

# LIPID TOPICS

by Mark Deeg, MD, PhD

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## Variations in Lipid Values

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There are two major inherent reasons lipid test results vary, even in the very short-term. The first is biological and the second is analytical. Large changes in lipid values may also be due to secondary causes of dyslipidemia.

### **Biological variation**

Biological variation, i.e., the normal day-to-day variation of total cholesterol, LDL cholesterol, and HDL cholesterol, is on the order of 3-5%. Many studies have also documented a seasonal variation. Although there is discordance between the studies, cholesterol levels tend to be higher in the winter months and lower in the summer months independent of the country of origin, ethnicity, age, sex, and baseline lipids. The seasonal variation has been reported to be as high as 12%<sup>1</sup>.

Other biological factors that contribute to a patient's cholesterol level include:

Within-day variation – An individual's serum cholesterol values can vary about 2-3% within the same day. Cholesterol levels are also lower for shorter periods in response to severe pain, surgery, and short-term physical strain.

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 anticoagulated 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%.

Capillary vs. Venous sampling – Most point of care testing devices can utilize capillary blood (obtained with a finger stick) in addition to venous blood. Total cholesterol measured from capillary plasma (obtained via a finger stick) tends to run 2 to 4% higher than venous plasma cholesterol.<sup>2</sup>

In contrast to cholesterol, the normal day-to-day variation of triglycerides is 20 to 30%. The variation in triglycerides has two major clinical implications: 1) when patients are being treated for high triglycerides, it is important to follow the trend in triglycerides, i.e., a single high or low triglyceride value may be due to biological variation and not an improvement or reduction in the efficacy of the triglyceride lowering medication, and 2) if LDL cholesterol is calculated by the Friedewald equation [LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5], then the biological variation in triglycerides will add to the variability of the LDL calculation. Since triglycerides are divided by 5 in this equation, this minimizes the effect of triglyceride variation on LDL calculation but will add 4 to 6% variability in the LDL calculation on top of the 3 to 4% variation in LDL per se.

### **Analytical variation**

Analytical variation is the variability in measuring cholesterol and triglycerides that derives from the assay itself due to random error and/or systematic error (described below).

LDL cholesterol is the primary target for reducing risk for cardiovascular disease. The reference method or “gold standard” for measuring LDL cholesterol is the beta-quantification procedure. This method subjects serum to ultracentrifugation to separate the VLDL from LDL and HDL. With this method, the LDL cholesterol measured actually includes intermediate density lipoproteins (IDL), LDL, and lipoprotein(a). It is important to recognize that this method for measuring LDL cholesterol includes other lipoproteins. All other analytical tools to determine LDL cholesterol are compared to this method.

The current primary goal for measuring LDL cholesterol is that the total error be within 12% of the true value. For example, LDL cholesterol of 150 mg/dL may translate to a range of 132 to 168 mg/dL. The “total error” combines the imprecision of a given assay (random error) with the inaccuracy or bias (systematic error, i.e., a given assay will always be high or low compared to the true results).

Comparison of methods to measure LDL cholesterol - Although ultracentrifugation is the gold standard, it is very cumbersome to perform in high volume on a routine basis. In 1972, Friedewald developed his formula to estimate LDL cholesterol where  $\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \text{triglycerides}/5$ . It is important to know that this calculated LDL also includes IDL and lipoprotein(a). This method was quickly adopted as the routine means of calculating LDL. However, the overall variability of calculated LDL between different clinical laboratories was nearly 12%. The Friedewald equation is most accurate when triglycerides are less than 250 mg/dL and not accurate when triglycerides exceed 400 mg/dL. Hence, post-prandial samples can not be used to calculate LDL.

In the past 10 years, direct methods have been developed to determine LDL cholesterol. These “direct” LDL methods initially used chemical precipitation or antibodies to separate LDL from the other lipoproteins. Currently, the third generation of direct LDL methods utilizes various means to block or dissolve lipoproteins to isolate LDL cholesterol. Unlike the calculated LDL, these direct LDL measurements do not include IDL and lipoprotein(a). All things being equal, it is expected that the direct LDL will be 5 to 10 mg/dL lower than the calculated LDL. In general, these assays are also much less affected by triglycerides and can be used in post-prandial samples with little loss of accuracy.

## **Secondary causes of dyslipidemia**

Large changes in cholesterol and/or triglycerides may be due to secondary causes of dyslipidemia.

Diet and alcohol – Cholesterol levels are increased by consuming too much dietary fat (particularly saturated or transfat), cholesterol, and calories. For that reason, it is recommended testing be performed when a patient has been on their normal diet for the previous two weeks. Alcohol consumption raises triglycerides and HDL. This effect can last for 2 or 3 days after consuming the last drink.

Exercise – Regular vigorous exercise affects plasma lipid levels. Exercise lowers the concentration of triglycerides, VLDL cholesterol, and LDL cholesterol, and raises HDL cholesterol levels.

Drugs – Certain drugs, besides lipid lowering agents, can affect blood lipid levels. For example, some drugs used to treat high blood pressure may increase triglycerides and decrease HDL cholesterol; oral estrogens (birth control pills) can lower total cholesterol and raise HDL cholesterol. Drugs that alter lipid levels include  $\beta$  blockers (particularly unselective  $\beta$  blocker like propranolol), bile acid binding resins (which lower cholesterol and LDL but also raise triglycerides), most protease inhibitors, diuretics, retinoic acid derivatives, and glucocorticoids.

Recent heart attack and stroke – Cholesterol levels fall and triglyceride levels rise considerably after a myocardial infarction or stroke and remain altered for several weeks. Cardiac catheterization does not seem to have a significant effect on cholesterol levels.

Trauma and acute infection – Cholesterol levels can decrease by as much as 40% after severe trauma and remain depressed for several weeks.

Pregnancy – Cholesterol levels increase by as much as 20-35% during pregnancy because of increases in LDL and VLDL.

Altered glucose metabolism – Insulin resistance, metabolic syndrome, and type 2 diabetes are associated with elevations in triglycerides and low levels of HDL. If a patient has or develops this lipid phenotype, these conditions must be considered.

Other disease states – Hyper- and hypothyroidism results in a decrease and increase, respectively in cholesterol. Liver disease and renal failure are also associated with changes in lipid levels. Malnutrition and chronic disease states, e.g., cancer, are associated with low levels of cholesterol.

## **Obtaining the best results for lipid testing**

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

1. 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.
2. 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 post-prandially; 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.\*
3. The patient should sit quietly for about five minutes before venipuncture. If the sitting position is not possible, the same position should be used each time the patient is sampled.
4. 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 venipuncture. 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 venipuncture.

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)<sup>3</sup> recommends the following:

5. 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.
6. 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.
7. 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.

*Summary*

- 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.

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