table 4).

Using the Youden's J statistic to determine the cut-off point with the best performance for identifying persons with insulin resistance, NMR lipoproteins increased the performance of TG and HDL-cholesterol to a sensitivity of 67.7% and a specificity of 72.9%. The positive and negative likelihood ratios were 2.50 and 0.44, respectively. For the same level of specificity, the combination of TG and HDL-cholesterol had a sensitivity of 51.2%, and TG-to-HDL-cholesterol ratio of 46.1%.

Figure 2a presents Vickers' decision curves displaying the net benefit achieved by detecting insulin resistance (lowest S_I quartile) based on predictions by TG and HDL-cholesterol

Table 2 Pearson's correlation coefficients relating measures of obesity, apo, and conventional and NMR lipoproteins to log S_I

Characteristic	All	Non-Hispanic whites	African-Americans	Hispanics
n	882	349	218	315
Measures of obesity				
BMI	−0.50***	−0.51***	−0.45***	−0.51***
Waist circumference	−0.55***	−0.56***	−0.49***	−0.58***
Lipoproteins				
Total cholesterol	−0.05	0.05	−0.12	−0.10
LDL-cholesterol	−0.07	0.02	−0.16*	−0.09
HDL-cholesterol	0.29***	0.33***	0.25***	0.29***
Non-HDL-cholesterol	−0.15***	−0.09	−0.22**	−0.17**
Estimated remnant cholesterol	−0.15***	−0.20***	−0.20**	−0.13*
Log TG	−0.30***	−0.30***	−0.30***	−0.32***
Log TG-to-HDL-cholesterol ratio	−0.34***	−0.37***	−0.34***	−0.35***
LDL size by gradient-gel electrophoresis	0.13***	0.14**	0.11	0.14*
Apolipoproteins				
ApoA-I	0.10**	0.15**	0.07	0.07
ApoB	−0.24***	−0.24***	−0.34***	−0.15**
ApoB-to-apoA-I ratio	−0.22***	−0.28***	−0.29***	−0.12*
Lipoprotein heterogeneity (NMR)				
Total VLDL particles	−0.08*	−0.03	−0.21**	−0.11
Large	−0.20***	−0.20***	−0.24***	−0.24***
Medium	−0.01	0.01	−0.13	−0.03
Small	−0.09**	−0.02	−0.20**	−0.11
Total LDL particles	−0.28***	−0.27***	−0.33***	−0.27***
IDL	−0.11***	−0.02	−0.29***	−0.08
Large	0.12***	0.11*	0.12	0.12*
Small	−0.28***	−0.27***	−0.33***	−0.27***
Total HDL particles	0.05	0.00	0.00	0.11
Large	0.24***	0.28***	0.25***	0.24***
Medium	−0.03	0.00	−0.01	−0.07
Small	−0.06	−0.17**	−0.14*	0.05
Particle size				
VLDL	−0.24***	−0.28***	−0.18**	−0.28***
LDL	0.27***	0.26***	0.31***	0.28***
HDL	0.24***	0.34***	0.29***	0.13*

*$p<0.05$; **$p<0.01$; ***$p<0.001$

levels with and without the addition of NMR lipoproteins. Likelihood ratio test for these two nested models was statistically significant (likelihood ratio χ^2 8.5; $p<0.001$). NMR lipoproteins had an added net benefit to TG and HDL-cholesterol levels if a person's predicted probability of having insulin resistance fell between 10% and 50%. As a point of reference, insulin-resistance probabilities of 0.12, 0.27 and 0.50 matched probabilities of having insulin resistance at BMI of 25, 30 and 35 kg/m^2, respectively in IRAS participants without diabetes. In a model that included, sex, ethnicity, clinic, BMI, and levels of fasting glucose, TG and HDL-cholesterol, NMR lipoproteins added a net benefit

(likelihood ratio χ^2 4.2; $p<0.001$) if a person's predicted probability of having insulin resistance fell between 30% and 70% (Fig. 2b).

