

Cholesterol Fasting or Non Fasting? Patient Variables in Cholesterol Testing Values

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Abstract: Fasting for at least eight hours prior to a lipid test has been standard practice in India and internationally for many years. However, a growing body of evidence and international expert opinion suggests that a non-fasting lipid profile can be used in most situations. Factors That Contribute to an Individual's Usual Cholesterol Level: The National Cholesterol Education Program (NCEP) has the following recommendations to ensure that individual lipid measurements are clinically useful. Individuals should be on their regular diet and their weight should be stable for at least 2 weeks before their lipids or lipoproteins are measured. Patient preparation and blood collection procedures should be standardized according to these guidelines: Variation in lipid values- Age and gender, Posture, Venous Occlusion, Anticoagulants. Prolonged tourniquet application (2–5 min) can increase cholesterol from 5 to 15%. Biological variation is <5% for cholesterol, LDL cholesterol, and HDL cholesterol and 20 to 30% for triglycerides, Considerable variation can occur from one assay to another between clinical laboratories, For patient care, it is important to know if the LDL is calculated or is measured directly, In order to compare results from different laboratories, it is important to know which assay method is utilized, If patient is non-fasting, a direct LDL test is recommended, Sudden changes in lipid values may indicate a change in diet, medications, or onset of a new disease state. When attempting to answer whether fasting or non fasting lipids are most appropriate, it is important to first think carefully about the clinical scenario and consider what question is to be answered with the results.

Keywords: Lipid profile, Cholesterol, National Cholesterol Education Program, European guidelines, Framingham equation.

INTRODUCTION

Some guidelines continue to promulgate the conventional practice of measuring the lipid profile in the fasting state, although other organizations endorse non-fasting lipid profile. One reason among others for preferring fasting lipid profile is the increase in triglyceride concentration seen during a fat tolerance test; however, the increase in plasma triglycerides observed after habitual food intake in most individuals is much less than that observed during a fat tolerance test [1-7].

Ideally, there should be one standard for reporting lipid profile in each country as also accreditation bodies should be aware of the present consensus statement. Fasting for at least eight hours prior to a lipid test has been standard practice in India

and internationally for many years. However, a growing body of evidence and international expert opinion suggests that a non-fasting lipid profile can be used in most situations.

This includes [1, 3, 4]: Calculating cardiovascular risk, Testing for hyperlipidaemia, Monitoring response to statin treatment

At present, the majority of guidelines recommend a fasting serum lipid test. This recommendation is based on achieving consistency between patients and over multiple tests by ensuring a relatively standardized metabolic state. It is also because the majority of research has been performed using fasting lipids, therefore it was assumed that making comparisons and analyzing risk would be less precise if using non-fasting tests [7].

DISCUSSION

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It is usually done in fasting blood specimen. Fasting refers to 12–14 h overnight complete dietary restriction with the exception of water and medication. This may hold true due to two main reasons: 1. postprandial triglycerides remain elevated for several hours [8, 9] 2. most reference values for serum lipids are established on fasting blood specimen. NCEP [10] and European guidelines [11] also recommend doing lipid profile in fasting blood specimen for assessment of cardiovascular risk. However, these guidelines allow total and HDL cholesterol in the non-fasting specimen as these lipids are not much different in fasting and non-fasting specimens. In addition, non-HDL cholesterol (total cholesterol- HDL cholesterol), a secondary target of therapy in adult treatment panel III, may also be used in the non fasting state [10].

As a fast-food meal consisting of e.g. a burger, a shake, and fries might be considered a fat tolerance test, in areas where fast-food consumption is especially high patients may be advised to avoid high-fat, fast-food meals on the day of lipid profile testing. Also, as LDL cholesterol is often calculated by the Friedewald equation, which includes the triglyceride concentration, calculated LDL cholesterol has been thought to be affected substantially by food intake; however, directly measured and calculated LDL cholesterol values are similar using both fasting and non-fasting lipid profile [12, 13].

If this Friedewald equation is employed, there may be some underestimation of LDL cholesterol when chylomicrons are present, which may even be circumvented if a modification of this equation is used [14]. Lipid-lowering trials have used fasting lipid measurements and, in order to follow evidence-based practice, fasting blood sampling has often been the standard in everyday risk assessment. To improve patient compliance with lipid testing, we therefore recommend that non-fasting lipid profile be used in the majority of patients, while with non-fasting plasma triglyceride $.5 \text{ mmol/L}$ (440 mg/dL), fasting sampling may be considered. Life-threatening or extremely abnormal test results deserve special attention and reactions of the clinical biochemical laboratory. In this regard, the following extreme hyper lipidaemias should be noted: triglycerides 10 mmol/L (880 mg/dL) because of risk of acute pancreatitis [15] LDL cholesterol $.5 \text{ mmol/L}$ (190 mg/dL) in adults or $.4 \text{ mmol/L}$ (155 mg/dL) in children and particularly $.13 \text{ mmol/L}$ (500 mg/dL) because of suspicious heterozygous and homozygous familial

hypercholesterolaemia [16-18], respectively, and Lp(a) 150 mg/dL (99th percentile) for very high risk of myocardial infarction and aortic valve stenosis [19-21].

