Fasting versus Nonfasting Lipids for Cardiovascular Disease Risk Estimation among healthy adults in Ibadan, Nigeria: A cross-sectional study

Nonfasting lipids for CVD risk assessment
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Abstract
Objective: Risk assessment at the individual level requires incorporating lipid profile results into several cardiovascular disease (CVD) risk estimating equations. Traditionally, fasting is usually required before the lipid profile studies. Recent guidelines have recommended the acceptability of non-fasting lipids for this assessment based on reports from several countries. We aimed to compare the agreement between 10-year risk estimates obtained using fasting and non-fasting lipids from apparently healthy Nigerians.

Methods: This was a cross-sectional study of 111 participants. Serum blood lipids were measured after a 12-hour overnight fast and after a standard local Nigerian meal. Risk estimations with the pooled cohort equations (PCE) and the Framingham risk score (FRS) equation were done with fasting and non-fasting lipid results. Correlations were done with Pearson's coefficient and agreement of proportions with McNemar's test.

Results: Comparing fasting versus non-fasting values, total cholesterol was within 10% for 58 (52.3%), triglycerides were >30% for 65 (58.6%), and high-density lipoprotein cholesterol was <30% in 60 (70.0%) participants. An increase in Low-density lipoprotein cholesterol was seen in 93 (82.8%) participants. With the PCE, 1 (0.8%) persons, had borderline risk with both their fasting and non-fasting samples. With the FRS, 1 participant who was categorised as moderate risk with the fasting sample was classified as low risk with the non-fasting sample. There was no significant difference in risk categorisation by the equations, p =1.0.

Conclusion: Risk categorisation by two (2) CVD risk estimating equations was not significantly affected by the fasting or non-fasting status of a healthy population.

Keywords: Lipids, Nonfasting, Cardiovascular, Risk Assessment

Plain English Summary
People are usually requested to fast for twelve hours before their blood samples may be collected for cholesterol tests. The results of such tests are then used to calculate their risk of developing diseases such as heart attack and stroke. This study shows that fasting may not be required before samples are collected for blood cholesterol tests in apparently healthy individuals as it has minimal effect on the calculations done to determine their risk of the aforementioned diseases.
Introduction
Atherosclerotic cardiovascular diseases (ASCVD) like ischemic heart disease, stroke and peripheral artery disease are the largest contributors to the increasing cardiovascular disease (CVD) burden in sub-Saharan Africa (1). According to a country report, a Nigerian aged between 30 and 70 years of age has a 22% chance of dying from a major noncommunicable disease, with CVD accounting for over half of that risk (2). Primary prevention at the individual level has made important contributions to the reduced incidence rates that are responsible for the declining mortality trends from CVD over the past 2 decades in high-income countries (3). At the very heart of primary prevention is the identification of individuals at risk of atherosclerotic cardiovascular disease. The use of estimation equations to predict ASCVD risk is a major advance in the older practice of identifying and treating individual risk factors, such as raised blood pressure and raised blood cholesterol. Inputting individual risk factors into any of the several cardiovascular risk estimation equations is the approach recommended by the World Health Organization to stratify patients into mild, moderate and high risk of ASCVD (4). Management options aimed at risk factor reduction may then be implemented to prevent or delay the onset of CVD in moderate and high-risk patients.

Several hundred CVD risk prediction equations have been published (5). There are notable variations concerning populations used for their development and validation, the specific 10-year risk reported, the population in which they are applicable and the parameters required for the execution of the equation. The most widely used equations for assessing risk in the general population include as a required parameter one or more components of a standard plasma lipid profile (6). The American College of Cardiology/American Heart Association pooled cohort equations (PCEs) require plasma total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C), the European Society of Cardiology’s Systematic COronary Risk Evaluation (SCORE) requires TC, HDL-C and low-density lipoprotein cholesterol (LDL-C) while the German Prospective Cardiovascular Münster risk score requires LDL-C, HDL-C, and triglycerides (TG) (7, 8, 9).

