## Editorial

# Determination of Fasting and Non-Fasting Cholesterol Levels of Low- and High-Density Lipoproteins with Homogenous Assays: A Promising Reliable Way to Assessment of Dyslipidemia

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Framingham Heart Study (FHS), started in 1948, has provided a lot of pivotal results and findings that help us understand and solve problems associated with atherosclerotic disease risk[1]. This FHS found that high levels of low-density lipoprotein-cholesterol (LDL-C) and low levels of high-density lipoprotein-cholesterol (HDL-C) were the causes of coronary heart disease (CHD), and the term "risk factor" also was set up due to the impetus of the FHS evidence[1-3]. As FHS findings have shown the significant relevance of dyslipidemia to CHD risk, the accurate measurement of LDL-C and HDL-C is of significant importance for the diagnosis of dyslipidemia and assessment of CHD risk without any dispute.

LDL-C has been measured in clinical practice using Friedewald formula, which is inapplicable for blood samples of non-fasting individuals or those with ≥400 mg/dL of triglyceride (TG)[4, 5]. There are no significant differences in total cholesterol (TC) levels between fasting and non-fasting states, however, TG usually increases postprandially. The Friedewald formula (TC−HDL-C−TG/5) comes into effect, as the value of TG/5 is nearly equal to that of very low-density lipoprotein-cholesterol. However, LDL-C estimated using the Friedewald formula tends to be lower than that determined using the ultracentrifugation method, if serum TG level is ≥200 mg/dL. Whence, a variety of modified methods with different TG coefficient in the formula have been reported, but most of these modified methods do not necessarily overwhelm the Friedewald formula with regard to accuracy, precision,

and convenience of LDL-C estimation[6-8].

In the meantime, a number of homogeneous assay methods for LDL-C measurement were developed in Japan because the limitations of the Friedewald formula (i.e., requirements of fasting blood samples and serum TG level <400 mg/dL) may interfere with routine clinical assessment of lipid metabolism in patients. Although commercial homogeneous assays have passed the precision and accuracy requirements defined by the National Cholesterol Education Program (NCEP), several earlier studies revealed that some homogenous assays exhibited poor analytical performance in patients with common diseases and even in disease-free subjects; some of these homogenous assays were thus withdrawn from the Japanese market[9-12]. Hypertriglyceridemia often provides a plus assay bias in LDL-C measurements, but the homogeneous assays have been expected to be applicable to postprandial samples even with the presence of much TG-rich lipoproteins in contrast to the Friedewald formula[9, 11, 13]. However, the accuracy of homogeneous assays has never been compared between non-fasting and fasting samples using the reference measurement procedures in disease-free and diseased subjects.

Miida, *et al*. demonstrated the availability of LDL-C and HDL-C homogeneous assays in non-fasting samples derived from disease-free and diseased subjects[14]. Fresh blood samples were collected from 59 disease-free and 109 diseased subjects and non-fasting samples constituted 72.9% and 42.9% of these samples, respectively. LDL-C and HDL-C concentrations were measured using homogeneous assays of four manufacturers. These four homogeneous assays for LDL-C and HDL-C met the NCEP requirements in terms of coefficient of variation, indicating that these assays are as accurate for non-fasting samples as for fasting samples in both disease-free and diseased subjects. Japan Atherosclerosis Society guideline recommends the assessment of non-HDL-C (TC−HDL-C) instead of LDL-C when using non-fasting samples

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**Table 1.** Cut-points of abnormal serum lipid levels in fasting and non-fasting states exhibited by the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine

| | non-fasting | | fasting | |
|---|---|---|---|---|
| | mmol/L | mg/dL | mmol/L | mg/dL |
| TC | ≥5 | ≥190 | ≥5 | ≥190 |
| TG | ≥2 | ≥175 | ≥1.7 | ≥150 |
| HDL-C | ≤1 | ≤40 | ≤1 | ≤40 |
| Non-HDL-C | ≥3.9 | ≥150 | ≥3.8 | ≥145 |
| LDL-C | ≥3 | ≥115 | ≥3 | ≥115 |

Abbreviations: TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Non-HDL-C means TC minus HDL-C. All the cut-points are referred to the special reports presented by the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine [20, 21].

or samples with TG level ≥400 mg/dL. The present study demonstrates that non-fasting non-HDL-C is also considered to be accurate because HDL-C measured by the homogenous assay is accurate in non-fasting samples.

Scientific interest in TG-rich lipoproteins has fluctuated over the past many years, ranging from causal risk factor for atherosclerotic cardiovascular diseases to innocent bystanders, but new insights from recent studies strongly support that high concentrations of TG-rich lipoproteins are causally associated with atherosclerotic cardiovascular diseases [15, 16]. For many years, a lipid profile has been hitherto routinely determined in the clinical laboratory using a fasting blood sample in principle. The rationale for such a requirement includes 1) postprandial increase in TG concentrations, 2) no application of Friedewald formula when using non-fasting samples, and 3) use of fasting samples for lipid measurement in many clinical trials and epidemiological studies. However, most of our lives are spent in the postprandial state. Recent evidence has demonstrated that non-fasting TG concentrations are a better predictor for future coronary events compared with fasting TG, in both men and women [16-19].

In 2016, the European Atherosclerosis Society and the European Federation of Clinical Chemistry and Laboratory Medicine presented a similar recommendation with regard to the clinical significance of non-fasting lipid profile [20, 21]. They recommended that laboratory reports should flag abnormal values of serum lipids for non-fasting samples (**Table 1**). The majority of these cut-point values correspond to desirable concentrations from guidelines and consensus statements in western countries. However, a desirable concentration cut-point for non-fasting TG was documented according to the Women's Health Study, which found that this cut-point was optimal for cardiovascular disease risk prediction [20-22]. The TG cut-points based on assays with correction for endogenous glycerol presumably in accordance with TG data in Japan [23]. In most laboratories of western countries, however, TG concentrations are measured without subtraction of the glycerol blank. Hence, non-fasting determination of serum lipids for the appropriate assessment of CHD risk should also be discussed in Japan.

Eventually, determination of LDL-C and HDL-C levels with the homogenous assays is certain to be a promising reliable way to assessment of dyslipidemia. Based on this perspective, the present study may contribute to the clinical reliability of non-fasting determination of serum lipids for dyslipidemia diagnosis and CHD risk assessment in the future.

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