



## Patient Variables in Lipid Testing

There are many factors that can influence the results of lipid tests so that they do not reflect the individual's usual lipid levels. These variations are often caused by blood collection technique, pre-existing blood collection factors, blood storage conditions and laboratory transportation factors. In order to ensure accurate results, it is important to understand and control these factors as much as possible.

### Factors That Contribute to an Individual's Usual Cholesterol Level

- **Age and gender** Cholesterol levels vary with age and sex. Adult males between 20 and 44 years of age have slightly higher levels than females of the same age on average, but older females have higher levels than males. Under the age of 20, females tend to have slightly higher cholesterol levels than males. Cholesterol levels tend to rise with age in both males and females.<sup>1,2</sup>
- **Day to day variation** On average, an individual's daily cholesterol level varies by 6.1%.<sup>3</sup> For a person with an average total cholesterol value of 200 mg/dL, this means that if the cholesterol were measured repeatedly over many days, weeks, or months, 95% of the values could range between 176 and 224 mg/dL and some values could even be outside of this range. And for some individuals, the day to day variation can be even greater.<sup>3</sup>
- **Within day variation** An individual's serum cholesterol values can vary up to 3% within the same day.<sup>3</sup>
- **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.<sup>3</sup>
- **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.<sup>3</sup>
- **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.<sup>3</sup>
- **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.<sup>3</sup>
- **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.<sup>4</sup>
- **Fasting** Total and HDL cholesterol can be measured in nonfasting 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 nonfasting, only total and HDL cholesterol will be usable<sup>1</sup> when evaluating medical decision points dependent on fasting levels.
- **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 also been observed after only 2 minutes.<sup>4</sup>
- **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%.<sup>4</sup> Lipid tests on the Alere Cholestech LDX® System should not be run on samples anticoagulated with fluoride, citrate, or oxalate. Refer to the package insert that accompanies each box of cassettes for information on appropriate sample types.
- **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.<sup>4</sup>
- **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.<sup>4</sup>

- **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.<sup>3</sup>

## NCEP Recommendations

The National Cholesterol Education Program (NCEP) has the following recommendations to ensure that individual lipid measurements are clinically useful:<sup>4,5</sup>

- While certain preanalytical 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 metabolic steady state; otherwise the values may not represent the patient's usual levels.
- 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.
- Lipid measurements should be made no sooner than 8 weeks after occurrence of myocardial infarction, or any form of trauma, including surgical trauma, acute bacterial or viral infection or illness, and short term physical strain.
- 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.
  - If only total cholesterol and HDL cholesterol are to be measured, either fasting or nonfasting samples can be used. 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.
- The patient should sit quietly for about 5 minutes before venipuncture. If the sitting position is not possible, the same position should be used each time the patient is tested.
- Prolonged venous occlusion should be avoided. If a tourniquet is used, the sample should be obtained within 1 minute of tourniquet application. Release the tourniquet as soon as possible during venipuncture. If difficulties are encountered, use the other arm, or release the tourniquet for a few minutes before attempting a second venipuncture.

## References

1. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. NIH Publication No. 02-5215, 2002.
2. Hickman TB et al. Distributions and trends of serum lipid levels among United States children and adolescents ages 4-19 years: data from the Third National Health and Nutrition Examination Survey. *Prev Med* 1998; 27(6):879-890.
3. Rifai N et al. Preanalytical variation in lipid, lipoprotein, and apolipoprotein testing. In: Rifai N et al (ed). *Handbook of Lipoprotein Testing*. 2nd ed. Washington, DC: AACC Press; 2000 p. 161-187.
4. Laboratory Standardization Panel of the National Cholesterol Education Program. Recommendations for improving cholesterol measurement. NIH Publication No. 90-2964, 1990.
5. Working Group on Lipoprotein Measurement, National Cholesterol Education Program. Recommendations on lipoprotein measurement. NIH Publication No. 95-3044, 1995.