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Establishing non-fasting lipid reference intervals and clinical decision limits in a senegalese adult cohort

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Abstract

Non-fasting lipid profiling is now widely recommended; however, reference intervals (RIs) are notably lacking in sub-Saharan Africa, particularly for emerging lipid biomarkers (non-HDL-C, sdLDL-C, TRL-C). This study aimed to establish these values in Senegal and propose pragmatic clinical decision thresholds. As part of the SEN-GENOME project, we conducted a study involving 746 adults, including a reference subgroup of 241 strictly healthy individuals. Non-fasting specimens were collected to analyse conventional and emerging lipid parameters using the Sampson-NIH equation. RIs were calculated in accordance with the CLSI EP28-A3c protocol. The upper reference limit for triglycerides 2.73 mmol/L confirms the clinical tolerance of the non-fasting state. However, the statistical RIs (2.5–97.5th percentiles) for total cholesterol and LDL-C significantly exceeded the NCEP ATP III risk thresholds. Furthermore, the general population's values exhibited a marked metabolic drift compared to the healthy reference group. Strict reliance on statistical RIs risks normalising atherogenic profiles. We advocate for the adoption of clinical decision thresholds based on the median (optimal threshold) and the 75th percentile (alert threshold) for extended lipid profiling. This approach, including emerging lipid parameters, is essential for improving early cardiovascular risk detection.

Keywords Reference intervals · Clinical decision limits · Non-fasting lipid profile · Sampson-NIH equation · Cardiovascular risk

Introduction

Cardiovascular diseases (CVDs), a leading cause of global morbidity and mortality, are rising alarmingly in sub-Saharan Africa, driven by rapid urbanisation and the nutritional transition[1–3]. The pivotal role of dyslipidaemia in cardiovascular pathophysiology has been underscored by large-scale population studies, notably the landmark Framingham and PROCAM trials[1,2]. Dyslipidaemias represent a heterogeneous group of plasma lipid

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and lipoprotein abnormalities, typically characterised by decreased high-density lipoprotein cholesterol (HDL-C) and/or elevations in low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG)[4].

Whether primary or secondary to smoking, alcohol consumption, obesity, diabetes or renal disease, dyslipidaemias constitute a major modifiable risk factor for CVD[4]. In particular, the reduction of LDL-C remains the primary target for cardiovascular risk mitigation[4].

Traditionally, CVD risk assessment relied on strictly fasting lipid profiles (8–12 h) to minimise the impact of postprandial lipaemia[5]. However, this paradigm has been challenged. The non-fasting (postprandial) state more accurately reflects daily physiology ("real-life") and better captures the residual atherogenic risk associated with triglyceride-rich lipoproteins (TRL) and their remnants[6–8]. Consequently, non-fasting lipid testing became the standard in Denmark in 2009, was adopted in the UK in 2014 and has been recommended for routine assessment in the USA since 2018[6,7,9].

This methodological shift presents two primary challenges. Firstly, it limits the applicability of the Friedewald formula for LDL-C calculation, which is sensitive to postprandial hypertriglyceridaemia and circulating chylomicrons, necessitating direct measurement or the use of more robust equations, such as Martin-Hopkins or Sampson-NIH[10–13]. Secondly, it potentially renders fasting-derived clinical decision limits and RIs obsolete. Simultaneously, interest is growing in emerging biomarkers, non-HDL cholesterol (non-HDL-C), small dense LDL cholesterol (sdLDL-C), large buoyant LDL (lbLDL-C) and very low density lipoprotein cholesterol (VLDL-C), TRL cholesterol (TRL-C), which offer a more nuanced view of residual atherogenic risk[14–16].

This interpretative challenge is particularly critical in Senegal, where the transition toward non-fasting lipid profiling has yet to be fully debated. More fundamentally, most laboratories lack local RIs, even for fasting profiles, and few utilise clinical decision thresholds for result interpretation. Instead, they rely on manufacturer inserts or foreign data established in disparate populations, disregarding the genetic, environmental and nutritional specificities of the local population. Medical biology in Senegal, therefore, faces a double deficiency: the absence of validated local RIs and clinical decision thresholds, combined with a lack of standards adapted to modern postprandial testing. This situation increases the risk of clinical misinterpretation. To address these gaps, this study sought to establish RIs and clinical decision thresholds for an extended lipid profile (including non-HDL-C, lbLDL-C, sdLDL-C, VLDL-C, TRL-C) in supposedly healthy Senegalese adults in a non-fasting state. Furthermore, it aims to determine the typical values of these parameters within a population that includes individuals with potential metabolic and cardiovascular risk factors.

Materials and methods

Study population

According to the most recent census, 98.9% of the Senegalese population are national citizens with a balanced sex ratio of 50.6% male to 49.4% female[17]. Nearly half of the population (47.0%) resides along the Dakar-Thiès-Diourbel axis with the latter two regions comprising the country's primary groundnut basin[17]. The regional nutritional context is defined by a high consumption of cereals such as rice and millet, vegetable oils including groundnut and palm oil and animal proteins from fish and meat.

The study population was derived from the SEN-GENOME project, a research initiative designed to establish a reference genome for the Senegalese population and document its genetic diversity[18]. The primary objective of SEN-GENOME is to develop a local genomic resource to support precision medicine tailored to regional specificities. Recruitment was conducted between November 2022 and May 2023, encompassing all 14 administrative regions of Senegal and 31 ethno-linguistic groups[18]. Eligible participants were required to be of Senegalese nationality, provide informed consent, and have parents and all four grandparents native to the same village. Furthermore, participants had to belong exclusively to one ethno-linguistic group and be the sole representative of their family within the study[18].

Data collection

Phenotypic data were collected via a structured questionnaire administered on digital tablets using KoboToolbox software. This tool allowed for the recording of socio-demographic details (age, sex, ethno-linguistic group, region), anthropometric measurements (weight, height), clinical data (blood pressure) and lifestyle factors (smoking, alcohol consumption, physical activity, time of last meal and meal frequency). Additionally, personal and family medical histories, blood donation status and viral screening results for HIV, HBV and HCV were documented[18].

Ethical consideration

The study received ethical approval from the Senegal's National Ethics Committee for Health Research (CNERS; Approval No. SEN22/63). All methods were performed in accordance with the relevant guidelines and regulations, and the research was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

Selection criteria and cohort definition

Participants were categorised into two distinct groups: the "typical values" cohort and the "reference" cohort. The typical values group comprised adults aged 20 years and older, recruited and sampled without fasting requirements regardless of the time of day. Conversely, the reference cohort, representing the healthy sub-population, was established through *a posteriori* selection in accordance with CLSI EP28-A3c international guidelines[19]. To be included in this reference group, subjects had to be free from any pathology at the time of recruitment, specifically excluding individuals with underweight, overweight, obesity, diabetes or hypertension. Normal anthropometry was defined by a Body Mass Index (BMI) between 18 and 25 kg/m², a systolic blood pressure between 90 and 140 mmHg and a diastolic blood pressure between 60 and 90 mmHg. Participants were also required to maintain a healthy lifestyle, defined as being a non-smoker (or ex-smoker abstinent for >1 year) with zero or occasional alcohol consumption (<2 times/week).

Non-inclusion criteria applied to pregnant women, individuals who had donated blood within the preceding three months and those with known HIV, HBV or HCV infections. *A posteriori* exclusions for both cohorts included participants who had fasted for more than 12 h, those with incomplete data or specimens showing signs of haemolysis or icterus. For the reference group specifically, individuals presenting any metabolic or cardiovascular risk factor, such as chronic kidney disease, personal history of stroke or myocardial infarction, active smoking or frequent alcohol consumption, were excluded from the final analysis.