It is also important to refer patients with very low concentrations of LDL cholesterol, apolipoprotein B, HDL cholesterol, or apolipoprotein A1 to a specialist lipid clinic for further evaluation of a major monogenic disorder of lipid metabolism. Each country, state, and/or province in individual countries should adopt strategies for implementing routine use of non-fasting rather than fasting lipid profile as well as flagging of abnormal values based on desirable concentration. A decrease in total cholesterol, HDL and LDL is observed for up to four hours after a standard meal [22]. A decrease in lipid levels after a meal is perhaps converse to what would be expected, but this occurs due to the dilutionary effect of water contained in the food.

Factors That Contribute to an Individual's Usual Cholesterol Level

Age and gender Cholesterol levels vary with age and sex. Cholesterol levels tend to rise with age in both males and females. Day to day variation On average, an individual's daily cholesterol level varies by 6.1%. Within day variation an individual's serum cholesterol values can vary up to 3% within the same day. Seasonal variation Cholesterol levels vary by 2.5% on average, depending on the season. Levels tend to be lower in the summer and higher in the winter. High density lipoprotein cholesterol (HDL) levels follow a similar trend. Diet and alcohol Cholesterol levels are increased by eating too much saturated dietary fat, cholesterol and calories. Alcohol can have different effects on lipid levels that can result in either harmful or beneficial effects on cardiovascular risk depending on the amount of intake and other factors [22]. Exercise Regular vigorous exercise affects lipid levels. Exercise lowers the concentration of triglycerides and low density lipoprotein cholesterol (LDL) and raises HDL levels over time [22]. Disease several endocrine, metabolic, renal, hepatic, and storage diseases can cause alterations in lipids. Hypothyroidism and diabetes are common causes of hyperlipidemia. Cancer, infections, and inflammatory diseases can also result in abnormal lipid metabolism [22].

Drugs Certain drugs, besides lipid lowering agents, can affect blood lipid levels. These include diuretics, some beta-blockers, sex steroids (e.g. birth control pills), glucocorticoids, and cyclosporine. Use of any of these drugs should be suspended, if possible, prior to lipid testing and noted with test results [22]. Posture Cholesterol levels can decrease significantly when a person goes from a standing to a sitting or lying down position. There can be a 6% decrease after sitting for 10-15 minutes [22, 23]. Fasting Total and HDL cholesterol can be measured in non fasting individuals

and recent food intake has only modest effects. However, triglycerides can increase markedly after eating. Since LDL is calculated from total and HDL cholesterol and triglycerides, the LDL level will change as a result of increases in triglycerides due to food intake. Therefore, if the testing opportunity is non fasting, only total and HDL cholesterol will be usable when evaluating medical decision points dependent on fasting levels [24]. Anticoagulants some anticoagulants, such as fluoride, citrate and oxalate dilute the plasma with water from the red cells in the sample. They can decrease cholesterol levels by up to 10%. Heparin has a negligible effect on cholesterol concentration and EDTA decreases cholesterol and triglyceride levels by about 3% [25]. Lipid tests on the Alere Cholestech LDX, System should not be run on samples anticoagulated with fluoride, citrate, or oxalate.

Recent heart attack or stroke Cholesterol and LDL levels fall considerably after a myocardial infarction or stroke and remain low for several weeks. Cardiac catheterization does not seem to have a significant effect on cholesterol levels [25]. Trauma and acute infection Cholesterol levels can decrease by as much as 40% after severe trauma and remain depressed for several weeks. Cholesterol levels are also lower for shorter periods in response to severe pain, surgery and short term physical strain. Acute bacterial and viral infection leads to temporarily altered cholesterol levels which return to the usual levels upon recovery [22]. Pregnancy Lipid levels can increase significantly during pregnancy, mainly in the second and third trimesters. They usually return to normal within 10 weeks postpartum except in women that are breast feeding [22]. The National Cholesterol Education Program (NCEP) has the following recommendations to ensure that individual lipid measurements are clinically useful [25, 26]. Individuals should be on their regular diet and their weight should be stable for at least 2 weeks before their lipids or lipoproteins are measured.

