Measured Versus Calculated Small Dense LDL-Cholesterol and Cardiometabolic Traits in a South African Population

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Abstract Small-dense low density lipoprotein (sdLDL) is increasingly viewed as a marker for evaluating atherogenic risk, however its clinical uptake is hampered by the cumbersome of available methods. Consequently, a number of alternative methods for the estimation of sdLDL have been developed and none have been tested in a population from Africa. We evaluated an equation to estimate sdLDL-C from classic lipid parameters in South Africans. This is a cross-sectional study involving 1550 participants in which direct measurement of sdLDL in 237 participants was performed using a homogeneous enzymatic assay. Their mean age (standard deviation, SD) was 54.2 (14.7) years. 156 (65.8%) were normotolerant, 29 (12.2%) pre-diabetes, 17 (7.2%) screen detected diabetes and 35 (14.8%) known diabetes. Measured sdLDL values ranged from 0.17 to 3.39 versus—1.85 to 2.52 mmol/L calculated sdLDL. There was a significant positive correlation between the two measurements with a Pearson correlation coefficient of 0.659 (95%CI: 0.581–0.726). In a regression model, the adjusted R^2 was 0.440 after adding age, 0.441 after further adding gender, then 0.443 with dysglycemia

and lastly 0.447 upon adding body mass index. With the exception of HDL-cholesterol levels that decreased across increasing quintiles of calculated sdLDL, our data showed significant correlations between sdLDL and cardiometabolic risk factors, all p values < 0.0001. In conclusion, this study has shown that calculated sdLDL can be efficiently used to approximate population levels of sdLDL; however the modest correlation indicate that at the individual level, it will poorly approximate true sdLDL levels, with possible implications for risk stratification.

Keywords Africa · Diabetes · Metabolic syndrome · Lipids · Small-dense low density lipoprotein

Introduction

Higher-than-optimal low density lipoprotein cholesterol (LDL) level is a well-established major risk factor for cardiovascular disease (CVD), and thus a therapeutic target for both primary and secondary prevention of CVD [1]. Based on particle size, LDL is divided into two distinct sub-classes—one composed predominantly of large, buoyant LDL particles and the other characterized by an abundance of small dense LDL (sdLDL) particles [2]. Compared to buoyant LDL, the latter are considered to be particularly atherogenic as a result of their greater ease of penetrating the arterial wall, decreased binding affinity for the LDL receptor, prolonged plasma half-life (thus increased interaction with matrix components) and lower resistance to oxidative stress on account of their higher content of polyunsaturated fatty acids [3, 4]. As such, the measurement of sdLDL is increasingly viewed as a potent tool for evaluating atherogenic risk and has been accepted

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as an emerging cardiovascular risk factor by the National Cholesterol Education Program [1].

The potential clinical utility notwithstanding, sdLDL particles are seldom measured routinely although there are methods available for this purpose including ultracentrifugation, gradient gel electrophoresis, nuclear magnetic resonance and a recently developed homogeneous enzymatic assay [2, 5]. Each of these methods, however, suffers from one or more limitations relating to exorbitant running costs, technical complexity and a requirement for elaborate instrumentation thus far, hampering their usefulness for routine clinical practice or population screening. Subsequently, in 2011, Srisawasdi et al. [6] developed an equation to estimate sdLDL-C from classic lipid parameters. The authors also indicated that regardless of differences in sex, age group, creatinine level or state of glucose intolerance, the equation provides a good estimate of sdLDL-C levels in healthy Thai individuals [6]. However, when tested in a different population, the equation was found not applicable in a Korean population, particularly with metabolic syndrome (MetS) indicating a need for extensive evaluation of the equation across broader ethnic and disease groups [7]. Thus, we evaluated the performance of the Srisawasdi et al.'s sdLDL-C formula in a sample of mixed ancestry South Africans with varying degrees of glucose tolerance. Additionally, we assessed the correlation between measured sdLDL and cardiometabolic risk profile in this population.