Laboratory analysis and biochemical procedures

Venous blood specimens were collected in plain tubes and centrifuged locally at regional hospitals. Serum aliquots were then frozen and transported to the Human Genetics Laboratory of Cheikh Anta Diop University (UCAD) for storage at −80 °C until the day of analysis. All assays were performed at the Biochemistry Laboratory of the Albert Royer National Children's Hospital using a BA400 automated analyser (Biosystems, Barcelona, Spain). Lipid parameters were either directly measured by spectrocolorimetry or calculated using validated formulae. Triglycerides (TG) were measured via the enzymatic lipase/glycerol kinase/glycerol 3-P oxidase/peroxidase method while total cholesterol (TC) was quantified using the enzymatic cholesterol esterase/cholesterol oxidase/peroxidase method. Both HDL-C and LDL-C were determined through direct enzymatic methods which selectively mask other lipoproteins prior to the enzymatic reaction.

Extended lipid profiling included the calculation of LDL-C using the Friedewald, Martin-Hopkins, and Sampson-NIH Eqs[10–12]. LDL sub-fractions, specifically lbLDL-C and sdLDL-C, were determined using the

Sampson-NIH equations[15]. VLDL-C was estimated using the Sampson-NIH formula[12]. Non-HDL-C was calculated as TC − HDL-C and TRL-C was determined as TC − (HDL-C + LDL-C). Internal quality control (IQC) at normal and pathological levels was performed at the start and midpoint of each run to ensure analytical validity.

All lipid concentrations are reported in International System (SI) units (mmol/L). To facilitate clinical interpretation across different regions, mass units can be derived from mmol/L using the following conversion factors: for cholesterol parameters, 1 mmol/L is equivalent to 0.3867 g/L and 38.67 mg/dL; for triglycerides, 1 mmol/L corresponds to 0.8857 g/L and 88.57 mg/dL.

Statistical analysis

Data analysis was conducted using STATA version 14.0 (Stata Corporation, College Station, TX). Continuous variables are reported as mean ± SD (standard deviation); for biological parameters, lipid profiles are further detailed using medians with interquartile ranges (P25–P75) and minimum–maximum values to provide a comprehensive distribution overview. Categorical variables are reported as n (%) and compared using the chi-square test or Fisher's exact test. The normality of lipid parameter distributions was assessed through visual inspection of histograms and confirmed by the Kolmogorov-Smirnov test. To manage outliers, a Box-Cox transformation was applied to normalise skewed data, followed by Tukey's method for exclusion of outliers. Sex-based partitioning was evaluated using the Wilcoxon-Mann-Whitney test on both raw and cleaned datasets with statistical significance set at $p < 0.05$. In compliance with CLSI EP28-A3c directives, RIs were determined non-parametrically using the 2.5th and 97.5th percentiles of the reference distribution[19]. The 90% confidence intervals (CI) for these limits were estimated using the Bootstrap technique (5 000 iterations)[19]. The same statistical procedures were applied to the typical values group to establish population-wide typical ranges.

Rationale for clinical decision limits

Recognising the well-established limitation that statistical RIs derived from healthy populations are descriptive in nature and may include values that are statistically common but clinically suboptimal, we derived a complementary set of pragmatic decision limits from the distribution of our strictly healthy reference subgroup ($n = 241$). This empirically motivated approach has subsequently been endorsed by the 2024 ADLM (Association for Diagnostics and Laboratory Medicine) Guidance Document on the Measurement and Reporting of Lipids and Lipoproteins, published after the design and analytical phases of the present study, which formally recommends defining "desirable" lipid values between the 50th and 75th percentiles of healthy populations[20].

Three operational zones were defined for each lipid parameter: an optimal target zone, corresponding to values below the median (50th percentile); an intermediate "limit zone" between the median and the 75th percentile (or between the 25th and the 50th percentile for HDL-C, for which the risk logic is reversed); and a high-risk zone, defined by values above the 75th percentile (or below the 25th percentile for HDL-C).

This three-tier model is conceptually consistent with the IFCC-LM doctrine that distinguishes reference values (descriptive, statistical) from clinical decision limits (interpretative, outcome-related), and with the framework adopted by the EAS/EFLM joint consensus and explicitly endorsed by the 2024 ADLM Guidance Document[6,20]. The external coherence of the resulting thresholds with the NCEP ATP III, ESC/EAS and ACC/AHA guidelines is examined in the Discussion Sects[4,21,22].

Results

Baseline characteristics of the study population

From an initial recruitment of 1 115 participants, 813 underwent lipid profiling. Following the selection process, 746 subjects were included in the "typical values" cohort, from which a sub-population of 241 strictly healthy individuals was selected to constitute the "reference" subgroup. The reference subgroup exhibited an optimal metabolic and haemodynamic profile, with body mass index (BMI) and blood pressure values falling within the healthy ranges, and the postprandial sampling window averaging two hours (Table 1, Supplementary Table S1).

Lifestyle analysis confirmed the healthy profile of the reference subgroup, in which the vast majority of participants had never smoked or consumed alcohol, and physical activity was evenly distributed across the three levels (Table 1, Supplementary Table S1). Significant sex-specific differences were noted for smoking (more prevalent in men) and physical activity (lower in women) (Table 1, Supplementary Table S1). In the typical values cohort, the lifestyle profile was more heterogeneous, with sedentary behaviour prevalent and a sex difference for both smoking and alcohol consumption (Table 1, Supplementary Table S1).

Regarding medical history, no participants in the reference subgroup had personal chronic pathologies; family histories of cardiovascular disease (CVD), type 2 diabetes, type 1 diabetes and chronic kidney disease were nonetheless documented (Table 1, Supplementary Table S1). In the typical values cohort, 13.7% of participants declared existing CVD, and the prevalences of family-history items were broadly similar to those of the reference subgroup (Table 1, Supplementary Table S1).

Geographically, participants in the reference subgroup primarily originated from the regions of Ziguinchor, Thiès and Saint-Louis, and the ethno-linguistic distribution was dominated by the Wolof, Serer and Pular (Supplementary Table S2). The typical values cohort showed a partly similar distribution, dominated by Thiès, Ziguinchor and Diourbel and by the same three ethno-linguistic groups (Supplementary Table S2). No statistically significant sex-based differences were detected for either characteristic in either cohort.

Lipid profile

Descriptive statistics for the 13 lipid parameters are reported in Table 2 for both cohorts. The typical values cohort showed systematically higher means and medians for atherogenic parameters than the reference subgroup (Table 2; full sex-stratified statistics in Supplementary Table S3).

Sex influence on lipid parameters in both cohorts

The effect of outlier removal on the sex-partitioning decision is detailed in Table 3 for both cohorts; the raw vs. cleaned values and full p-values are reported in Supplementary Table S4. In the reference subgroup, sex partitioning was statistically justified for TG, HDL-C, the TC/HDL-C ratio and TRL-C. In the typical values cohort, the list extended to TC, all four LDL-C estimates, non-HDL-C and lbLDL-C. Women showed lower TG concentrations and higher HDL-C concentrations than men in both cohorts after outlier removal.

Lipid parameters distribution and normalisation

Data distribution analysis revealed a notable difference in statistical behaviour between the two cohorts. In the reference subgroup, TC, HDL-C, non-HDL-C and the LDL-C estimates initially followed approximately normal distributions, whereas sdLDL-C, the TC/HDL-C ratio and triglyceride-related parameters (TG, TRL-C, VLDL-C) showed a right-skewed distribution. A Box-Cox transformation successfully normalised all skewed distributions prior to outlier removal. In the typical values cohort, most parameters required Box-Cox transformation. Box-Cox λ values, Kolmogorov-Smirnov diagnostics and graphical illustrations are reported in Supplementary

Table 1 Sociodemographic, anthropometric, lifestyle and medical-history characteristics of the reference subgroup and the typical values cohort.