Discussion

In IRAS participants without diabetes, the ability of the TG-to-HDL-cholesterol ratio to identify insulin-resistant individuals is similar to that of TG and HDL-cholesterol levels. Apolipoprotein concentrations (both apoA1 and apoB) and estimated remnant cholesterol in the fasting state do not

Table 3 Predictive discrimination of lipoproteins and apolipoproteins for detecting insulin resistance[a]

Characteristic	AUC	p value
Conventional lipoproteins, apo and LDL particle size		
Log TG and HDL-cholesterol	0.676	Referent
Log TG	0.641	0.020
HDL-cholesterol	0.651	0.012
Non-HDL-cholesterol	0.549	<0.001
Estimated remnant cholesterol	0.604	<0.001
Log TG-to-HDL-cholesterol ratio	0.673	0.685
ApoA-I and apoB	0.626	0.010
LDL particle size by gradient-gel electrophoresis	0.572	<0.001
Log TG, HDL-cholesterol, estimated remnant cholesterol, apoA-1, apoB and LDL size by gradient-gel electrophoresis	0.691	0.063
TG and HDL-cholesterol vs NMR lipoproteins		
NMR lipoproteins	0.745	Referent
Log TG and HDL-cholesterol	0.676	<0.001
Log TG, HDL-cholesterol and NMR lipoproteins	0.764	0.011
TG, HDL-cholesterol and NMR lipoproteins vs a clinical model		
Clinical model[b]	0.828	Referent
+Log TG and HDL-cholesterol	0.840	0.073
+NMR lipoproteins	0.857[†]	<0.001
+Log TG, HDL-cholesterol and NMR lipoproteins	0.860[‡]	<0.001

[a] Insulin resistance defined as the lowest S_I quartile

[b] Clinical model included age, sex, ethnicity, clinic, BMI and fasting glucose as independent variables

[†] $p=0.025$ for test of comparison with the clinical model that included log TG and HDL-cholesterol; [‡] $p=0.002$ for test of comparison with the clinical model that included log TG and HDL-cholesterol

add discriminative value to TG and HDL-cholesterol levels. However, NMR lipoproteins have greater predictive discrimination than TG and HDL-cholesterol levels or the TG-to-HDL-cholesterol ratio.

TG concentration has long been used to identify individuals with insulin resistance [6, 7]. The McAuley Index, which is computed using TG and fasting insulin levels, may have a predictive discrimination that is comparable with more sophisticated indices based on the OGTT [37]. Since insulin concentration is not readily available in most clinical settings and many epidemiological studies, other indices derived from TG and/or

HDL-cholesterol levels have been proposed to identify insulin-resistant individuals [7, 8]. In the current report, the combination of TG and HDL-cholesterol levels correctly classified two-thirds of apparently healthy individuals according to their insulin resistance status (Table 3). This ability to detect insulin resistance is similar to that of the TG-to-HDL-cholesterol ratio. Consequently, in line with previously reports [8, 10], our data suggest that the TG-to-HDL-cholesterol ratio is a simple surrogate index of insulin resistance.

Insulin resistance is associated with apolipoproteins and LDL-cholesterol particles [2, 12, 13]. While this is also found

Fig. 1 Receiver operating characteristic curves for predictive discrimination of HDL-cholesterol plus TG levels (continuous line) or NMR lipoproteins (dotted line) (**a**) in the absence and (**b**) within the context of a clinical model that included age, sex, ethnicity, clinic, BMI and fasting glucose
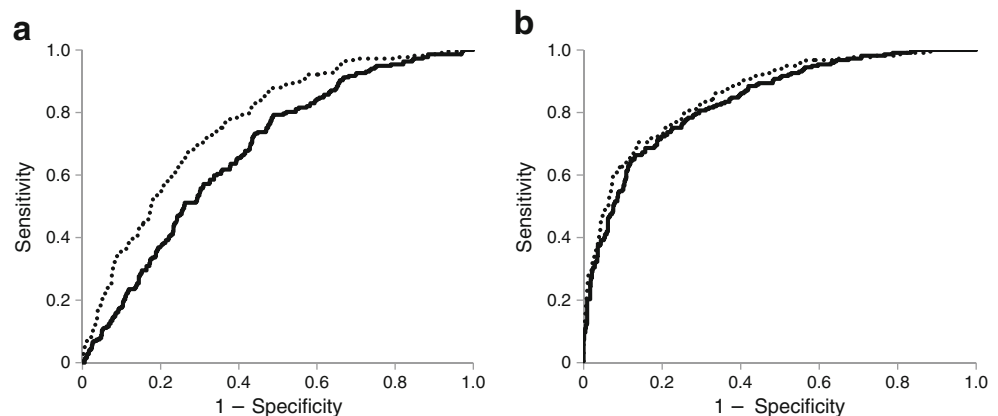
Table 4 Added predictive value
of NMR lipoproteins to a clinical
model that included TG and
HDL-cholesterol for detecting
insulin resistance