Materials and Methods

Ethical Approval of the Study

This investigation is based on the Cape Town Vascular and Metabolic Health (VMH) study from Cape Town that has been approved by the Research Ethics Committees of the Cape Peninsula University of Technology (CPUT) and Stellenbosch University (respectively, NHREC: REC - 230 408 – 014 and N14/01/003). For this sub-study, ethical approval was also obtained from the CPUT Health and Wellness Sciences Research Ethics Committee (CPUT/HW-REC 2014/H08). The study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants signed written informed consent after all the procedures had been fully explained in the language of their choice.

Study Design and Procedures

This was a cross-sectional study involving participants from the ongoing Cape Town Vascular and Metabolic Health (VMH) study. VMH is an extension of the Cape

Town Bellville South study, which has been described in details previously [8]. Data collection was based on a standardised questionnaire available in the electronic form on a password-protected personal digital assistant (PDA). Upon completion of the assessment of each participant, data were automatically encrypted and transmitted via mobile internet connection to a dedicated server, from which they were checked for completion, downloaded and stored for future use. Physical examination involved data collection on blood pressure (BP) which was measured according to the World Health Organisation (WHO) guidelines [9], using a semi-automatic digital blood pressure monitor (Omron M6 comfort-preformed cuff BP Monitor) on the right arm in sitting position and at rest for at least 10 min. Body weight (to the nearest 0.1 kg) was measured with the subject in light clothing and without shoes, using an Omron body fat meter HBF-511 digital bathroom scale, which was calibrated and standardized using a weight of known mass. Height to the nearest centimetre was measured with a stadiometer, with subjects standing on a flat surface. Body Mass Index (BMI) was calculated as weight per square meter (kg/m^2). Waist circumference was measured with a non-elastic tape at the level of the narrowest part of the torso, as seen from the anterior view. BP and anthropometric measurements were performed three times and their average used for analysis.

Biochemical Assays

Blood samples were collected from all participants after an overnight fast and from those with no history of doctor diagnosed diabetes mellitus, a further sample after a 2-h 75 g oral glucose tolerance test (OGTT), following the WHO recommendation [10]. Routine biochemical parameters were analyzed at an ISO 15,189 accredited Pathology practice (PathCare, Reference Laboratory, Cape Town, South Africa). Blood glucose (mmol/L) was measured using the enzymatic hexokinase method (Beckman AU, Beckman Coulter, South Africa). Insulin (mmol/L) was determined by a paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa). HDL-C (mmol/L) was by enzymatic immunoinhibition—End Point (Beckman AU, Beckman Coulter, South Africa). Low-density lipoprotein cholesterol (LDL-C) (mmol/L) was measured by enzymatic selective protection—End Point (Beckman AU, Beckman Coulter, South Africa). Triglycerides (TG) (mmol/L) were estimated by glycerol phosphate oxidase—peroxidase, End Point (Beckman AU, Beckman Coulter, South Africa). Glycated haemoglobin (HbA1c) was measured by High Performance Liquid Chromatography (Biorad Variant Turbo, BioRad, South Africa). Small-dense low density lipoprotein (sdLDL) (mmol/L) was measured directly, using a homogenous

enzymatic assay (Randox sLDL-EX“SEIKEN”) on an ABX Pentra 400 analyser (HORIBA ABX, Montpellier, France) as well as calculated sLDL, using the formula proposed by Srisawasdi et al. [6] which is $\text{sLDL-C}_{\text{mmol/L}} = 0.580 \text{ (non-HDL-C)} + 0.407 \text{ (dLDL-C)} - 0.719 \text{ (cLDL-C)} - 0.312$.