		Reference subgroup (*n* = 241)				**Typical values cohort (*n* = 746)**			
Variable	Category	Combined	**Men (*n* = 106)**	**Women (*n* = 135)**	*p*-value	Combined	**Men (*n* = 335)**	Women (*n* = 411)	*p*-value
Anthropometric and clinical characteristics — mean ± SD									
Age (years)	—	38.51 ± 12.92	37.65 ± 12.85	39.19 ± 12.98	0.612	44.30 ± 14.73	45.90 ± 15.99	43.00 ± 13.50	**0.020***
BMI (kg/m²)	—	21.70 ± 1.86	21.47 ± 1.78	21.87 ± 1.91	0.090	24.00 ± 5.16	22.63 ± 3.93	25.13 ± 5.75	**< 0.001***
SBP (mmHg)	—	122.20 ± 9.84	124.44 ± 9.59	120.44 ± 9.70	**0.003***	132.07 ± 18.80	133.99 ± 16.47	130.50 ± 20.39	**< 0.001***
DBP (mmHg)	—	77.61 ± 6.80	77.09 ± 6.70	78.01 ± 6.87	0.292	83.56 ± 12.24	83.73 ± 12.03	83.42 ± 12.42	0.826
Hours since last meal	—	2.75 ± 2.15	2.82 ± 2.08	2.70 ± 2.21	0.348	2.91 ± 2.39	2.99 ± 2.31	2.84 ± 2.46	0.347
Lifestyle — n (%)									
Smoking	Never	220 (91.3)	88 (83.0)	132 (97.8)	**< 0.001***	616 (82.6)	214 (63.9)	402 (97.8)	**< 0.001***
	Former	21 (8.7)	18 (17.0)	3 (2.2)	—	87 (11.7)	78 (23.3)	9 (2.2)	—
	Current	0 (0.0)	0 (0.0)	0 (0.0)	—	43 (5.8)	43 (12.8)	0 (0.0)	—
Alcohol consumption	Never	213 (88.4)	91 (85.8)	122 (90.4)	0.376	606 (81.2)	255 (76.1)	351 (85.4)	**< 0.001***
	Occasional	28 (11.6)	15 (14.2)	13 (9.6)	—	95 (12.7)	43 (12.8)	52 (12.7)	—
	Regular (≥ monthly)	0 (0.0)	0 (0.0)	0 (0.0)	—	45 (6.0)	39 (11.7)	8 (1.9)	—
Physical activity	High	72 (29.9)	43 (40.6)	29 (21.5)	**0.001***	183 (24.5)	101 (30.1)	82 (20.0)	**0.010***
	Moderate	84 (34.9)	37 (34.9)	47 (34.8)	—	251 (33.6)	110 (32.8)	141 (34.3)	—
	Low	85 (35.3)	26 (24.5)	59 (43.7)	—	309 (41.4)	123 (36.7)	186 (45.3)	—
Meal frequency	Once daily	139 (57.7)	66 (62.3)	73 (54.1)	0.252	436 (58.4)	202 (60.3)	234 (56.9)	0.394
	More than once	102 (42.3)	40 (37.7)	62 (45.9)	—	310 (41.6)	133 (39.7)	177 (43.1)	—
Medical history — n (%)									
Personal history of CVD	Yes	0 (0.0)	0 (0.0)	0 (0.0)	—	102 (13.7)	41 (12.2)	61 (14.8)	0.621
Family history, type 1 DM	1st-degree relative	13 (5.4)	1 (0.9)	12 (8.9)	**0.024***	41 (5.5)	11 (3.3)	30 (7.3)	0.051
Family history, type 2 DM	1st-degree relative	24 (10.0)	5 (4.7)	19 (14.1)	0.053	91 (12.2)	22 (6.6)	69 (16.8)	**< 0.001***
Family history, CVD	1st-degree relative	95 (39.4)	45 (42.5)	50 (37.0)	0.640	253 (33.9)	116 (34.6)	137 (33.3)	0.621
Family history, CKD	1st-degree relative	6 (2.5)	3 (2.8)	3 (2.2)	0.954	15 (2.0)	6 (1.8)	9 (2.2)	0.861

BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; SD, Standard Deviation; CVD, Cardiovascular Disease; DM, Diabetes Mellitus; CKD, Chronic Kidney Disease. Continuous variables are reported as mean ± SD and compared using the Wilcoxon-Mann-Whitney test; categorical variables are reported as n (%) and compared using the Chi² test or the Fisher's exact test. The "—" symbol indicates non-applicable comparisons (i.e., when one cell is uniformly zero by selection criteria, or when only one modality exists). Significant p-values (< 0.05) are highlighted in bold red and marked with an asterisk (*). The exhaustive sex-stratified report, including geographic and ethnolinguistic distribution, is provided in Supplementary Tables S1 and S2.

Table 2 Baseline lipid profiles of the reference subgroup and the typical values cohort.

	Reference subgroup ($n=241$)				Typical values cohort ($n=746$)			
Lipid parameter (mmol/L)	*n*	Mean ± SD	Median (P25–P75)	Min–Max	*n*	Mean ± SD	Median (P25–P75)	Min–Max
Classical lipid parameters								
Triglycerides (TG)	240	1.14 ± 0.61	0.96 (0.75–1.38)	0.26–3.35	744	1.33 ± 0.79	1.10 (0.80–1.63)	0.11–6.05
Total cholesterol (TC)	241	5.48 ± 1.55	5.30 (4.37–6.39)	2.35–12.46	746	5.88 ± 1.74	5.67 (4.66–6.81)	2.10–14.95
HDL-C	241	1.86 ± 0.54	1.76 (1.47–2.15)	0.72–3.90	746	1.87 ± 0.60	1.74 (1.48–2.12)	0.52–6.32
Direct LDL-C	226	3.08 ± 1.11	2.90 (2.30–3.83)	0.70–6.46	691	3.34 ± 1.24	3.19 (2.51–3.99)	0.70–11.84
Friedewald LDL-C	241	3.10 ± 1.34	2.92 (2.15–3.80)	0.39–10.24	743	3.42 ± 1.48	3.24 (2.38–4.22)	0.03–11.84
Martin-Hopkins LDL-C	240	3.08 ± 1.34	2.92 (2.15–3.83)	0.18–10.21	744	3.45 ± 1.48	3.26 (2.41–4.25)	0.13–11.84
Sampson-NIH LDL-C	240	3.15 ± 1.34	2.97 (2.17–3.88)	0.31–10.24	744	3.47 ± 1.48	3.29 (2.44–4.30)	0.05–11.71
TC/HDL-C ratio	241	3.09 ± 1.00	2.94 (2.42–3.60)	1.28–8.76	746	3.34 ± 1.07	3.18 (2.60–3.95)	1.11–8.76
Emerging lipid parameters								
Non-HDL-C	241	3.62 ± 1.42	3.41 (2.61–4.42)	0.62–11.04	746	4.02 ± 1.63	3.86 (2.88–4.97)	0.62–12.77
lbLDL-C (NIH)	240	2.28 ± 1.01	2.17 (1.55–2.84)	0.05–7.24	744	2.46 ± 1.09	2.36 (1.74–3.06)	0.00–8.06
sdLDL-C (NIH)	240	0.88 ± 0.41	0.78 (0.59–1.11)	0.23–3.00	743	1.01 ± 0.49	0.93 (0.65–1.24)	0.05–3.76
VLDL-C (NIH)	240	0.47 ± 0.28	0.39 (0.28–0.57)	0.08–1.42	744	0.57 ± 0.39	0.44 (0.31–0.70)	0.05–3.68
TRL-C	241	0.49 ± 0.39	0.39 (0.31–0.57)	0.18–5.20	745	0.57 ± 0.41	0.44 (0.34–0.65)	0.18–5.21

TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; lbLDL-C, large-buoyant LDL-cholesterol; sdLDL-C, small-dense LDL-cholesterol; VLDL-C, Very-Low-Density Lipoprotein Cholesterol; TRL-C, Triglyceride-Rich Lipoprotein Cholesterol; NIH, Sampson-NIH equation. The exhaustive sex-stratified report is provided in Supplementary Table S3. Conversion factors: cholesterol, 1 mmol/L = 0.3867 g/L = 38.67 mg/dL; triglycerides, 1 mmol/L = 0.8857 g/L = 88.57 mg/dL.

