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Changing Recommendations on the Use of Non-Fasting Versus a Fasting Lipid Profile: A Review of Current Literature

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Abstract **Review Article**

Cardiovascular disease is a major cause of death all over the world. Various tests included in the lipid profile testing panel are used to predict the risk of cardiovascular disease, for monitoring treatment and for setting treatment goals. This review article revisits the various parameters in the lipid profile, the current clinical recommendations, and the recent guideline changes of testing requirements from a fasting to a non-fasting sample. The impact on various parameters when a non-fasting lipid testing is performed along with the criteria that should be used while using the new recommendations of non-fasting lipid testing are discussed. The article also reviews the different equations used for the calculation of low density lipoprotein cholesterol and its clinical applications in the current clinical guideline settings. It also briefly describes the increasing clinical importance of parameters like non-high density lipoprotein cholesterol, apolipoprotein-B and remnant cholesterol. Recent literature reviews conclude that risk prediction is similar and, in many cases, better with non-fasting lipid profile testing compared to a fasting state test. Hence it is recommended to use non fasting lipid profile testing for better risk prediction, patient and laboratory convenience, keeping in mind the cut off and flagging criteria for a repeat fasting test.

Keywords: Non-fasting lipid profile, fasting lipid profile, cholesterol, triglycerides, LDL-C, Non-HDL-C, HDL-C.

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of non-communicable disease deaths in the developed world [1–4]. Diabetes, obesity, and dyslipidemia are known risk factors for CVD [5–11]. A standard lipid profile, which includes total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) is the most common test used for cardiovascular risk prediction [12,13].

Lipid profile has been conventionally performed on a fasting specimen in patients. Lipid profile with 8-12 hours of overnight fasting except for water and medication was generally recommended. One of the major reasons for this recommendation was because the post prandial triglycerides remain elevated for several hours in the blood [14] mainly due to presence of chylomicrons and as a result, can cause variability and negatively impact the estimation of the LDL-C using the Friedewald's formula [15]. Since many established reference values for serum lipids were created based on a fasting specimen, previous guidelines also recommended testing for LDL-C in fasting specimens for assessing cardiovascular risks and to monitor patients' response to lipid lowering therapy [12].

Recent observational studies and guidelines from different countries have recommended the use of a non-fasting lipid profile testing for initial risk screening for CVD in most scenarios [16–18]. A non-fasting state is more reflective of our natural physiologic state.

There have been extensive reviews based on studies conducted over more than 300,000 individuals for predicting cardiovascular events with non-fasting lipids and no diminution of correlation between the two were observed [17]. Also, many studies with large populations have shown that there is minimal or no significant change in the values of different lipid parameters in fasting and non-fasting state (Table 1).

Table-1: Mean Maximal change between fasting and non-fasting state specimens for parameters of lipid profile

		Mean maximal change (mmol/L)				
Name of the Study	N	Triglyceride	Total Cholesterol	LDL-Cholesterol	HDL-Cholesterol	Remnant Cholesterol
Copenhagen general population study [19]	108,245	+0.3	-0.2	-0.2	-0.1	+0.2
Women's Health Study [20]	26,330	+0.2	-0.1	-0.2	0.0	-
NHANES [21]	12,744	+0.1	-0.1	-0.1	0.0	-
Calgary Laboratory Services [22]	209,180	+0.3	0.0	-0.1	0.0	-

The possible advantages of the non-fasting lipid testing are many including

- Patient convenience, especially with habitual food intake in non-fasting state.
- Better compliance to testing.
- Reduced visits to the laboratories as the tests can be conducted anytime during the day.
- Reduction in patient volume builds up in the mornings compared to a fasting lipid profile.
- Improved turnaround time and reduced patient waiting time for laboratories due to non-crowding and spread of patients throughout the day.
- Reduction in review and decision making at a later stage by clinicians and reduction in follow up visits since the tests can be done on the day of visit itself as there are no special preparations needed.
- Reflects natural physiological state.
- It is safer for patients with diabetes as it is less likely to cause iatrogenic hypoglycemic incidents.
- Evidence based review of recent literature shows that prediction of incident events is like that of fasting lipid levels [17].
- Genetically elevated non-fasting triglycerides and calculated remnant cholesterol are causal risk factors for myocardial infarction [23].

Aims and objectives

This article aims to review current literature on the changing practice of performing a non-fasting lipid profile testing instead of a fasting lipid profile pillared on the recent guidelines and to provide guidance for laboratories and physicians on when and how these are applicable, the advantages of such testing, conditions in which such testing may not be fit and how test results are to be reported and flagged while using the non-fasting lipid profile. The review highlights the impact of non-fasting testing on major analytes of the lipid profile and summarizes the newer equations available for calculating the low-density lipoprotein-cholesterol.