Statistical Analysis

Data were analysed using the R statistical software version 3.2.2 (2015-08-14) (The R Foundation for Statistical Computing, Vienna, Austria). Variables are summarized as mean and standard deviation, median and minimum–maximum, and count and percentages. The Shapiro–Wilk W test was employed to determine whether the sd-LDL values were normally distributed, based on probability thresholds of $p > 0.1$; while the kurtosis of its distribution was assessed with the Anscombe–Glynn test [11], an skewness assessed with the D’Agostino test [12]. The continuous association between measured and estimated sd-LDL values was assessed using Pearson’s correlation coefficient and linear regression models. Systematic bias in the two measurements was then examined using Bland and Altman plots [13], implemented with the use of ‘Research Methods’ package of R. Paired-sample t test was used to compare differences in means of Measured and Estimated sd-LDL. To assess the clinical importance of statistically significant differences between measured and calculated sd-LDL, the total allowable error (TEa) was used which was based on within- and between-subject variation [14]. The mean of the calculated measurement was then compared with the reference (measured) and should be within clinical range of reference mean $\pm$ TEa. The correlation between estimated sdLDL and cardio-metabolic risk profile was assessed via Spearman’s correlation, but also by assessing differences and monotonous trends in the levels of cardio-metabolic risk factors across participants grouped by increasing fifths of measured sLDL. Monotonous trends assessment used the Cochran-Armitage trend test for proportions and Jonckheere-Terpstra trend test for medians.

Results

General Characteristics of the Study Population

The starting sample comprised 1550 participants with data available on all variables required to estimate sLDL levels. They were all included in the studies of the correlation between calculated sLDL and cardiometabolic risk profile. Of this total population, a sub-sample of 237 participants had concomitant data on measured and calculated

sLDL and were included in the validation studies of calculated against measured sLDL. The characteristics of this sub-sample are shown in Table 1. In all, 55 (23.2%) were men and 182 (76.8%) women. Their mean age (standard deviation, SD) was 54.2 (14.7) years. Among them, 156 (65.8%) were normotolerant, 29 (12.2%) had prediabetes, 17 (7.2%) had screen-detected diabetes and 35 (14.8%) had known diabetes. Their BMI was 31.2 (8.6) kg/m² and 54.7% were obese whilst another 20.3% were overweight. The 2-hour glucose levels, BMI, HDL cholesterol, waist and hip circumferences were significantly higher in females compared to their male counterparts, all $p \leq 0.0302$ (Table 1).

Measured Versus Calculated sLDL in the Sub-Sample of 237 Participants

Values (in mmol/l) ranged from 0.17 to 3.39 [mean 0.95 (SD 0.51), median 0.84] for measured sLDL and -1.85 to 2.52 [0.91 (0.45), 0.87] for calculated sLDL. Both measurements did not strictly follow a normal distribution (both $p < 0.0001$ for the Shapiro–Wilk test), in spite of the apparent bell-shape curves (Fig. 1a). The skewness coefficient (p value for the Agostino test) and kurtosis coefficient (p value for the Anscombe test) were 1.315 (< 0.0001) and 5.494 (< 0.0001) for measured sLDL; and -0.418 (0.0091) and 8.835 (< 0.0001) for calculated sLDL.

The mean difference (measured – calculated) in sLDL was 0.04 mmol/l [95%CI -0.01 to 0.09 ; paired sample t test p value = 0.122]. There was a significant positive correlation between the two measurement with a Pearson correlation coefficient of 0.659 (95%CI: 0.581–0.726). The linear regression curves depicting this association is shown in Fig. 1b. The related equation was of the form ‘Calculated sLDL = 0.580* measured sLDL + 0.358 and the adjusted coefficient of determination for this equation was $R^2 = 0.435$. The adjusted R^2 was 0.440 after adding age to the model, 0.441 after further adding gender, then 0.443 with dysglycemic status and lastly 0.447 upon adding body mass index (BMI). The Bland–Altman plots showed an acceptable agreement between the methods used to determine sLDL (Fig. 1c). Based on total TEa, the allowable range of sLDL (mmol/l) was 0.80–1.10, which comprised the mean value of calculated sLDL of 0.91 mmol/l, indicating that, difference if any, with the measured sLDL was not clinically significant.