Table 3 Sex-based comparison of lipid parameters in the reference (RI) subgroup and the typical values (TV) cohort after outlier removal.

	Reference subgroup			**Typical values cohort**			
Parameter (mmol/L)	**Men (n=106)**	**Women (n=135)**	**p-value**	**Men (n=335)**	**Women (n=411)**	**p-value**	**Outliers removed (RI / TV)**
Classical lipid parameters							
Triglycerides	1.26 ± 0.64	1.09 ± 0.58	**0.024***	1.38 ± 0.71	1.29 ± 0.75	**0.013***	2 / 6
Total cholesterol	5.30 ± 1.34	5.48 ± 1.47	0.502	5.66 ± 1.55	5.97 ± 1.55	**0.006***	4 / 14
HDL-C	1.71 ± 0.47	1.89 ± 0.44	**< 0.001***	1.76 ± 0.54	1.86 ± 0.47	**< 0.001***	8 / 14
Direct LDL-C	2.97 ± 1.01	3.10 ± 1.09	0.467	3.18 ± 1.03	3.41 ± 1.14	**0.007***	3 / 16
Friedewald LDL-C	3.00 ± 1.14	3.03 ± 1.22	0.999	3.21 ± 1.27	3.49 ± 1.32	**0.007***	5 / 15
Martin-Hopkins LDL-C	2.97 ± 1.11	3.03 ± 1.24	0.978	3.21 ± 1.27	3.49 ± 1.32	**0.005***	4 / 16
Sampson-NIH LDL-C	3.03 ± 1.14	3.08 ± 1.24	0.959	3.26 ± 1.27	3.54 ± 1.32	**0.006***	5 / 17
TC / HDL-C ratio	3.19 ± 0.89	2.97 ± 0.83	**0.034***	3.31 ± 0.97	3.33 ± 1.03	0.783	5 / 8
Emerging lipid parameters							
Non-HDL-C	3.54 ± 1.22	3.57 ± 1.37	0.747	3.85 ± 1.47	4.09 ± 1.50	**0.033***	3 / 10
lbLDL-C (NIH)	2.15 ± 0.85	2.25 ± 0.93	0.526	2.28 ± 0.93	2.56 ± 0.98	**< 0.001***	4 / 16
sdLDL-C (NIH)	0.91 ± 0.39	0.85 ± 0.39	0.276	0.98 ± 0.44	1.01 ± 0.47	0.441	1 / 6
VLDL-C (NIH)	0.49 ± 0.28	0.44 ± 0.26	0.058	0.57 ± 0.34	0.54 ± 0.36	0.088	1 / 4
TRL-C	0.52 ± 0.26	0.44 ± 0.23	**0.008***	0.57 ± 0.31	0.54 ± 0.41	**0.008***	1 / 1

TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; lbLDL-C, large-buoyant LDL-cholesterol; sdLDL-C, small-dense LDL-cholesterol; VLDL-C, Very-Low-Density Lipoprotein Cholesterol; TRL-C, Triglyceride-Rich Lipoprotein Cholesterol; NIH, Sampson-NIH equation.

Conversion factors: cholesterol, 1 mmol/L = 0.3867 g/L = 38.67 mg/dL; triglycerides, 1 mmol/L = 0.8857 g/L = 88.57 mg/dL.

Outliers were identified on the Box-Cox transformed scale using Tukey's fences(CLSI EP28-A3c) and removed prior to between-sex comparison. p-values from the Wilcoxon-Mann-Whitney test (Men vs Women); * $p < 0.05$ — drives sex-partitioning for the corresponding reference interval. Sex partitioning is therefore retained for: HDL-C, the TC/HDL-C ratio, TG and TRL-C in the reference subgroup; and for TG, TC, HDL-C, all four LDL-C estimates, non-HDL-C, lbLDL-C and TRL-C in the typical values cohort. Raw (pre-outlier-removal) data are reported in Supplementary Table S4; λ values and Kolmogorov-Smirnov diagnostics for the Box-Cox transformation are reported in Supplementary Table S5.

Table S5 and Supplementary Figures S1 to S6. Figure 1 illustrates the typical normalisation achieved for four representative parameters (TG, TC, TC/HDL-C ratio, sdLDL-C) in the reference subgroup.

Reference intervals versus typical values

A clear discrepancy was observed between the RIs derived from the strictly healthy subgroup and the typical values derived from the general cohort (Table 4 and Table 5). Reference intervals were consistently narrower and lower; typical values were markedly shifted upwards. Sex-based partitioning was required for HDL-C, the TC/HDL-C ratio, TG and TRL-C in the reference subgroup (Table 4; Supplementary Table S6), while only the TC/HDL-C ratio, sdLDL-C and VLDL-C remained sex-independent in the typical values cohort (Table 5; Supplementary Table S7).

Clinical decision limits

Applying the three-tier framework to our data, the clinical decision limits derived from the reference subgroup are summarised in Table 4. For total cholesterol, the optimal target is < 5.30 mmol/L (limit zone 5.30–6.39; high risk > 6.39); for LDL-C (Sampson-NIH), optimal < 2.97 mmol/L (limit zone 2.97–3.88; high risk > 3.88); for triglycerides, optimal < 0.96 mmol/L (limit zone 0.96–1.38; high risk > 1.38); for HDL-C, the inverted logic yields an optimal target > 1.76 mmol/L (limit zone 1.47–1.76; high risk < 1.47). Sex-stratified thresholds, applied where the cleaned-data sex comparison reached statistical significance (Table 3), are also reported in Table 4; the