Historically and traditionally, lipid profile measurements have required a sample collected after a 12-hour fast. This requirement was deemed necessary by the proponents of the now widely used Friedewald formula, LDL-C = TC – (HDL-C + TG/5), which allowed the accurate calculation of LDL-C from TC, HDL-C and TG, without the use of the laborious preparative ultracentrifugation process (10). They observed that the triglyceride to cholesterol ratio in Very Low-Density Lipoprotein (VLDL) is relatively constant in normal subjects and nearly all patients with dyslipidaemia at about 5:1 as well as that when chylomicrons are not detectable in the blood, most of the triglyceride in plasma is from VLDL (10). The inference from these observations is that when chylomicrons are not detectable in the blood, as occurs in the fasting state, most of the triglyceride in plasma is from VLDL and the ratio and formula apply. Following on from this, when chylomicrons are detectable in the blood, in the post-prandial state, most of the triglyceride in plasma will not be from VLDL alone, the ratio will not be 5:1 and the formula should not apply. Thus, to use the Friedewald formula, a 12-hour fast has been required to ensure that all the dietary sourced chylomicrons have been cleared from circulation (10).

Large population studies over the last 2 decades have shown that serum lipid levels show only minor variation between the fasting and the postprandial state (11, 12). From the reports, postprandial serum triglyceride levels increased by about 20%, at most. This is within the scope of biological variation estimates for TG at 20 – 30% (13, 14). Differences between fasting and post-prandial values for TC, HDL-C and LDL-C have been reported to be less than 5%. The implications of these variations have also been explored on CVD risk estimation. Using the 2013 PCEs equation, Mora et al demonstrated nearly 94% concordance in the ASCVD risk scores obtained for the same individual using fasting and non-fasting samples (15).

There are no known local studies that have investigated the impact of the use of non-fasting lipids on CVD risk estimation in Nigeria. This study aims to examine the degree of agreement or differences between CVD risk estimates obtained using fasting versus non-fasting samples.

Materials and Methods
Study site and participants
This was a cross-sectional study of one hundred and eleven (111) apparently healthy staff of the University College Hospital, Ibadan aged between 30 and 65 years. Pregnant and lactating women were excluded from the study as well as those on any hypolipidemic medication or a special diet. The University College Hospital is a tertiary health facility located in the heart of Ibadan, Oyo state. It is a 1,000-bed hospital with over 5,000 staff.

Data/Sample Collection Protocol
A semi-structured questionnaire was used to collect information on demographic, social and
Clinical history. Blood pressure (BP) was measured before the fasting sample was collected. BP measurement was taken after the participant was seated for about 10 minutes with hospital-provided Welch Allyn 767 Mobile Aneroid Sphygmomanometer (Baxter Incorporated, Alabama, United States of America). Participants were told to fast overnight for a minimum of 12 hours from 8 pm the previous day to 8 am the next day. A fasting blood sample was taken at 8 am and a standard meal (477 grams (9.5 tablespoon servings)) of jollof rice prepared according to Oguntano protocol (16) was served along with 5 pieces of fried plantain and 2 pieces of beef. Another sample was drawn after 4 hours for a postprandial lipid profile. Both samples were collected into plain bottles. Samples were allowed to clot and retract before centrifuging at 3000g for 15 minutes using Uniscope Laboratory centrifuge, model SM112 (Surgifriend Medicals, England) to obtain sera which was stored at -20°C in plain bottles. The centrifuge was validated using a strobe tachometer. Analysis was carried out within 1 week of collection.

Laboratory Analyses
Plasma total cholesterol, HDL-C and TG were assayed using the enzymatic method on the automated chemistry platform Landwind C100 plus (Shenzhen Landwind Biomedical Technology Co., Ltd, Shenzhen 518040, Guangdong, China). LDL-C was calculated using Martin-Hopkins equation (17).