Table 1 Participants general characteristics

Characteristics	Overall	Males	Females	<i>p</i> value
n (%) ^a	237 (100)	55 (23.2)	182 (76.8)	
Glucose tolerance, n (%) ^a				0.3576
Normal	156 (65.8)	40 (74.1)	116 (63.4)	
Prediabetes	29 (12.2)	4 (7.4)	25 (13.7)	
New diabetes	17 (7.2)	2 (3.7)	15 (8.2)	
Known diabetes	35 (14.8)	8 (14.8)	27 (14.7)	<0.0001
BMI categories				
Normal weight	59 (25)	23 (43.6)	36 (19.8)	
Overweight	48 (20.3)	19 (35.2)	29 (15.9)	
Obese	129 (54.7)	12 (22.2)	117 (64.3)	
Age, years ^b	54.2 (14.6)	52.7 (16.2)	54.6 (14.1)	0.4387
Fasting glucose (mmol/l) ^b	6.0 (3.4)	5.6 (2.6)	6.1 (3.6)	0.1852
2-hour glucose (mmol/l) ^b	6.8 (3.2)	5.7 (3.3)	7.2 (3.0)	0.0119
Fasting insulin (IU/l) ^c	7.5 (4.7–12.2)	5.5 (3.6–8.9)	7.9 (5.1–12.6)	0.0016
2-hour insulin (IU/l) ^c	46.1 (27.9–82.4)	29.6 (12.9–61.1)	50.7 (31.3–89.7)	0.0033
Body mass index (kg/m ²) ^b	31.2 (8.6)	26.2 (7.8)	32.7 (8.3)	< 0.0001
Waist circumference (cm) ^b	97 (17)	92 (19)	98 (17)	0.0302
Hip circumference (cm) ^b	110 (16)	101 (14)	113 (16)	< 0.0001
Systolic blood pressure (mmHg) ^b	129 (24)	129 (26)	129 (23)	0.9796
Diastolic blood pressure (mmHg) ^b	82 (12)	81 (12)	82 (12)	0.4392
Total cholesterol (mmol/l) ^b	5.1 (1.2)	5.0 (1.3)	5.1 (1.1)	0.3510
HDL cholesterol (mmol/l) ^b	1.3 (0.3)	1.1 (0.3)	1.3 (0.3)	0.0002
Measured LDL cholesterol (mmol/l) ^b	3.2 (1.0)	3.2 (1.1)	3.2 (1.0)	0.9391
Calculated LDL cholesterol (mmol/l) ^b	3.2 (1.0)	3.2 (1.1)	3.2 (1.0)	0.7930
Non-HDL cholesterol (mmol/l)	3.8 (1.2)	3.8 (1.2)	3.8 (1.1)	0.9500
Triglycerides (mmol/l) ^b	1.4 (0.7)	1.3 (0.6)	1.5 (0.7)	0.1642

^aCount and percentages, ^bMean and standard deviation, ^cMedian and minimum-maximum

Correlation of Calculated sdLDL with Cardiometabolic Risk Profile in the Overall Sample of 1550 Participants

The profile of the overall 1550 study participants shown in Table 2 is mostly similar to that of the sub-sample included in the validation study. Of these 1550 participants, 377 (24.3%) were men, and 642 (41.9%) were obese. Calculated sdLDL values (in mmol/l) ranged from −1.85 to 3.89 (median 0.87). Across increasing quartiles of calculated sdLDL, significant differences were observed in the levels of all assessed cardio-metabolic risk factors (all $p < 0.0001$), with always significant linear trend (all $p < 0.0001$ for linear trend). With the exception of HDL-cholesterol levels that decreased across increasing quintiles of calculated sdLDL, levels of all other cardiometabolic risk factors increased. Accordingly, there was a positive and significant correlation between calculated sdLDL and all these risk factors, and a negative correlation with HDL-cholesterol. Positive Spearman's correlation coefficients

ranged from 0.132 ($p < 0.0001$) for male gender to 0.896 ($p < 0.0001$) for non-HDL cholesterol, Table 2.