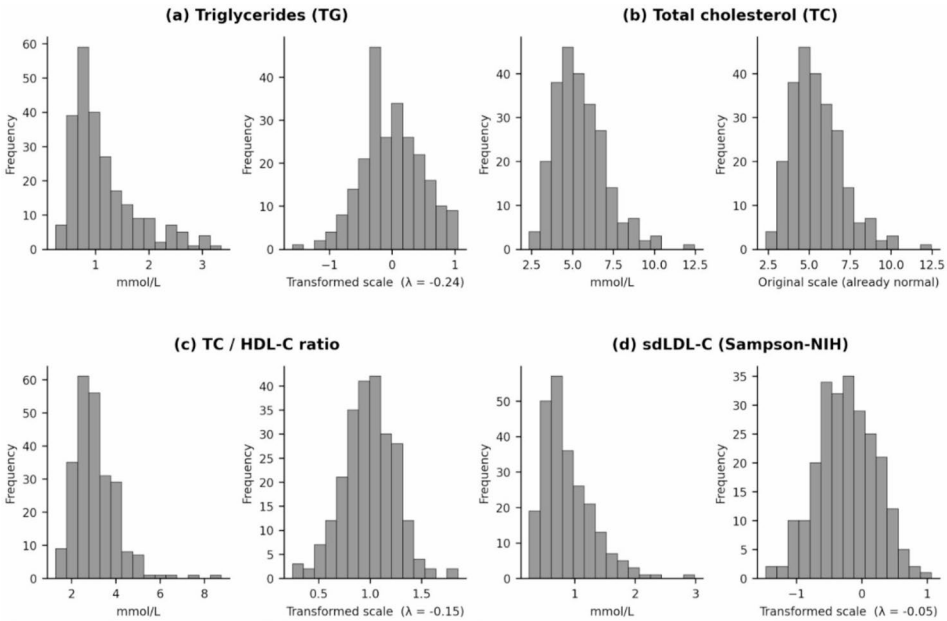

Fig. 1 Distribution of triglycerides (TG), total cholesterol (TC), the TC/HDL-C ratio and small-dense LDL-C (sdLDL-C, Sampson-NIH equation) before and after Box-Cox transformation in the reference subgroup. Each panel shows the original (left) and the Box-Cox-transformed (right) distribution with the corresponding power parameter λ. TG, Triglycerides; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; sdLDL-C, small-dense LDL Cholesterol computed using the Sampson-NIH 2021 equation. The original distributions of TG, the TC/HDL-C ratio and sdLDL-C were significantly skewed (Kolmogorov-Smirnov, $p < 0.001$), whereas TC was already-normal. Box-Cox transformation successfully normalised all skewed distributions ($p > 0.05$ after transformation), enabling Tukey-based outlier removal prior to non-parametric percentile estimation. Diagnostic Box-Cox plots for all 13 lipid parameters in both cohorts are provided in Supplementary Figures S1 to S6.

Table 4 Reference intervals and clinical decision limits for the strictly healthy reference subgroup.

Parameter (mmol/L)	Group	*n*	Lower limit (P2.5) [90% CI]	Upper limit (P97.5) [90% CI]	Optimal (<P50)	Limit zone (P50–P75)	High risk (>P75)
Classical lipid parameters							
Triglycerides	*Combined*	239	0.47 [0.42–0.50]	2.73 [2.51–3.01]	<0.96	0.96–1.38	>1.38
	Men	105	0.50 [0.47–0.59]	2.81 [2.51–3.14]	<1.04	1.04–1.50	>1.50
	Women	134	0.43 [0.37–0.49]	2.70 [2.26–3.05]	<0.90	0.90–1.26	>1.26
Total cholesterol	*Combined*	237	3.13 [2.98–3.42]	8.66 [8.08–8.94]	<5.30	5.30–6.39	>6.39
HDL-C[a]	*Combined*	233	1.14 [1.04–1.19]	2.77 [2.69–2.88]	>1.76	1.47–1.76	<1.47
	Men	102	1.09 [0.93–1.14]	2.64 [2.56–2.75]	>1.66	1.40–1.66	<1.40
	Women	130	1.19 [1.14–1.27]	2.82 [2.72–2.95]	>1.91	1.58–1.91	<1.58
Direct LDL-C	*Combined*	223	1.32 [1.11–1.53]	5.39 [4.90–5.83]	<2.90	2.90–3.83	>3.83
Friedewald LDL-C	*Combined*	236	1.01 [0.73–1.32]	5.54 [5.16–5.96]	<2.92	2.92–3.80	>3.80
Martin-Hopkins LDL-C	*Combined*	236	0.91 [0.70–1.35]	5.67 [5.18–5.96]	<2.92	2.92–3.83	>3.83
Sampson-NIH LDL-C	*Combined*	235	1.01 [0.73–1.32]	5.60 [5.18–6.04]	<2.97	2.97–3.88	>3.88
TC/HDL-C ratio	*Combined*	236	1.79 [1.75–1.85]	4.92 [4.73–5.26]	<2.94	2.94–3.60	>3.60
	Men	103	1.56 [1.38–1.95]	4.97 [4.67–5.26]	<3.13	3.13–3.75	>3.75
	Women	133	1.77 [1.36–1.83]	4.55 [4.27–4.88]	<2.79	2.79–3.39	>3.39
Emerging lipid parameters							
Non-HDL-C	*Combined*	238	1.37 [1.11–1.71]	6.53 [5.98–6.81]	<3.41	3.41–4.42	>4.42
lbLDL-C (NIH)	*Combined*	236	0.62 [0.39–0.88]	4.17 [3.70–4.48]	<2.17	2.17–2.84	>2.84
sdLDL-C (NIH)	*Combined*	239	0.34 [0.31–0.39]	1.81 [1.68–1.94]	<0.78	0.78–1.11	>1.11
VLDL-C (NIH)	*Combined*	239	0.16 [0.13–0.18]	1.22 [1.04–1.27]	<0.39	0.39–0.57	>0.57
TRL-C	*Combined*	240	0.21 [0.21–0.23]	1.09 [1.01–1.22]	<0.39	0.39–0.57	>0.57
	Men	106	0.23 [0.21–0.26]	1.14 [1.01–1.27]	<0.41	0.41–0.62	>0.62
	Women	135	0.21 [0.18–0.23]	1.14 [0.91–1.27]	<0.36	0.36–0.52	>0.52

TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; lbLDL-C, large-buoyant LDL-cholesterol; sdLDL-C, small-dense LDL-cholesterol; VLDL-C, Very-Low-Density Lipoprotein Cholesterol; TRL-C, Triglyceride-Rich Lipoprotein Cholesterol; NIH, Sampson-NIH equation.

Conversion factors: cholesterol, 1 mmol/L = 0.3867 g/L = 38.67 mg/dL; triglycerides, 1 mmol/L = 0.8857 g/L = 88.57 mg/dL.

Reference intervals computed according to CLSI EP28-A3c using the non-parametric percentile method (P2.5 and P97.5) with 90% bootstrap confidence intervals (n = 5000 iterations). Decision limits derived from the strictly healthy reference subgroup distribution: Optimal target (<P50), Limit zone (P50–P75), High risk (>P75). P50 corresponds to the median; the lower bound of the Optimal column therefore reads the median value of the distribution. [a] HDL-C inverted logic: Optimal (>P50), Limit zone (P25–P50), High risk (<P25). Sex partitioning is applied only when the Wilcoxon-Mann-Whitney test on cleaned data showed $p < 0.05$ (Table 3): TG, HDL-C, TC/HDL-C ratio and TRL-C. The exhaustive sex-stratified report — including reference values for both sexes for every parameter, irrespective of the statistical significance of the sex difference — is provided in Supplementary Table S6.

exhaustive sex-stratified report, irrespective of statistical significance, is provided in Supplementary Tables S6 and S7.

Comparison of Senegalese reference intervals with published international data

Table 6 summarises the Senegalese non-fasting lipid RIs derived from the present study and compares them with RIs reported in published international data[23–37]. The literature comparison contextualises our findings within the global landscape of lipid reference data and reveals substantial inter-population heterogeneity in both the median values and the upper percentile limits.