	Clinical model	Clinical model+NMR lipoproteins
AUC (95% CI)	0.840 (0.810, 0.870)	0.860 (0.832, 0.888); $p=0.002$
Hosmer–Lemeshow χ^2 statistic	3.0; $p=0.934$	7.7; $p=0.456$
NRI, %		
Overall	Referent	14.0% (95% CI 6.2, 21.9); $p<0.001$
For insulin-resistant persons	Referent	9%; $p=0.013$
For non-insulin-resistant persons	Referent	5%; $p=0.008$
Category-free NRI, %		
Overall	Referent	46.1% (95% CI 31.1, 61.1); $p<0.001$
For insulin-resistant persons	Referent	19%; $p=0.005$
For non-insulin-resistant persons	Referent	27%; $p<0.001$
IDI		
Overall	Referent	0.045 (95% CI 0.029, 0.61); $p<0.001$
For insulin-resistant persons	Referent	0.034
For non-insulin-resistant persons	Referent	−0.011
Integrated sensitivity	0.480	0.514; $p<0.001$
Integrated 1 − specificity	0.170	0.159; $p<0.001$

Clinical model included age, sex, ethnicity, clinic, BMI, fasting glucose, log TG and HDL-cholesterol as independent variables

NRI, NRI for persons with events+NRI for persons without events; Category-free NRI, category-free NRI for persons with events+category-free NRI for persons without events

in the current report, neither apoA-I or apoB levels nor LDL size by gradient-gel electrophoresis increases the predictive discrimination of TG and HDL-cholesterol levels. However, a more comprehensive evaluation of lipoprotein composition (lipoprotein size and particle and subclass concentrations) is useful for detecting insulin-resistant individuals. NMR lipoproteins have greater predictive discrimination than the combination of TG and HDL-cholesterol levels (correct classification of three-quarters vs two-thirds of apparently healthy individuals; $p<0.001$). NMR lipoproteins allow for the correct reclassification of an additional 40% of individuals (Table 4), with net benefit across a broad range on insulin resistance (between 10% and 50% by Vickers' decision analysis) (Fig. 2). In addition, NMR lipoproteins have a discrimination advantage (correct reclassification of an additional 14% of individuals) even after considering the effect of clinical factors that are associated with insulin resistance such as BMI and fasting glucose.

The current findings suggest that the addition of a basal measure of lipoprotein heterogeneity (e.g. NMR) can improve the prediction of insulin resistance even over the commonly used measures such as the TG-to-HDL-cholesterol ratio, which are already useful [8, 9]. This finding could be useful in clinical research since many methods to determine insulin resistance are time-consuming and expensive (e.g. hyperinsulinaemic clamp or MINMOD model). This approach could also be useful in clinical practice although no pharmacologic agent that improves insulin sensitivity has

yet been shown to definitively reduce cardiovascular disease in non-diabetic individuals.

Estimated remnant cholesterol in the fasting state (which reflects VLDL and IDL content) does not add to the predictive discrimination of TG and HDL-cholesterol levels. However, the type of remnant cholesterol that has been associated with both chronic inflammation and cardiovascular disease is the one estimated in the non-fasting state (which also reflects chylomicron remnants) [11]. We cannot assess whether or not this type of remnant cholesterol adds predictive discrimination to TG and HDL-cholesterol because the IRAS only has lipoprotein data in the fasting state.