Patient preparation and blood collection procedures should be standardized according to these guidelines

Make note of whether the patient has fasted for at least 12 hours or has engaged in physical activity within the past 24 hours for any analysis other than total cholesterol. However, the variability of cholesterol fractions may be increased postprandially. Thus, if triglycerides and lipoproteins are to be measured, the patient should be instructed to take nothing by mouth (other than water and prescribed medications) for at least 12 hours before the blood sample is taken. For convenience, the fasting period should not be less than 9 hours. LDL may be underestimated slightly (2–4%) in individuals who have fasted 9 hours compared to those who have fasted for 12 hours or more.

Variation in lipid values

Age and gender – Cholesterol levels vary with age and sex. Under age 20, females have higher cholesterol levels than males. Adult males between 20 and 45 years of age generally have higher levels than females of the same age. Total cholesterol, LDL, and triglycerides increase with age for both sexes. Peak lipid levels for men generally occur between the ages of 40 and 60 and for women, between the ages of 60 and 80. Several factors that occur before or during blood collection, during storage, or shipping to the laboratory may affect the lipid results. It is important to understand and control these factors as much as possible in order to get accurate results.

Posture – Cholesterol can decrease significantly after a person has been sitting for five minutes. Changes as large as 10-15% have been observed. Venous occlusion – Cholesterol concentrations have been found to increase an average of 10-15% after a tourniquet was applied for five minutes. Increases of 2-5% have been observed after only two minutes. Anticoagulants – Some anticoagulants, such as fluoride, citrate, and oxalate, dilute the plasma with water from the red cells in the sample. These can decrease plasma cholesterol levels by up to 10%. Cholesterol tests should not be done on samples anti coagulated with fluoride, citrate, or oxalate. Heparin (blood collection tubes with heparin have a green top) has a negligible effect on cholesterol concentration, and EDTA (blood collection tubes with EDTA have a purple top) decreases cholesterol levels by about 3%.

Guidelines

- Make note of whether the patient has fasted for at least 12 hours or has engaged in physical activity within the past 24 hours for any analysis other than total cholesterol.
- If only total cholesterol and HDL cholesterol are to be measured, either fasting or non-fasting samples can be used. However, the variability of cholesterol fractions may be increased postprandial.
- The patient should sit quietly for about five minutes before veni puncture. If the sitting position is not possible, the same position should be used each time the patient is sampled.
- Prolonged venous occlusion should be avoided. If a tourniquet is used, the sample should be obtained within one minute of tourniquet application. Release the tourniquet as soon as possible during veni puncture. If difficulties are encountered, use the other arm, or, if this is not feasible, release the tourniquet for a few minutes before attempting a second veni puncture. In order for the patient's cholesterol value to be more clinically useful, the influence of pre-analytical factors must be appreciated. The Laboratory Standardization Panel

of the National Cholesterol Education Program (NCEP) 3 recommends the following:

- a) While certain pre-analytical factors are not entirely controllable (e.g., state of health, dietary habits, activities, medication), every effort must be made to measure a person's lipids and lipoproteins only when the person is in a steady state; otherwise the values may not represent the patient's usual cholesterol level.
- b) Individuals should be on their regular diet and their weight should be stable for at least two weeks before their lipids or lipoproteins are measured.
- c) Cholesterol measurements should be made no sooner than eight weeks after occurrence of myocardial infarction, or any form of trauma, acute bacterial or viral infection or illness, and short-term physical strain. The Friedewald formula was subsequently introduced to allow calculation of a low density lipoprotein cholesterol (LDL-C) level using fasting data [22].

The guidelines appropriately emphasize the general utility of a fasting lipid panel, they also indicate that obtaining lipids in the fasting state is preferred, rather than mandatory, depending on the clinical scenario [27]. The definition of LDL-C estimated by the traditional Friedewald formula includes cholesterol contained in biological low density lipoprotein (LDL), intermediate-density lipoprotein, and lipoprotein (a). When assessing residual risk, it is important to remember that other factors beyond cholesterol (e.g., lifestyle, smoking status, blood pressure, and blood glucose) play an important role [28]. A discussion of non HDL-C, whether or not it is focused on a specific target number, should first emphasize intensive lifestyle interventions with particular attention to medication adherence, improved diet, and healthy activity. During the last few years efforts have been made to simplify blood sampling by replacing fasting lipid profile with non-fasting lipid profile as it has been found that lipids, lipoproteins and apolipoproteins were not much different in fasting and non-fasting state with the exception of triglycerides which were higher in non-fasting state and all these were associated with cardiovascular risk prediction [29]. However, a fasting sample is preferred if cardiovascular disease (CVD) risk assessment is based on total cholesterol, LDL cholesterol or non-HDL cholesterol but HDL cholesterol, triglycerides, total/HDL cholesterol ratio and apolipoprotein A-1 predict CVD when measured non fasting [30]. The most interesting part is that non-fasting triglycerides levels may be even better predictor of cardiovascular risk as compared to fasting triglycerides [31, 32].