Table 5 Reference values for lipid parameters in a supposedly healthy Senegalese population with potential cardiovascular risk factors (Typical values cohort)

Parameter (mmol/L)	Group	n	Median (P50)	Lower limit (P2.5) [90% CI]	Upper limit (P97.5) [90% CI]
Classical lipid parameters					
Triglycerides	*Combined*	738	1.10	0.49 [0.48–0.53]	3.12 [3.01–3.40]
	Men	331	1.20	0.53 [0.49–0.59]	3.07 [2.93–3.40]
	Women	407	1.03	0.46 [0.45–0.50]	3.14 [2.97–3.89]
Total cholesterol	*Combined*	732	5.67	3.16 [3.08–3.49]	9.52 [9.05–9.83]
	Men	327	5.49	3.13 [3.05–3.36]	9.49 [8.56–10.19]
	Women	405	5.80	3.52 [3.05–3.67]	9.52 [9.03–9.96]
HDL-C[a]	*Combined*	732	1.74	1.14 [1.09–1.16]	3.00 [2.90–3.18]
	Men	325	1.66	1.06 [0.98–1.14]	3.13 [2.82–3.36]
	Women	407	1.81	1.19 [1.16–1.22]	2.95 [2.82–3.03]
Direct LDL-C	*Combined*	675	3.19	1.53 [1.45–1.63]	5.77 [5.43–6.00]
	Men	307	3.06	1.42 [1.32–1.58]	5.30 [5.09–5.84]
	Women	368	3.29	1.63 [1.50–1.71]	5.97 [5.53–6.13]
Friedewald LDL-C	*Combined*	728	3.24	1.27 [1.03–1.40]	6.23 [5.97–6.62]
	Men	323	3.06	1.16 [0.98–1.42]	5.92 [5.25–6.80]
	Women	405	3.37	1.32 [1.03–1.50]	6.44 [6.08–6.62]
Martin-Hopkins LDL-C	*Combined*	728	3.26	1.16 [1.01–1.40]	6.18 [5.95–6.57]
	Men	325	3.11	1.09 [0.93–1.37]	5.90 [5.30–6.78]
	Women	403	3.37	1.29 [1.09–1.53]	6.41 [6.05–6.75]
Sampson-NIH LDL-C	*Combined*	727	3.29	1.19 [1.03–1.42]	6.34 [6.03–6.65]
	Men	323	3.16	1.14 [0.93–1.42]	6.03 [5.43–6.85]
	Women	404	3.45	1.32 [1.11–1.55]	6.52 [6.15–6.65]
TC/HDL-C ratio	*Combined*	738	3.18	1.76 [1.68–1.81]	5.74 [5.50–5.88]
Emerging lipid parameters					
Non-HDL-C	*Combined*	736	3.86	1.66 [1.45–1.76]	7.21 [6.96–8.02]
	Men	329	3.70	1.58 [1.29–1.76]	7.19 [6.47–8.20]
	Women	407	3.91	1.71 [1.53–1.86]	7.24 [6.88–8.12]
lbLDL-C (NIH)	*Combined*	724	2.36	0.85 [0.75–0.93]	4.63 [4.42–4.81]
	Men	318	2.20	0.83 [0.70–0.98]	4.22 [3.90–4.73]
	Women	406	2.44	0.91 [0.65–1.06]	4.76 [4.45–4.91]
sdLDL-C (NIH)	*Combined*	737	0.93	0.36 [0.36–0.39]	2.17 [1.99–2.40]
VLDL-C (NIH)	*Combined*	740	0.44	0.18 [0.16–0.18]	1.47 [1.34–1.60]
TRL-C	*Combined*	744	0.44	0.21 [0.21–0.23]	1.34 [1.27–1.63]
	Men	333	0.47	0.23 [0.21–0.26]	1.24 [1.19–1.40]
	Women	411	0.41	0.21 [0.18–0.23]	1.50 [1.29–1.81]

TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; lbLDL-C, large-buoyant LDL-cholesterol; sdLDL-C, small-dense LDL-cholesterol; VLDL-C, Very-Low-Density Lipoprotein Cholesterol; TRL-C, Triglyceride-Rich Lipoprotein Cholesterol; NIH, Sampson-NIH equation.

Conversion factors: cholesterol, 1 mmol/L = 0.3867 g/L = 38.67 mg/dL; triglycerides, 1 mmol/L = 0.8857 g/L = 88.57 mg/dL.

Reference intervals computed according to CLSI EP28-A3c using the non-parametric percentile method (P2.5 and P97.5) with 90% CI estimated by bootstrap (n = 5 000 iterations). Important caveat: the typical values cohort includes adults with latent metabolic and cardiovascular risk factors (smoking, alcohol use, sedentary lifestyle, elevated BMI). The reference intervals derived from this cohort therefore reflect the actual distribution of the Senegalese adult population and should not be used as 'normal' or 'healthy' thresholds; the strictly healthy reference subgroup (Table 4 and Supplementary Table S6) provides those values. Table 5 retains only the statistically justified sex partitions, the exhaustive sex-stratified report is shown on Supplementary Table S6.

Discussion

To our knowledge, this study is the first to establish reference intervals (RIs) for an extended lipid profile in Senegalese adults in a non-fasting state. By aligning with the EAS/EFLM joint consensus and with the position of the AHA/ACC and the National Lipid Association, our findings confirm the technical feasibility of non-fasting lipid

Table 6 Comparison of reference intervals from the present study with published global literature.

Authors/Reference	Study Location	Sex	*n*	Age (years)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Current Study (Reference Intervals)	*Senegal*, *2026*	Combined	241	20–79	3.13–8.66	1.14–2.77	1.01–5.60	0.47–2.73
		Women	135		3.10–8.77	1.19–2.82	1.06–5.97	0.43–2.81
		Men	106		3.13–8.07	1.09–2.64	1.09–5.17	0.50–2.70
Current Study (Typical Values)	*Senegal*, *2026*	Combined	746	19–80	3.16–9.52	1.14–3.00	1.19–6.34	0.49–3.12
		Women	411		3.52–9.52	1.19–2.95	1.32–6.52	0.45–3.11
		Men	335		3.13–9.49	1.06–3.13	1.14–6.03	0.52–3.04
Ayemoba et al. [23]	*Nigeria*, *2021*	Combined	6 169	18–26	2.40–6.20	0.50–2.70	0.80–4.50	0.50–1.80
		Women	237		2.20–6.30	0.40–2.30	0.90–4.70	0.40–1.60
		Men	5 932		2.40–6.20	0.50–2.70	0.80–4.50	0.50–1.80
Achila et al. [24]	*Eritrea*, *2017*	Combined	255	—	2.99–7.69	0.80–2.06	—	—
		Women	106	60–80	3.35–7.64	0.85–2.06	—	—
		Men	119		2.58–8.00	1.06–2.06	—	—
Tang Tsana et al. [25]	*Cameroon*, *2022*	Combined	422	18–65	2.94–6.02	0.90–2.06	1.37–4.13	0.35–1.36
		Women	248		3.12–5.99	1.00–2.01	1.44–3.95	0.31–1.20
		Men	194		2.87–6.02	0.80–2.01	0.52–1.67	0.34–1.48
Koumaré et al. [26]	*Burkina Faso*, *2015*	Women	140	15–50	2.93–5.86	0.79–1.68	1.16–4.18	—
		Men	139		2.93–5.81	0.65–1.77	1.18–3.99	—
Abebe et al. [27]	*Ethiopia*, *2018*	Combined	1 175	18–61	2.06–5.34	—	—	0.41–2.47
		Women	—		2.17–5.24	—	—	0.90–2.30
		Men	—		2.01–5.47	—	—	0.41–2.54
Noma et al. [28]	*Japan*, *1991*	Women	467	21–60	3.48–5.96	0.93–2.12	—	0.35–1.54
		Men	677		3.51–5.80	0.70–2.00	—	0.46–1.70
Jia et al. [29]	*China*, *2023*	Combined	42 978	18–78	4.33–5.25	1.23–1.46	2.56–3.38	0.90–1.59
		Women	21 905		3.27–6.87	0.99–2.27	1.63–4.74	0.43–3.10
		Men	21 073		3.32–6.77	0.85–1.94	1.78–4.76	0.55–4.49
Sairam et al. [30]	*India*, *2014*	Women	3 187	20–70	3.02–6.50	0.75–1.81	1.57–4.39	0.59–2.37
		Men	7 478		2.97–6.55	0.64–1.57	1.55–4.54	0.63–3.05
Borai et al. [31]	*Saudi Arabia*, *2016*	Combined	804	18–65	3.50–6.36	—	1.80–4.34	—
		Women	—		—	0.98–2.19	—	0.39–1.60
		Men	—		—	0.74–1.76	—	0.50–3.58
Rahmani et al. [32]	*Iran*, *2019*	Women	594	20–73	3.04–6.09	0.93–2.16	1.29–4.15	0.43–2.10
		Men	548		3.12–6.73	0.80–1.85	1.39–4.52	0.54–3.44
Tietz Textbook [33]	*USA*, *2018*	Combined	—	20–29	3.00–5.91	—	—	—
		Combined	—	30–39	3.81–6.89	—	—	—
		Combined	—	40–79	3.60–7.10	—	—	—
		Women	—	15–79	—	0.90–2.30	—	—
		Men	—	15–79	—	0.80–1.80	—	—
		Women	—	25–49	—	—	1.30–4.60	—
		Men	—	25–49	—	—	1.60–4.90	—
		Combined	—	50–79	—	—	1.90–4.90	—
		Women	—	30–79	—	—	—	0.40–2.40
		Men	—	30–79	—	—	—	0.50–3.40
Carroll et al. [34]	*USA*, *1993*	Women	4 114	20–74	3.69–7.87	0.85–2.06	2.08–5.68	0.56–3.01
		Men	4 572		3.71–7.50	0.74–1.75	2.06–5.37	0.63–3.56
Connelly et al. [35]	*Canada*, *1992*	Women	8 571	18–74	2.94–7.29	0.73–2.08	1.24–4.90	0.36–3.16
		Men	8 348		3.06–7.29	0.89–1.78	1.40–5.03	1.04–4.50