Discussion

Although there is increased evidence of the usefulness of sdLDL in the identification of individuals at high cardiovascular risk, a standardised methodology for its assessment is lacking. In the absence of a suitable, routinely applicable method for sdLDL measurement, the main aim of this study was to evaluate the alternative approach for estimating sdLDL by assessing the performance of measured sdLDL against an equation derived from routine lipid measurements. Overall we observed a good agreement between the two methods as shown by the Bland–Altman plot. Although comparisons between measured and calculated sdLDL yielded more than half less R^2 values (0.432) compared to that derived by Srisawasdi et al. [6] (0.878), we similarly observed that the adjusted R^2

Fig. 1 Relationship of measured vs. calculated small dense LDL cholesterol. **a** Density plot showing the distribution curve for measured sdLDL (continuous curve) and the superimposed distribution curve for calculated sdLDL (dotted line). **b** Linear regression curves showing the continuous association of measured with estimated sd-LDL (continuous line). The dotted diagonal line is the line of perfect agreement. The intercept, beta coefficient and adjusted R^2 for the model are also shown. **c** Bland and Altman plots comparing measured with estimated sd-LDL distribution curves for measured sd-

LDL. The difference Measured minus Estimated value for each participant is plotted against the mean of the two measurements. The horizontal dashed line through 0 (zero) is the line of perfect agreement between the two measurements. The parallel solid black line is the *bias line* (mean difference), and the colored band about it for the 95% confidence interval, while the shaded area delineate the zone of agreement between the two measurement. The lines of best fit from linear regressions are also shown (dotted superimposed curves)

values were improved in models enriched with age, gender, dysglycaemia or BMI. Our data showed significant correlations between sdLDL and cardiometabolic risk factors with all p values < 0.0001 , corroborating previous report on sdLDL as a marker of CVD [15].

Previous studies have used different equations to establish possible reliable methods to replace measured sdLDL with reliable calculated sdLDL values. For example, Hattori et al. [16] used the LDL-C/LDL-apolipoprotein B ratio as a predictive value for the level of sdLDL in a Suita population with CVD and found the formulae to be useful in lipoprotein disorders. These findings were later confirmed by Wagner et al. [17], but a study that tested the use of the formula against LDL particle size as measured by gradient-gel electrophoresis (GGE) was not in agreement with the use of the LDL-C/LDL-apolipoprotein B ratio [18]. The equation used in this study to evaluate measured versus calculated sdLDL, was originally proposed by Srisawasdi et al. [6] and reported a strong linear relationship between measured and calculated sdLDL-C values across a wide range of sdLDL values (0.18–4.84 mmol/L) in a Thai population [6]. In our study which comprised of obese individuals with varying degrees of glucose tolerance, measured sdLDL-C concentrations

ranged between 0.17 and 3.39 mmol/L and showed a modest agreement with the calculated sdLDL. Other studies have shown that sdLDL should be evaluated together with LDL-C as a risk factor for the assessment for CAD in T2DM [19, 20]. This notion is further buttressed by the findings in Indian patients with established CAD, where higher sdLDL levels compared to individuals without CAD were observed despite all individuals had comparable LDL levels [21]. Similarly, Hoogeveen et al. [22] reported that they found sdLDL-C to be a better predictor of risk assessment for incident CHD even in subjects at low cardiovascular risk as measured by LDL-C. In our study, enriching the model with age, gender, dysglycaemia or BMI improved the prediction of sdLDL, but the effect remained modest.