DISCUSSION

Triglycerides and non-fasting state – current guideline recommendations

Most guidelines including the latest European and US guidelines [17, 24] recommend a non-fasting sample for initial screening and have defined specific cut off values for non-fasting sample for triglycerides. Currently there is no consensus on the cutoff values of triglycerides for which a repeat fasting lipid profile is recommended [25].The European Atherosclerosis Society/European Federation of Clinical Chemistry and Laboratory Medicine (EAS/EFLM) guidelines state that if the triglyceride is > = 440mg/dl (5 mmol/L) the test should be repeated in the fasting state while the American Heart Association/American College of Cardiology (AHA/ACC) guideline recommends the cutoff value of > 400mg/dl (4.5 mmol). The National Institute for Health and Care Excellence (NICE) guidelines recommend a fasting specimen if the triglycerides are > 880mg/dl (10mmol/l). These are primarily applicable because the high triglyceride values can impact the calculation of the LDL using the Friedewald's equation. The ACC further recommends estimation of baseline LDL-C using the newer equation of Martin- Hopkins [16-18].

Many recent studies suggest that the changes in levels of triglycerides in non-fasting state against a fasting state are clinically insignificant [17]. The fact that most patients have normal triglycerides (150 mg/dl) even in the non-fasting state is also beneficial for performing a non-fasting lipid profile test [26–28].

The ACC recommends a fasting lipid profile test in the following -

- To establish the diagnosis of metabolic syndrome since TGL >150mg/dl is one of the criteria for diagnosing metabolic syndrome.
- To identify lipid disorders in persons with a family history of premature Atherosclerotic Cardiovascular Disease (ASCVD) or genetic lipid disorders
- To identify and monitor individuals with a risk of hypertriglyceridemia induced pancreatitis and

monitoring of patients on lipid-lowering medication [18].

The recent NICE guidelines recommend a non-fasting Lipid Profile testing as initial baseline assessment in all cases except when triglycerides are between 10-20 mmol/l [16]. Table 2 summarizes the NICE guideline recommendations on actions to be done for various levels of abnormal triglycerides and non-HDL-C.

Table-2: NICE Guideline Recommendation on abnormal Triglycerides and Non-HDL Cholesterol [16]

Triglyceride/Non-HDL Level	Action
TGL >20 mmol/l (~ >1770 mg/dl)	Refer the patient to a lipid clinic or to urgent specialist review.
TGL Between 10-20mmol/l (between ~ 885 -1770 mg/dl)	Repeat Triglyceride measurement with a fasting test after 5 days but <14 days and assess the values. If the values persist above 10 mmol/l then the patient is considered at risk for acute pancreatitis.
Non-HDL-C >7.5 mmol/l (~ 290mg/dl)	To be referred to a specialist for advice

Non-Fasting Triglyceridemia and risk for CVD

Interestingly, while association between elevated fasting triglycerides and risk of heart disease is minimal, growing evidence suggests that elevated non fasting triglyceride concentrations confer significant risks. One study concluded that in women over 12 years who fall in the highest tertile with elevated triglycerides on a non- fasting sample , after adjustment for age, BP, history of smoking and total cholesterol, the risk of adverse cardiac events was almost double (Hazard ratio 1.98 95% confidence interval) compared to women in the highest tertile of triglycerides when tested with fasting triglyceride samples (the hazard ratio was 1.09 , confidence interval 95%) and there was no additional risk for women in the highest tertile for fasting triglycerides for cardiac events [29].

Multiple studies on patients with Type 2 diabetes mellitus have also found higher incidence of cardiac events with high non-fasting triglycerides compared to high fasting triglycerides [30–33].

High Density Lipoprotein-Cholesterol (HDL-C)

This is also called 'good cholesterol" and high levels of HDL-C are said to protect against development of CVD or stroke.

HDL-C is measured directly by using precipitation techniques to separate Apolipoprotein-B from the HDL-C and thereafter estimating the HDL-cholesterol in the supernatant [19]. Apart from these, other methods like ultracentrifugation, HPLC, direct enzymatic colorimetric methods etc. can also be used for the measurement of HDL-C [20].

Various studies show that concentrations of HDL-C are not affected by fasting or non-fasting status of the individual [21, 22, 26, 34, 35].