Table 6 (continued)

Authors/Reference	Study Location	Sex	*n*	Age (years)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Maatela et al. [36]	*Finland, 1994*	Women	299	30–99	4.10–8.57	0.95–2.19	2.50–6.53	0.46–2.31
Marniemi et al. [37]		Men	292		4.22–8.41	0.80–1.91	2.67–6.56	0.52–2.84

TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TG, Triglycerides. Reference intervals are reported as Lower Limit – Upper Limit (P2.5 – P97.5). "—" indicates that the parameter was not reported in the original study or that the corresponding subgroup count was not specified. Conversion factors: cholesterol, 1 mmol/L = 0.3867 g/L = 38.67 mg/dL; triglycerides, 1 mmol/L = 0.8857 g/L = 88.57 mg/dL. Cross-study comparisons should be interpreted with caution: the studies summarised here span more than three decades and use heterogeneous lipids determination (beta-quantitation, the reference method and direct enzymatic methods) as well as different fasting protocols, all of which may influence the reported upper reference limits.

profiling in a sub-Saharan African setting and provide the first locally derived non-fasting reference framework for Senegal[6,7,9].

We acknowledge, however, that the question of fasting versus non-fasting profiling remains debated. The most recent international guidelines—the 2025 ESC/EAS focused update, the 2026 ACC/AHA/Multisociety guideline on the management of dyslipidemia, and the joint French SFE/SFD/NSFA/SFC consensus—still endorse fasting cut-points for triglycerides and acknowledge the relevance of fasting blood glucose as part of dyslipidaemia screening, while the 2024 ADLM Guidance Document explicitly states that fasting is no longer routinely required for lipid panels[20–22,38]. Our position is therefore not to advocate for the abandonment of fasting profiling, but rather to provide a parallel, locally derived framework for non-fasting screening, which is particularly relevant to West-African outpatient practice where fasting compliance is challenging. The choice between fasting and non-fasting profiling should remain context-specific and guided by the clinical question, in line with prevailing guidelines.

Such cross-study comparisons should be interpreted with caution: the studies summarised in Table 6 span more than three decades and use two different determination methods (beta-quantitation, which remains the reference method (Finland) and enzymatic method (all the other studies listed)) which may influence the reported upper reference limits[23–37]. With this methodological reservation in mind, comparative analysis of our results with global literature reveals a dual specificity within the Senegalese population, suggesting the influence of both genetic factors and the nutritional transition[39–41]. On one hand, we identified the protective lipid phenotype frequently described in Black African populations, characterised by relatively low TG and elevated HDL-C compared to Caucasian standards (Table 2)[41,42]. This metabolic profile (low TG/high HDL-C) is classically attributed to higher plasma lipoprotein lipase activity which promotes TG clearance, coupled with lower hepatic lipase activity which preserves HDL particles[40–42]. The observed median TG (1.10 mmol/L), significantly lower than the European threshold of 1.98 mmol/L, attests to efficient postprandial clearance and corroborates the theory of increased lipoprotein lipase (LPL) activity in Black African subjects[6]. On the other hand, our upper limits for TC and LDL-C differ markedly from those reported in other recent African (Nigeria, Eritrea, Cameroon, Burkina Faso, Ethiopia) and Asian (Japan, China, India, Saudi Arabia, Iran) studies, which display lower thresholds (Table 6)[23–32]. Our values align more closely with North American (USA, Canada) or European (Finland) standards (Table 6)[33–37]. This finding likely reflects a marked Westernisation of lifestyle in Senegal coupled with specific genetic susceptibility. Consequently, using reference values imported from other African nations or manufacturer inserts based on disparate populations would pose a significant risk of misclassifying dyslipidaemias in Senegal.

Consistent with established physiology, a marked sex influence was observed. Women in the reference subgroup exhibited a more protective lipid profile, with higher HDL-C and a lower atherogenic index (TC/HDL-C ratio) than men (Table 3). Notably, sex also influenced concentrations of triglycerides and cholesterol associated with TG-rich lipoproteins (TRL); men displayed significantly higher concentrations of TG and TRL-C than women (Table 3). These differences justify the establishment of sex-specific reference intervals for HDL-C, the TC/HDL-C ratio, TG and TRL-C in the reference subgroup. However, analysis of the typical values cohort showed a partial reversal of this trend, with women displaying a more atherogenic profile than men, characterised by higher concentrations of TC, non-HDL-C and LDL-C (Table 3). This shift probably reflects a higher

prevalence of obesity and sedentary behaviour among Senegalese women as suggested by our anthropometric and lifestyle data (higher BMI and lower physical activity levels) (Table 1).

A major finding of this study is the significant discrepancy between reference intervals and typical population values. Typical values show a marked upward drift; in this group, the upper limits (97.5th percentile) for TC and LDL-C considerably exceed the therapeutic intervention thresholds defined by the European consensus or NCEP ATP III[6,43]. The inclusion of pathological profiles within the typical values reflects the high prevalence of latent metabolic and cardiovascular risk factors, such as smoking, alcohol consumption, sedentary lifestyle, obesity, and hypercaloric diets, among clinically asymptomatic subjects. This underscores the necessity of applying strict exclusion criteria, in accordance with the CLSI EP28-A3c protocol, to define reference intervals[19]. Relying on raw population statistics alone would lead to the misclassification of pathological values as "normal".

Furthermore, our results highlight a major discordance between statistical reference intervals and clinical decision limits or cardiovascular health targets. Despite the rigorous selection of our reference subgroup, the upper limits (97.5th percentile) for several parameters exceed the risk limits accepted by the NCEP ATP III[43]. This is particularly evident for TC and LDL-C (Sampson-NIH), where the upper limits (8.66 mmol/L and 5.60 mmol/L, respectively) included manifestly atherogenic concentrations. Building on the locally derived three-tier framework defined in the Methods and applied in the Results (Table 4), the relevance of these decision limits is now examined against the recommendations of the NCEP ATP III, the 2025 ESC/EAS focused update and the 2026 ACC/AHA/Multisociety guideline on the management of dyslipidemia[21,22,43].