Our study has several strengths. The IRAS is a well-characterised large, and ethnically diverse population. Sophisticated measures of insulin sensitivity (derived from the FSIGTT) and lipoprotein heterogeneity (acquired by NMR spectroscopy and gradient-gel electrophoresis) were obtained [17, 30]. Using decision curve analysis [24], the addition of NMR lipoproteins to TG and HDL-cholesterol improved the detection of insulin resistance across a broad range of a person's predictive probability of insulin resistance. The relationships between lipoproteins and apolipoproteins and insulin sensitivity was comparable across categories of ethnic groups; these relationships hold even though African-Americans tend to have both more insulin resistance and less lipoprotein abnormalities (lower TG concentration, higher HDL-cholesterol concentration and larger LDL particle size) than non-Hispanic whites [38–40]. A higher predictive
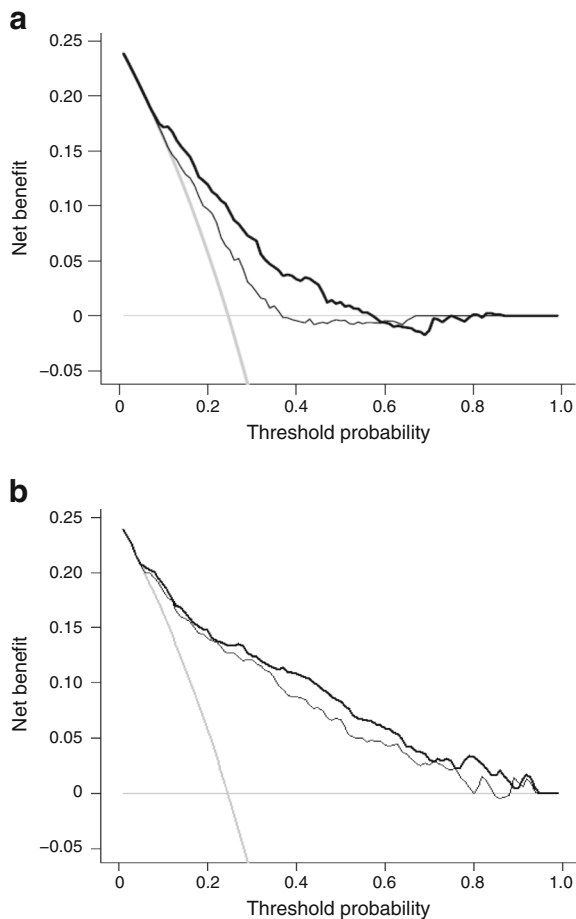
a

b

Fig. 2 Vickers' decision curves to assess incremental predictive ability. Incremental predictive ability of NMR lipoproteins to HDL-cholesterol and TG levels (**a**) in the absence and (**b**) within the context of a clinical model that included age, sex, ethnicity, clinic, BMI and fasting glucose. None of the participants treated as insulin resistant (thin grey line); everyone treated as insulin resistant (thick grey line); decision curves for the predicted probabilities with TG and HDL-cholesterol levels (thin black line) and with the addition of NMR lipoproteins (thick black line)

add information across a broad range of insulin resistance. This tool can be applied to fasting blood samples. Therefore, our results may be important for detecting insulin resistance in intervention and epidemiological studies.

Contribution statement CL contributed to the conception and design of the study, analysis and interpretation of data, and drafting the article. AJH, MJR and AF contributed to the analysis and interpretation of data, and revised the manuscript critically for important intellectual content. SMH contributed to the acquisition of data, conception and design of the study, analysis and interpretation of data, and drafting the article. All authors approved the final version of the article for publication. CL is responsible for the integrity of the work as a whole.

Duality of interest SMH has been a consultant for Liposcience Inc, Raleigh, NC, USA. All of the other authors have no duality of interest associated with this manuscript.

discrimination of NMR lipoproteins as compared with HDL-cholesterol plus TG concentrations was demonstrated across categories of ethnicity, sex, adiposity and glucose tolerance. A potential limitation is that we assessed lipoprotein heterogeneity by only NMR spectroscopy. Other methods, such as gradient-gel electrophoresis and density gradient ultracentrifugation [12, 14], were not used in the IRAS.

In summary, as previously shown [8, 10], the TG-to-HDL-cholesterol ratio does predict insulin resistance status in non-diabetic individuals. ApoA-I and apoB do not improve the predictive discrimination of TG and HDL-cholesterol levels. However, a more comprehensive assessment of lipoprotein composition (using NMR spectroscopy) adds discriminative value to conventionally measured lipoproteins. NMR lipoproteins allow for the correct reclassification of an additional 14–40% of individuals, depending on which other risk factors of insulin resistance are also considered, and

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