LDL-C in the fasting and non-fasting state

LDL-C often termed as "bad cholesterol 'is considered as an important cause for atherosclerosis and CVD. It is also an important marker used to monitor patients' response to lipid lowering therapy. Lipoproteins increase in size and reduce in density from HDL to LDL to IDL (intermediate density lipoproteins) and VLDL (very low-density lipoproteins) and finally to chylomicrons [19].

The gold standard method for measuring LDL-C is Beta quantification. However, it uses ultracentrifugation to separate the lipid components and is very labor intensive, expensive and may not be available in all the laboratories [36].

Different guidelines recommend LDL-C levels to be used for starting lipid lowering therapy and focus on lowering LDL-C levels. Hence to estimate LDL-C accurately is of prime importance.

Interestingly, the NICE guidelines advise using non-HDL cholesterol, rather than LDL cholesterol levels for treatment targets since measurement of LDL-C requires a fasting sample and triglyceride levels <4.5mmol/L while non-HDL cholesterol does not [16].

Equations used for LDL-C calculation

Friedewald's equation for calculation of LDL-C

LDL-C has traditionally been calculated for the last 50 years using the Friedewald's equation when lipid profile is done in the fasting state (when TGL levels <4.5mmol/l or < 400mg/dl) as follows –

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TGL}}{2.2} \text{ (mmol/l)}$$

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TGL}}{5} \text{ (mg/dl) [15]}$$

The biggest disadvantage of using this equation is the fact that high levels of triglycerides and low LDL-C may underestimate the calculation of LDL-C.

Following can be summarized as the limitations of Friedewald's equation

- Accuracy depends on measurement of three other lipids used in the calculation (Total Cholesterol, HDL-C, triglycerides).

- It includes cholesterol from lipoprotein (a) which may be higher in patients of nephrotic syndrome and other races like African Americans.
- This includes "one size fits all" calculation of VLDL since a fixed ratio is used and can result in inaccurate VLDL calculations.
- In cases where LDL-C is low (as is often seen in patients on lipid lowering medication) and TGL is high, there is a risk of underestimating LDL-C and misclassifying patients in lower risk category [37–42].

Martin Hopkins equation for LDL-C calculation

Several equations tried to improvise on the Friedewald's equation by modifying the fixed TGL/5 or TGL/2.2 ratio. Out of these, Martin and colleagues derived an equation which uses variable ratio for VLDL-C depending on the patient's TGL and non-HDL-C values. This ratio is variable and ranged from 3.1-11.9 and is personalized to the specific lipid panel. The equation is as follows-

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TGL}}{\text{Adjustable Factor}}$$

Several studies have tested for the accuracy of Martin-Hopkins equation compared to the Friedewald's equation. It was found that this was about 92% accurate in contrast to 85% accuracy for Friedewald's estimation. It was also found to be accurate when the LDL-C values were lower [43].

The 2018 AHA/ACC guidelines have acknowledged the strength of the Martin-Hopkins equation specifically for those with LDL-C < 70mg/dl and TGL > 150mg/dl. A very large database of patients was studied for lipid profiles and the findings were as follows-

- Accuracy of LDL-C was 94% with Martin-Hopkins equation as compared to 77% for Friedewald's equation.
- The results were similar when the triglyceride levels were higher in patients.

As per the guidelines of 2018 AHA/ACC, LDL-C cut-off of 70 mg/dl is considered very important to categorize patients for initiation of lipid lowering therapy or statins or to add non-statin therapy in very high risk ASCVD patients. As per the guidelines, it is important to accurately estimate LDL-C values also to monitor the patients on statin therapy.

However, the guidelines failed to endorse Martin-Hopkins equation in patients with higher LDL-C values [23]. Another important update in the 2018 AHA/ACC guidelines was the utility of using non-fasting samples. These were of much value in certain at-risk populations such as diabetics, wherein overnight fasting may need adjustments of oral medications or insulin thereby placing patients at risk for hypo or hyperglycemia. Also, non-fasting samples ensure better patient compliance and reduce the patient burden on laboratories in the morning shifts.

Martin-Hopkins equation further proved advantageous even in the non-fasting samples with increased triglycerides since the equation can adapt by adjustment of the VLDL-C ratio [44].

In a separate study done on a very large database, Martin-Hopkins equation performed significantly better with an accuracy of 87-94% compared to 71-93% for Friedewald's equation [43].

Several health care systems and national laboratories have adopted the Martin-Hopkins equation across the world. It has proved advantageous especially for those patients with values of LDL-C < 70 mg/dl.

Even though there are several chemical assay methods to directly estimate LDL-C, there is a lack of standardization in these methods [45].