Although the terms non-fasting and postprandial can be considered synonyms but there is some difference as non fasting sample means blood draw at any time without knowledge of the time of previous meal while postprandial implies a sample at a fixed time after a standard meal. Moreover, triglycerides increase step wise after fat diet, therefore, non-fasting triglycerides would vary depending on time after meal with highest levels 4–5 h postprandial [32]. Further, the cut off levels of non-fasting triglycerides for cardiovascular risk have not yet been defined. It is important to compare serum lipid profile in fasting and at different time interval after a representative meal in terms of prediction of cardiovascular risk. As is true for fasting triglycerides, postprandial lipemia can be affected by ethnicity, alcohol consumption, and menopausal status, and thus these factors should be considered in clinical practice [33]. Thus, a lot has yet to be done in this area and then if the use of non-fasting lipid profile could be included in recommended guidelines then the sampling for lipid profile would be simplified and this will improve the compliance for lipid lowering treatment. Till then we have to believe in fasting lipid profile for assessment and management of cardiovascular risk.

In addition to fasting/non-fasting state there are other factors (pre-analytical) which may affect lipid components:

- A change from an upright to a supine position due to dilutional effect can reduce the cholesterol levels by 10% and triglycerides by 12% [34].
- Prolonged tourniquet application (2–5 min) can increase cholesterol from 5 to 15% [35, 36].
- Cholesterol is slightly higher in winter than in summer and the opposite is true for triglycerides [34, 36, 37].
- The disease conditions like nephrotic syndrome increase total cholesterol, LDL cholesterol and VLDL cholesterol [38] and hypothyroidism increases LDL cholesterol and total cholesterol. Infection and inflammation may decrease total cholesterol and HDL cholesterol and increase triglycerides [39]. Lipids alter following myocardial infarction [40, 41] and these changes may persist for several weeks. That is why it is better to do lipid profile in such patients within 24 h of myocardial infarction. The study by Nawaz *et al.*, [42] showed that all individual values of the lipid profile in patients admitted with acute illness vary significantly during and after hospital stay, whereas the ratio of total cholesterol to HDL remains relatively stable. It is important that all these factors should be kept in mind while interpreting the lipid profile.

In general, a non-fasting lipid test would be appropriate in the following clinical scenarios:

CVD risk assessment, Initial investigation of lipid levels (unless the patient has a history of familial hyperlipidaemia), Monitoring lipid levels over time, Monitoring response to lipid-lowering treatment (unless the patient has high triglycerides), Testing for any reason in patients who are “hard to reach” or have low motivation for undergoing a fasting test

Most evidence on calculating cardiovascular risk is based on fasting lipid test results, however, results from non-fasting lipid tests have also been shown to be strongly predictive of adverse cardiovascular events [43-45]. During a CVD risk assessment, specific values are entered into a risk calculator, such as the Framingham equation. The Framingham equation uses total and HDL cholesterol values, which have the lowest variation between fasting and non fasting samples, and a range of other factors, such as blood pressure, to calculate risk. The extra precision gained from a fasting result is therefore unnecessary [45]. Patients with a low cardiovascular risk and a non-fasting lipid-profile within the ideal range will not require further testing for five to ten years, provided there are no significant changes to lifestyle or diet, or no significant new information arises, e.g. significant family history or relevant new personal history. A non-fasting lipid test can be used as an initial investigation of hyperlipidaemia. A non-fasting sample is appropriate for subsequent tests, unless very high triglycerides have been identified.

CONCLUSION

There are two inherent sources of variability in cholesterol and triglycerides measurements: Biological and Analytical. Biological variation is <5% for cholesterol, LDL cholesterol, and HDL cholesterol and 20 to 30% for triglycerides, Considerable variation can occur from one assay to another between clinical laboratories, For patient care, it is important to know if the LDL is calculated or is measured directly, In order to compare results from different laboratories, it is important to know which assay method is utilized, If patient is non-fasting, a direct LDL test is recommended, Sudden changes in lipid values may indicate a change in diet, medications, or onset of a new disease state. We have shown that there are a number of clinical scenarios in which fasting lipids offer valuable clinical information, but that in others, non fasting lipids will suffice. To assess the initial risk of CVD in an untreated patient, fasting or non fasting total cholesterol and HDL-C levels provide all that is needed.

When assessing a patient’s response to lipid therapy, the 2013 guidelines note that fasting lipids and calculation of the change in LDL-C allow estimation of therapeutic response and adherence to therapy. In other

scenarios, however, including the one described in the introductory case, non fasting lipids can provide requisite information without further inconvenience. Therefore, when attempting to answer whether fasting or non fasting lipids are most appropriate, it is important to first think carefully about the clinical scenario and consider what question is to be answered with the results.

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