This dissociation echoes a general doctrine, formally established by the IFCC Committee on Reference Values and Decision Limits (IFCC-LM): statistical reference intervals are descriptive in nature, reflecting the distribution of a healthy population, and must not be confused with clinical decision limits, which are interpretative thresholds defined on the basis of the relationship between an analyte concentration and the risk of an adverse clinical outcome. Although particularly visible for lipid parameters, this principle applies broadly across clinical chemistry biomarkers and underpins the rationale for our three-tier framework.

Beyond this general doctrine, our three-tier model, based on the median and the 75th percentile of a strictly healthy reference subgroup, was developed empirically before the publication of the 2024 ADLM Guidance Document on the Measurement and Reporting of Lipids and Lipoproteins, which subsequently endorsed precisely the same stratification principle. In this guidance, the ADLM expert panel explicitly states that "desirable" lipid values are best defined between the 50th and 75th percentiles of healthy populations, a principle now formalised as recommendation of the document and endorsed for both laboratory professionals and clinicians[20]. The trans-Atlantic convergence between the European EAS/EFLM joint consensus and the North American ADLM guidance on this stratification principle reinforces the methodological soundness of our locally derived thresholds and supports their adoption as an interpretative framework for routine lipid reporting in Senegalese clinical biology[6,20].

As reported in Table 4 and Tables S6-7, application of this three-tier framework to our cohort yielded parameter-specific optimal targets, limit-zone bounds and high-risk thresholds. The external coherence of these locally derived thresholds with internationally recognised cardiovascular-prevention guidelines is examined below.

The relevance of this model is confirmed when compared with NCEP ATP III and EAS/EFLM recommendations[6,43]. Our local optimal target for LDL-C (2.97 mmol/L) corresponds exactly to the alert threshold recommended by the EAS/EFLM for moderate risk[6]. Moreover, our high-risk threshold (3.88 mmol/L) anticipates the 4.14 mmol/L intervention threshold of the NCEP ATP III[43]. The clinical utility of our stratification model is particularly highlighted by the analysis of calculated sdLDL-C. Our high-risk threshold (75th percentile) corresponds to a concentration of 1.11 mmol/L. This local threshold is remarkably consistent with the 1.19 mmol/L cut-off derived from the Sampson sdLDL-C equation, originally developed using NHANES data and subsequently validated independently within the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, highlighting a robust model convergence for detecting high-risk patients who elude classic criteria[15,44]. This consistency extends to other parameters; for triglycerides, our local risk threshold of 1.38 mmol/L offers increased sensitivity compared to the standard 1.69 mmol/L norm. For HDL-C, using the 25th percentile would set the alert at > 1.40

mmol/L for men and > 1.58 mmol/L for women, maintaining a higher protection requirement than the Western threshold of 1.03 mmol/L. These new benchmarks provide Senegalese clinicians with a framework aligned with local biological reality, fostering earlier detection of metabolic shifts, particularly metabolic syndrome associated with insulin resistance[41]. Finally, for total cholesterol, the European alert threshold of 4.91 mmol/L is lower than our median of 5.30 mmol/L[6]. Reliance on this criterion alone would lead to many healthy Senegalese adults being wrongly classified as dyslipidaemic. This "false hypercholesterolaemia" is explained by the specificity of the Senegalese lipid phenotype: high HDL-C concentrations arithmetically inflate the TC. Isolated use of TC is therefore misleading in Senegal, risking false positives due to high HDL. To bypass this bias, clinicians should shift their focus from TC to the TC/HDL-C ratio. Our results define specific alert thresholds for this ratio (> 3.75 in men and > 3.39 in women) offering risk stratification far truer to the local metabolic reality.

On the practical side, our decision-limit framework is intended to support an evolution in the layout of lipid laboratory reports, from a purely numerical reference-interval format toward a layered output combining the measured value, the corresponding optimal/limit/high-risk zone and a brief interpretative comment integrating the patient's overall cardiovascular risk profile. This evolution is fully consistent with the EAS/EFLM joint consensus and with the recent AHA/ACC scientific statements, and is in line with the increasing trend in clinical-chemistry practice to provide post-analytical decision support directly within the laboratory report[6,7].

It is essential to underline that the proposed decision limits are intended as a laboratory-side decision-support tool and not as a stand-alone diagnostic threshold. The clinical interpretation of the lipid profile cannot be reduced to the comparison of a single concentration with a single cut-point: it must remain embedded in a global, multivariable assessment of cardiovascular risk, ideally relying on validated risk-stratification scores such as the AHA-PREVENT equations, now formally endorsed by the 2026 ACC/AHA Multisociety dyslipidaemia guideline in place of the Pooled Cohort Equations, or the European SCORE2 and SCORE2-OP charts[22,45,46]. As no fully validated cardiovascular risk score is currently available for sub-Saharan African populations, the development and validation of a dedicated regional score, integrating local genetic, environmental and nutritional specificities, should be a priority for future research.

Our study has several limitations. Firt, the cross-sectional design precludes any direct causal link with the occurrence of cardiovascular events, which will require future longitudinal validation against incident outcomes. Second, the lack of meal standardisation may have influenced post-prandial lipid concentrations; however, this design choice was deliberate to capture real-world physiological variability and ensure that the proposed decision limits remain pragmatic and applicable in routine outpatient practice.

Third, fine age-stratification of the reference subgroup was not feasible given the CLSI minimum of 120 individuals per partition; the intervals reported here are therefore age-pooled adult values[19]. Although the male subgroup ($n = 106$) was slightly below this ideal target, the robustness of our intervals is ensured by the use of Bootstrap-based 90% confidence intervals.

Fourth, apolipoprotein B and lipoprotein(a), increasingly recommended by current international guidelines, were not measured within the SEN-GENOME cohort due to mainly budgetary and analytical-platform constraints[20–22]. Their incorporation is explicitly planned for the next phase of the project. Pending the establishment of local Apo-B and Lp(a) reference values, the non-HDL-C and TC/HDL-C-ratio thresholds reported here can be used as practical proxies in the Senegalese clinical context.

Finally, we acknowledge that no formal clinical validation of the proposed three-tier decision-limit framework involving an external panel of clinicians was conducted within the present work. The 2024 ADLM Guidance Document on the Measurement and Reporting of Lipids and Lipoproteins, which endorses the use of the 50th–75th percentiles of a strictly healthy reference subgroup as the optimal-desirable interval, was published after the design and analytical phases of our study, and its conceptual framework retrospectively supports the empirical approach we adopted. A prospective clinical validation of the locally derived thresholds, involving Senegalese specialists in internal medicine, cardiology, neurology and clinical biology, and longitudinal assessment against incident cardiovascular events, is explicitly planned as part of the next phase of the SEN-GENOME programme.

Conclusion

This study provides the first non-fasting lipid reference framework in Senegal, derived in strict accordance with the CLSI EP28-A3c protocol from a community-based cohort that encompasses all 14 administrative regions of the country. Our findings highlight the inadequacy of conventional statistical reference intervals (2.5–97.5th percentiles), which risk normalising latent atherogenic risk within the population. Consequently, we advocate for a paradigm shift toward pragmatic clinical decision thresholds based on the median and the 75th percentile—a model whose strong alignment with international cardiovascular guidelines is reinforced by the 2024 ADLM Guidance Document, published after the design of the present study. We specifically recommend the discontinuation of isolated total cholesterol interpretation in favour of the TC/HDL-C ratio, and the widespread adoption of non-fasting profiling for outpatient cardiovascular screening. Such a transition will ensure more effective, accessible, and culturally adapted primary cardiovascular prevention in Senegal.

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Data availability The data that support the findings of this study are available from the corresponding author, El Hadji Malick NDOUR, upon reasonable request. The data are not publicly available due to participant privacy and ethical restrictions governed by the Senegal's National Ethics Committee for Health Research (CNERS) within the framework of the SEN-GENOME project.

Declarations

Competing interests The authors declare no competing interests.

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