NIH equation for LDL-C calculation

The NIH equation is the most recent equation released in 2020 for the calculation of LDL-C in patients with normal lipid profile and /or those with hypertriglyceridemia [46]. It is said to perform equally well in both the fasting and non-fasting states.

The equation used Beta quantification results from a large patient population who had high triglyceride levels and can be used to estimate LDL-C for triglycerides <800 mg/dl. It is also said to be more accurate than the Friedewald's and Martin-Hopkins equations for patients with normal lipid profiles.

Another added advantage of this equation is it can classify more patients of dyslipidemia into different LDL-C treatment groups and can thus help further in the management of these patients. It has already been implemented in certain US laboratories however more studies are needed to further validate the equation and its clinical usefulness.

The equation is as follows and can be used when the triglyceride levels are less than 800 mg/dl.

$$\text{LDL-C} = \frac{\text{TC}}{0.948} - \frac{\text{HDL-C}}{0.971} - \left(\frac{\text{TG}}{8.56} + \frac{[\text{TG} \times \text{Non-HDL-C}]}{2140} - \frac{\text{TG}^2}{16100}\right) - 9.44$$

Non-fasting LDL-C and heart disease

CVD risk assessment is done using several scores such as Framingham, ACC/AHA pooled cohort equation, Reynolds scoring, European systematic coronary risk evaluation etc. [47].

None of these scores use lipid parameters such as triglycerides or LDL-C (calculated) which may be

impacted by the patient's fasting or non-fasting status, but they incorporate total cholesterol and HDL-C which are not impacted by patient's fasting status. Other parameters used in the scoring are age, sex, blood pressure, smoking etc.

It has been demonstrated that association of non-fasting lipid levels (LDL-C and triglycerides) with coronary events is similar to those for fasting lipid levels [20, 48, 49].

Non-HDL-C and its significance

Non-HDL cholesterol is calculated by subtracting HDL-C from the total cholesterol levels. It includes all the atherogenic apolipoprotein-B containing lipoproteins (LDL-C, VLDL-C, IDL-C, lipoprotein-A, Chylomicrons, and their triglyceride rich remnants [50].

Non-HDL-C is particularly important in obesity patients especially with adiposopathy, where increased body fat can cause dysfunction of adipose tissue leading to elevated triglycerides and elevated non-HDL-C. Since obesity is prevalent worldwide, the accompanying adiposopathic dyslipidemia supports non-HDL-C estimation [51].

In patients without elevated triglycerides or those with low cholesterol levels, there is a high degree of concordance between LDL-C and non-HDL-C in predicting risk of coronary heart disease [52].

Assessment of non-HDL-C is more important in patients with LDL-C < 70mg/dl and elevated triglyceride levels since estimation of LDL-C alone in these patients may result in the over or underestimation of ASCVD [53,54].

Non-HDL-C is unaffected by the fasting status of the patient. Also, it can be easily calculated from the standard lipid panel report at no extra cost to the patient.

The NCEP-ATP III guidelines also endorse use of non-HDL-C as secondary treatment target, especially in patients with increased TGL levels (> 200mg/dl] after attainment of LDL-C goals [12], while the NICE guidelines recommend use of non-HDL-C as a primary target for treatment [16].

In patients of metabolic syndrome, using LDL-C alone to predict risk for coronary artery disease may be less accurate than when used in conjunction with non-HDL-C and apolipoprotein-B [55]. Optimal level of non-HDL-C is <130mg/dl or 3.37mmol/L. The treatment goal is generally 30mg/dl above the LDL-C. In general, lower the non-HDL-C values; the better it is [12]. The NICE guidelines recommend a greater than 40% reduction in non-HDL cholesterol [16].

Apolipoprotein-B

Apolipoprotein-B is a structural protein which is a major component of VLDL, IDL and LDL lipoprotein. Each of these carries one molecule of apolipoprotein-B, hence total serum apolipoprotein-B corresponds to total number of LDL, VLDL and IDL particles. Thus apolipoprotein-B plasma levels reflect the concentration of the atherogenic particles while non-HDL cholesterol levels reflect the concentration of cholesterol transported by these particles [56].

Studies indicate that apolipoprotein-B is a better tool to assess cardiovascular risk than compared to LDL-C and non-HDL-C [57].

Furthermore, Apolipoprotein-B is an important parameter for assessing risk in settings of diabetes and in patients with metabolic syndrome since these patients have small dense LDL particles with relatively normal LDL-C but higher levels of Apolipoprotein-B [58].

Fasting or non-fasting status does not affect the Apolipoprotein-B concentrations of the patient [17]. Estimation of Apolipoprotein-B however is not routinely performed in standard lipid profiles.