CVD risk estimation
The 10-year risk for ASCVD was estimated using 2 equations. The first equation was the American College of Cardiology/American Heart Association pooled cohort equations (PCEs) accessed at www.msdmanuals.com/professional/multimedia/clinical-calculator/cardiovascular-risk-assessment-10-year-revised-pooled-cohort-equations-2018. The 10-year risk for ASCVD is categorized as low-risk (<5%), borderline risk (5% to <7.5%), intermediate risk (7.5% to <20%), and high risk (≥20%) (18). The other equation was the Framingham risk score equation was accessed at https://www.mdcalc.com/calc/38/framingham-risk-score-hard-coronary-heart. The 10-year risk for ASCVD is categorized as low-risk (<10%), moderate risk (10 to 20%), and high-risk (>20%) disease (19).

Data Analysis
Statistical analysis was done using IBM SPSS version 23 (IBM Corp., Armonk, NY, USA). Qualitative variables were presented as frequencies (percentages), while quantitative variables were presented as mean (SD). Pearson’s correlation was used to check for association between quantitative variables. Agreement of risk categorization between estimates from fasting versus non-fasting results was assessed using McNemar’s test for paired nominal data. Significance was set at p<0.05.

Results
A total of one hundred and eleven (111) persons were recruited for the study with a mean age of 41.7 (6.9) years, which was not statistically different from that of the male participants at 42.3 (7.7) years, p = 0.689. Table 1 shows specific clinical characteristics of the study population.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>52 (46.8%)</td>
<td>59 (53.2%)</td>
<td>111 (100%)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>42.3 (7.7)</td>
<td>41.7 (6.9)</td>
<td>42 (7.3)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120.8 (12.4)</td>
<td>121.7 (13.6)</td>
<td>121.3 (13)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.9 (10.1)</td>
<td>76.4 (9.9)</td>
<td>74.8 (10.1)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.42 (0.49)</td>
<td>4.63 (0.48)</td>
<td>4.53 (0.49)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.13 (0.25)</td>
<td>1.07 (0.21)</td>
<td>1.10 (0.23)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.25 (0.19)</td>
<td>1.27 (0.24)</td>
<td>1.26 (0.22)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.66 (0.49)</td>
<td>2.86 (0.49)</td>
<td>2.76 (0.50)</td>
</tr>
<tr>
<td>nTC (mmol/L)</td>
<td>4.46 (0.59)</td>
<td>4.57 (0.59)</td>
<td>4.52 (0.59)</td>
</tr>
<tr>
<td>nTG (mmol/L)</td>
<td>1.54 (0.38)</td>
<td>1.49 (0.33)</td>
<td>1.51 (0.35)</td>
</tr>
<tr>
<td>nHDL-C (mmol/L)</td>
<td>1.40 (0.25)</td>
<td>1.49 (0.27)</td>
<td>1.45 (0.26)</td>
</tr>
<tr>
<td>nLDL-C (mmol/L)</td>
<td>2.45 (0.58)</td>
<td>2.47 (0.57)</td>
<td>2.46 (0.57)</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation); SBP – Systolic Blood Pressure, DBP – Diastolic Blood Pressure, TC – Total Cholesterol, TG – Triglycerides, HDL-C – High-density lipoprotein cholesterol, LDL-C – Low-density
Ten (9%) of the participants had a fasting TC ≥ 200mg/dL, with 4 (3.6%) of these persons also having a postprandial TC ≥ 200mg/dL. One of the participants had postprandial TG of >200mg/dL but <400mg/dL. Thirty-one (52.5%) of the female participants had an HDL-C value ≥ 50 mg/dL, while 48 (92.3%) of the male participants had an HDL-C value ≥ 40 mg/dL. The highest LDL-C values in the fasting and postprandial state were 168mg/dL and 167mg/dL respectively.

Table 2 shows a classification of the percentage change in the measured lipid profile parameters.