Our data showed an increasing trend in dysglycaemia, MetS components except HDL-C with increasing sdLDL quintiles, further supporting the value of including sdLDL in determining CVD risk. Type 2 diabetes mellitus in an independent risk factor for CVD and the mixed ancestry population of South Africa has one of the highest prevalence rates of T2DM in Africa [8] with rapid progression from the pre-diabetic stage to diabetes [23] and a high 30-year cardiovascular disease risk profile [24]. Increased

Table 2 Cardio-metabolic risk profile of the 1550 participants in the total sample, across increasing quintiles of calculated small dense LDL cholesterol

Characteristics	Overall	Quintiles of calculated sdLDL cholesterol					<i>p</i> diff	<i>p</i> trend	σ (<i>p</i> value)
		Q1	Q2	Q3	Q4	Q5			
N (%) ^a	1550 (100)	312 (20.1)	308 (19.9)	311 (20.1)	309 (19.9)	310 (20.0)			
Median sdLDL, mmol/l [min–max]	0.87 (– 1.85 to 3.89)	0.42 (– 1.85 to 0.51)	0.66 (0.56–0.77)	0.87 (0.77–0.99)	1.10 (0.99–1.25)	1.46 (1.25–3.89)			
Men, n (%) ^a	377 (24.3)	108 (34.6)	78 (25.3)	69 (22.2)	72 (23.3)	50 (16.1)			
Women, n (%)	1173 (75.7)	204 (65.4)	230 (74.7)	242 (77.8)	237 (76.7)	260 (83.9)			
Glucose tolerance n (%) ^a							<0.0001	<0.0001	0.248 (< 0.0001)
Normal	861 (61.9)	207 (81.5)	189 (68.2)	175 (62.7)	157 (54.1)	133 (45.9)			
Prediabetes	243 (17.5)	26 (10.2)	47 (17.0)	44 (15.8)	64 (22.1)	62 (21.4)			
New diabetes	95 (6.8)	14 (2.8)	10 (3.6)	21 (7.5)	23 (7.9)	34 (11.7)			
Known diabetes	191 (13.7)	14 (5.5)	31 (11.2)	39 (14.0)	46 (15.9)	61 (21.0)			
BMI categories n (%) ^a							<0.0001	<0.0001	0.312 (< 0.0001)
Normal weight	532 (34.7)	185 (60.3)	134 (44.1)	98 (31.9)	72 (23.4)	43 (14.0)			
Overweight	358 (23.4)	50 (16.3)	65 (21.4)	71 (23.1)	75 (24.4)	97 (31.6)			
Obese	642 (41.9)	72 (23.5)	105 (34.5)	138 (44.9)	160 (52.1)	167 (54.4)			
Age, years ^b	49.2 (15.4)	39.9 (15.5)	47.3 (16.2)	50.2 (14.6)	54.5 (13.6)	54.2 (12.3)	<0.0001	<0.0001	0.342 (< 0.0001)
Fasting glucose (mmol/l) ^b	5.8 (2.8)	4.9 (1.4)	5.3 (2.1)	5.8 (2.8)	6.0 (2.7)	6.8 (4.0)	<0.0001	<0.0001	0.317 (< 0.0001)
2-hour glucose (mmol/l) ^b	6.6 (3.1)	5.5 (2.2)	6.1 (2.3)	6.8 (3.4)	7.2 (2.8)	7.8 (4.0)	<0.0001	<0.0001	0.312 (< 0.0001)
Fasting insulin (IU/l) ^c	6.7 [4.2–10.8]	5.1 [3.2–8.0]	5.7 [3.6–9.1]	6.6 [4.2–10.0]	7.6 [5.0–11.9]	9.3 [95.9–14.9]	<0.0001	<0.0001	0.293 (< 0.0001)
HbA1c (%) ^b	6.2 (1.6)	5.6 (0.9)	6.0 (1.2)	6.2 (1.6)	6.3 (1.6)	6.8 (2.1)	<0.0001	<0.0001	0.338 (< 0.0001)
Body mass index (kg/m ²) ^b	29.2 (8.1)	25.3 (7.2)	27.8 (8.2)	29.7 (8.1)	31.0 (7.5)	32.0 (7.5)	<0.0001	<0.0001	0.335 (< 0.0001)
Waist circumference (cm) ^b	92 (17)	82 (17)	88 (16)	92 (17)	97 (15)	99 (14)	<0.0001	<0.0001	0.380 (< 0.0001)
Systolic blood pressure (mmHg) ^b	128 (24)	118 (22)	124 (23)	129 (24)	135 (24)	133 (24)	<0.0001	<0.0001	0.259 (< 0.0001)
Diastolic blood pressure (mmHg) ^b	81 (14)	77 (14)	80 (13)	82 (15)	84 (13)	84 (13)	<0.0001	<0.0001	0.195 (< 0.0001)
Total cholesterol (mmol/l) ^b	5.1 (1.2)	3.9 (0.6)	4.6 (0.6)	5.1 (0.6)	5.6 (0.7)	6.5 (1.0)	<0.0001	<0.0001	0.818 (< 0.0001)
HDL cholesterol (mmol/l) ^b	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)	1.3 (0.3)	1.2 (0.3)	<0.0001	<0.0001	– 0.112 (< 0.0001)
Measured LDL cholesterol (mmol/l) ^b	3.2 (1.0)	2.0 (0.4)	2.7 (0.4)	3.2 (0.4)	3.7 (0.5)	4.5 (0.9)	<0.0001	<0.0001	0.879 (< 0.0001)
Calculated LDL cholesterol (mmol/l) ^b	3.2 (1.0)	2.1 (0.5)	2.7 (0.5)	3.2 (0.6)	3.6 (0.7)	4.2 (1.1)	<0.0001	<0.0001	0.739 (< 0.0001)
Non-HDL cholesterol (mmol/l) ^b	3.8 (1.1)	2.5 (0.5)	3.2 (0.4)	3.8 (0.5)	4.3 (0.6)	5.3 (0.9)	<0.0001	<0.0001	0.896 (< 0.0001)