Although the 2018 AHA/ACC guidelines mention apolipoprotein-B and non-HDL-C as being more accurate markers of cardiovascular risk than LDL-C, they are considered as risk enhancers and not primary targets for therapy [23].

The 2019 ESC/EAS guidelines recommend use of apolipoprotein-B and non-HDL-C in very high-risk groups with other coexisting diseases like diabetes, obesity, hypertriglyceridemia [59].

Apolipoprotein-B was found to be a better predictor of cardiovascular risk than non-HDL-C in epidemiological studies. Also, cardiovascular risk was higher when elevated levels of apolipoprotein-B were observed even when non-HDL-C was normal, and the risk was not high with elevated non-HDL-C along with a normal apolipoprotein-B level [60].

Remnant Cholesterol

Remnant cholesterol is all the cholesterol that is not HDL-C or LDL-C. It is also called as remnant lipoprotein and is very atherogenic [61]. It is composed of VLDL-C and IDL-C in the fasting state and in the non-fasting state; it is composed of VLDL-C, IDL-C and Chylomicrons. Remnant cholesterol is calculated as

Remnant Cholesterol =TC - HDL-C - LDL-C [17].

Various studies have showed that remnant cholesterol is a powerful contributor for risk of stroke and coronary artery disease independent of fasting or non-fasting status [62–66].

Flagging of abnormal values in laboratory reports

The laboratory report should mention the fasting or non-fasting status of the patient. It is recommended to flag values above or below the desirable cutoff values both in fasting and non-fasting state [17]. The recommended cutoffs for the various lipid parameters are provided in Table 3.

Table-3: Recommended Cutoff values of various parameters that should be flagged in test reports [17]

Analyte Name	Non-Fasting Specimen	Fasting Specimen
Triglycerides	≥ 2 mmol/l or ≥ 175 mg/dl	≥ 1.7mmol/l or ≥ 150mg/dl
Total cholesterol	≥5 mmol/l or ≥ 190 mg/dl.	≥5 mmol/l or ≥ 190 mg/dl
LDL-C	≥ 3mmol/l or ≥ 115mg/dl	≥ 3mmol/l or ≥ 115mg/dl
Remnant cholesterol	≥ 0.9 mmol/l or ≥ 35mg/dl	≥ 0.8 mmol/l or ≥ 30 mg/dl
Non-HDL-C	≥ 3.9 mmol/l or ≥ 150 mg/dl	≥ 3.8 mmol/l or ≥ 145 mg/dl
HDL-C	≤ 1 mmol/l or ≤ 40 mg/dl	≤ 1 mmol/l or ≤ 40 mg/dl

CONCLUSIONS

- One of the most important modifiable risk factors for cardiovascular disease is hypercholesterolemia. Lipid profile in fasting samples was widely accepted by physicians and patients alike to estimate the levels and make treatment decisions depending on the "good" and "bad" cholesterol levels.
- Several studies done subsequently, some of them on very large populations, showed that there is only minimal variation in the levels of triglycerides and LDL-C in non-fasting state and almost no variation in the levels of total cholesterol and HDL-C.
- Non-fasting lipid profiles have many advantages for better patient compliance due to convenience and improved laboratory turnaround times. They have been accepted as first line screening tests by several guidelines during the past few years. The acceptance has been relatively slower due to the concern about misclassifying patients into lower risk category when assessing their LDL -C using Friedewald's equation. However, many of the most recent guidelines accept non-fasting lipid profile as it reflects the atherogenic status of lipoproteins and is said to be a better indicator for predicting risk for cardiovascular events than LDL-C alone. Non-fasting lipids are widely accepted by many societies for baseline levels and for follow up studies.
- For calculated LDL-C, the newer equations such as Martin Hopkins should be considered (when LDL-C is < 70mg/dl) instead of the Friedewald's equation, and the NIH equation needs to be further validated before being accepted universally.
- Laboratories should indicate the fasting or non-fasting status of the individual and must flag values appropriately on the report.
- Non-HDL-C should be incorporated in the report of a standard lipid profile since it is merely calculated from total and HDL-C levels and is an important parameter in treatment monitoring.
- Non-fasting lipid profile can be done in most cases and all ages for initial lipid testing for any patient, for CVD risk assessment, for patients with diabetes to minimize hypoglycemic risk and for patients on stable drug therapy.
- Fasting lipid profile is still recommended when there are elevated triglycerides that may impact the calculation of LDL-C and for patients who are starting medications that may cause severe hypertriglyceridemia (e.g., prior to treatment with isotretinoin, antiretrovirals, corticosteroids, estrogen etc.)

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