Table 2: Percentage change in serum lipid profile following the standard meal

<table>
<thead>
<tr>
<th></th>
<th>Percentage change</th>
<th>&lt; -50%</th>
<th>-20 to -49.9%</th>
<th>-10 to -9.9%</th>
<th>0 to -9.9%</th>
<th>9 to 0%</th>
<th>10 to 9.9%</th>
<th>19 to 9.9%</th>
<th>20 to 19.9%</th>
<th>&gt; 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td></td>
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<td>TG</td>
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<tr>
<td>HDL-C</td>
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<tr>
<td>LDL-C</td>
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</table>

The majority (73, 65.8%) of the values obtained for TC in the non-fasting state were within 10% of the fasting value. A decline in TC was observed in 58 (52.3%) participants. Almost all (102, 91.8%) of the participants had at least a 10% increase in the level of TG. Sixty-five (65, 58.6%) had greater than a 30% increase in the postprandial value over the fasting value. Most (87, 78.4%) of the participants had a reduction in the HDL-C cholesterol value. Sixty (70.0%) of these participants had less than a 30% decline in the value of HDL-C. Similar to TG, a majority (93, 82.8%) of the participants had an increase in their LDL-C value. Three (2.7%) of these had LDL-C values greater by at least 50%.

ASCVD risk estimates using the PCE from the fasting and non-fasting samples ranged from 0.1 – 7.4% and 0.1 – 6.5%, respectively. There was a strong correlation between the 2 estimates with a Pearson correlation score of 0.988, p<0.001. The number (percentage) of persons with risk estimates less than 1% was 49 (44.1%) and 53 (47.7%) for the fasting and non-fasting samples, respectively. One hundred and eight (97.3%) participants of the study population had low risk, with estimates of less than 5% from both fasting and non-fasting samples. The remaining three (2.7%) persons, had borderline risk. The values ranged from 5.0 – 7.4% and 5.1 – 6.5% for fasting and non-fasting, respectively.

ASCVD risk estimates using the Framingham equation from the fasting and non-fasting samples ranged from 0.0 – 11.5% and 0.0 – 7.7%, respectively. There was a strong correlation between the 2 estimates with a Pearson correlation score of 0.930, p<0.001. The number (percentage) of persons with risk estimates less than 1% was 80 (72.1%) and 85 (76.6%) for the fasting and non-fasting samples, respectively. One hundred and ten (110, 99.1%) and 111 (100%) participants of the study population had low risk with estimates of less than 10% from results from the fasting and non-fasting samples, respectively. The single participant who had moderate risk with the fasting sample had a post-prandial decline of 30mg/dL (14.9%) in TC and an increase of 16mg/dL (39.0%) in HDL-C.

Table 3 shows the distribution of the participants into risk categories. There was no significant difference in the distribution, comparing fasting estimates with non-fasting estimates for either equation.
Table 3: Agreement between Nonfasting and Fasting Lipid Measurements for Classifying Participants into Categories of ASCVD Risk

<table>
<thead>
<tr>
<th>Equation</th>
<th>10-y risk category by fasting lipid measurements</th>
<th>10-y risk category by nonfasting lipid measurements</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled Cohort Equation</td>
<td>Low Risk</td>
<td>108 (97.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Borderline Risk</td>
<td>0 (0%)</td>
<td>3 (2.7%)</td>
</tr>
<tr>
<td></td>
<td>Intermediate Risk</td>
<td>Low Risk</td>
<td>Intermediate Risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>108 (99.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Framingham Risk Score</td>
<td>Low Risk</td>
<td>110 (99.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Equation</td>
<td>Intermediate Risk</td>
<td>0 (0%)</td>
<td>1 (0.9%)</td>
</tr>
</tbody>
</table>

**Discussion**

Requesting lipid profile studies in apparently healthy persons is primarily done for the assessment of risk for cardiovascular disease. This will involve the use of any of the severally available CVD risk estimating equations. Using two (2) very popular equations we have demonstrated that there is significant agreement in CVD risk estimates made from fasting and non-fasting lipid profile sample results for a largely low-risk population. For the participants who might have required some intervention based on their risk estimate from the PCE equation (10-year risk >5%), there was 100% agreement. Mora et al also demonstrated similarly high concordance between PCE estimates from fasting versus non-fasting lipids among those deemed low risk. Of the 1247 participants deemed low risk by fasting sample measurement in their population, 98.6% (1230) participants were also deemed low risk by nonfasting samples results (20). The agreement between the fasting and non-fasting sample estimates may be explained by the degree of variation in the specific lipid parameter, TC and HDL-C, used in the estimation equations. The mean of the maximal postprandial change in TC and HDL-C reported by several large prospective studies across several countries was 8mg/dL and 4 mg/dL respectively (11, 12, 21). Karmani et al conducted a systematic examination of the PCE to determine the risk factor levels of each variable in the equation required to exceed risk thresholds. For TC, using the entire range of values permitted by the risk assessment tool, increments of 10 mg/dl were made while holding all the other variables constant. The resulting changes in the CVD risk estimate for African Americans were modest (22).