Table 2 continued

Characteristics	Overall	Quintiles of calculated sdLDL cholesterol					<i>p</i> diff	<i>p</i> trend	σ (<i>p</i> value)
		Q1	Q2	Q3	Q4	Q5			
Triglycerides (mmol/l) ^b	1.4 (1.1)	0.8 (0.3)	1.0 (0.3)	1.2 (0.4)	1.6 (0.5)	2.4 (1.9)	<0.0001	<0.0001	0.784 (< 0.0001)

^aCount and percentages, ^bMean and standard deviation, ^cMedian and minimum-maximum

sdLDL levels have been reported in diabetes, obesity and MetS [25, 26], however a prospective nested case–control study involving Koreans, sdLDL was found inferior to the presence of diabetes in predicting future CVD event [27]. In South Africa and other African countries, diabetes is often undiagnosed [8, 28] and the inclusion of a highly atherogenic factor in risk stratification is highly recommended. However, further studies are required to improve the estimation of sdLDL in African populations.

This study has some limitations. Although the study sample number was sufficient for reliable statistical analysis, the number of males in the study population was rather low. The age of the study population was also high, which could have had an effect on the study results, as several of the parameters tested are known to increase with age. Our participants comprised essentially mixed-ancestry participants, it is therefore unknown if our findings are generalizable to a typical Black African population. Regardless of these limitations, we are not aware of other studies that have assessed the performance of sdLDL formulae in Africa. In conclusion, this study has shown a modest agreement between measured and calculated sdLDL using the equation proposed by Srisawasdi et al. [6] in mixed ancestry South Africans. This shows that calculated sdLDL can be efficiently used to approximate population levels of sdLDL; however the modest correlation indicate that at the individual level, it will poorly approximate true sdLDL levels, with possible implications for risk stratification.

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