The key attraction for the use of non-fasting samples for lipid profile studies is that it is convenient and resource-saving. The process of blood sampling would be simplified for the patients and the laboratories. The inconvenience as well as the resources required for returning on a separate date are avoided by the patient while the laboratory can conserve resources spent early in the day for more phlebotomy services to cope with a surge of patients needing a fasting sample. The former reason may enhance patient compliance with testing. There is also the patient safety component for persons who have diabetes mellitus who no longer have to face the risk of hypoglycaemia from an overnight fast. Nonfasting sampling may also be more convenient for children and the elderly (23, 24).

Beyond the convenience of the non-fasting sample, is that it may indicate CVD risk not obviously estimated from the fasting sample. This was the suggestion from a United States study of 26,509 women (fasting and non-fasting) enrolled in the Women's Health Study and followed up for about 14 years. Non-fasting triglyceride levels were independently associated with incident cardiovascular events while fasting triglyceride levels showed little independent relationship (25). This was similarly demonstrated in another prospective study of about 14,000 men and women in Denmark followed up for about 20 years. Increased levels of non-fasting TG were linked with increased risk of ASCVD and mortality in both genders (26). This association between non-fasting triglycerides and CVD risk is important to identify as most individuals will be in the postprandial phase for between 16 – 18 hours a day. Information in the postprandial phase may therefore be more reflective of the environment to which the cells and tissues are constantly exposed (27).

Given the above, it is unsurprising that the number of national societies and guidelines endorsing the use of non-fasting lipids for CVD risk assessment is increasing (28, 29). This study supports the adoption of this position as part of...
the primary preventive efforts to reduce the burden of ASCVD among Nigerians.

Study limitations
Our population of apparently healthy persons was selected from staff of a tertiary hospital who may be more aware of their health status than the general population.

Conclusion
We suggest that the inclusion of non-fasting lipids as an option for ASCVD risk assessment for apparently healthy Nigerians should be strongly considered. This will have a significant public health impact as it may improve compliance with and uptake of requests for CVD risk assessment.

List of Abbreviations
ASCVD: Atherosclerotic cardiovascular diseases
BP: Blood pressure
CVD: Cardiovascular diseases
DBP: Diastolic blood pressure
FRS: Framingham risk score
HDL-C: High density lipoprotein cholesterol
LDL: Low density lipoprotein
LDL-C: Low density lipoprotein cholesterol
nHDL-C: nonfasting high-density lipoprotein cholesterol
nLDL-C: nonfasting low-density lipoprotein cholesterol
nTC: non-fasting total cholesterol
nTG: non-fasting triglycerides
PCE: Pooled cohort equations
SBP: Systolic blood pressure
SCORE: Systematic COronary Risk Evaluation
SD: Standard deviation
TC: Total cholesterol
TG: Triglycerides
UI: University of Ibadan
UCH: University College Hospital
VLDL: Very low-density lipoprotein cholesterol

Availability of data and materials
The data sets used and analyzed during the current study are available from the corresponding author on request.

Competing interests.
The authors declare no competing interests.

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Authors’ contributions
Conceptualization: KMA, EBJ, BOT, OOA
Data acquisition: EBJ, OOA
Data analysis: KMA, OOA
Article writing: KMA, OOA
Manuscript review: KMA, EBJ, BOT, OOA
Supervision: EBJ
Final approval: KMA, EBJ, BOT, OOA

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