

Blood Cholesterol Screening: Influence of Fasting State, Biological Variation and the Single Cholesterol Assay on Total Cholesterol Level

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Summary

Postprandial changes in plasma total cholesterol (TC) are minimal and there is essentially no difference between fasting vs random TC concentrations, as reflected in the small diurnal coefficient of variation (CV) for TC of 2.5%. Similarly, a cholesterol-rich meal within the last 24 hours lacked an impact on plasma TC. Thus, random specimens are acceptable in blood cholesterol screening. The intraindividual biological CV (CV_b) for plasma TC as measured over a long period was estimated from the data of several published studies to be 6.0%, which, when combined with a probable analytical CV (CV_a) of 5% during screening, gave a total intraindividual CV (CV_t) of about 8% for the single cholesterol assay. There is consensus that 'high TC values' acquired during screening should be confirmed under the conventional laboratory setting capable of CV_a of 3% or less.

Key words: Cholesterol screening, fasting state, plasma total cholesterol, intraindividual biological variation, analytical variation.

Introduction

Public screening for high blood cholesterol using the opportunistic method at all government outpatient clinics, particularly in individuals who possess one or more other risk factors of coronary heart disease (CHD) like cigarette smoking, hypertension and family history of heart disease, is included in the plan of action for the Cardiovascular Screening Project of the Ministry of Health, Malaysia¹.

However, there is currently some confusion or uncertainty among the local medical community on whether a fasting specimen is necessary, or which analytical system to use, in blood cholesterol screening. This was reflected in the frequent debates on these issues during relevant meetings, erroneous statements on the nature of the blood specimen in the press by 'concerned' individuals, and requests from the private sector for information on the topic.

This article reviews several published findings on the impact of fed versus fasting states plasma TC, biological variation in plasma TC, and implications of the single assay in blood cholesterol screening. It is hoped that the findings presented will serve as useful information as well as provide appropriate guidelines to the Malaysian health authorities and other professionals involved in blood cholesterol screening.

What Constitutes Plasma TC?

The lipids in circulation e.g., cholesterol, triglycerides (TG) and phospholipids, are solubilised in the predominantly aqueous environment of the circulation by complexing with proteins as lipoproteins.

There are 4 major classes of lipoproteins viz.: chylomicrons, low-density lipoproteins (LDL), very-low density lipoproteins (VLDL), and high-density lipoproteins (HDL). Each of these lipoprotein species contains cholesterol (free plus esterified), TG, phospholipids and proteins (known as 'apoproteins') but in different proportions (Table I). The plasma TC is the sum of the cholesterol content in all these lipoprotein classes.

Table I
Composition of plasma lipoproteins (%)*

Lipoprotein	Protein	Phospholipid	Cholesterol	TG
Chylomicron	2	8	6	84
LDL	25	22	43	10
VLDL	10	10	20	60
HDL	48	30	18	4

*Modified from Anderson *et al*² and Dupont³

Postprandial Plasma Lipid Concentrations

During absorption, TG resynthesised in the intestinal mucosa are 'packaged' together with cholesterol and phospholipids into chylomicrons. These lipoprotein particles are then released into the lymphatic system and enter the circulation at the left subclavian vein through the thoracic duct, and from there, the various sites of utilisation in the body.

The influx of TG-rich chylomicrons in the circulation causes postprandial lipaemia which peaks 4 to 6 hours when plasma TG concentrations >100% of preprandial values have been encountered^{4,6}. However, postprandial hypertriglyceridaemia is usually of only modest proportions in normal subjects^{6,7} since circulating chylomicrons have a short half-life (less than 1 hour in man⁸) and are entirely cleared between 9 and 12 hours⁷.

In contrast, the contribution of chylomicron-cholesterol to the circulating cholesterol pool would be expected to be only marginal since the ratio of cholesterol to TG in chylomicrons is about 1:14 (Table I).

As the TG-rich chylomicrons enter the circulation and are catabolised, some changes in cholesterol distribution also occur in the other lipoproteins. Redgrave and Carlson⁹ and Cohn *et al*¹⁰ reported significant postprandial increases in VLDL-cholesterol but no change in LDL-cholesterol. Cohn's group also observed a decrease in the cholesterol ester content of HDL, while Demacker *et al*¹¹ reported a diurnal CV of 4.5% for HDL-cholesterol but no significant difference between fasting and non-fasting levels.

Overall, most investigators observed no significant difference between pre vs postprandial or fed vs fasting plasma TC concentrations^{5,6,9,10,11,12}. However, Havel⁴ demonstrated a slight increase of 10% plasma TC in healthy subjects after an unrealistically high-fat (2.0 g/kg body weight) meal (Fig 1). It is also noteworthy that there has been a great improvement in cholesterol methodology since Havel's study in 1957.

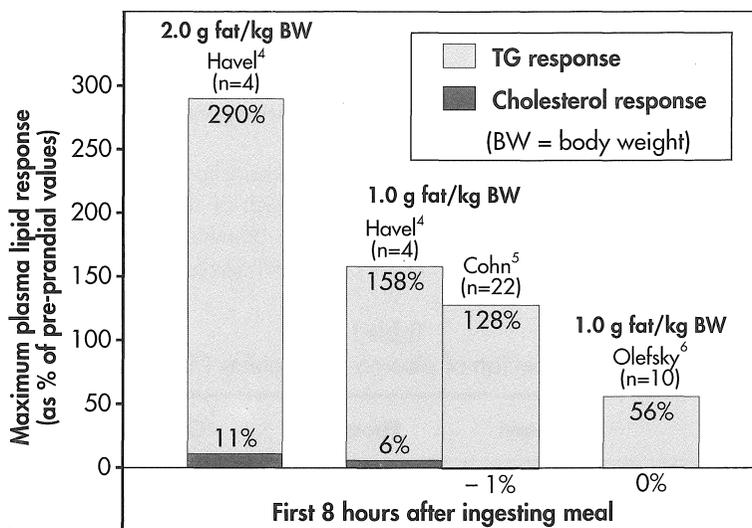


Fig 1: Postprandial plasma TG and cholesterol responses to different amounts of dietary fat in healthy adults.

The use of more realistic dietary fat levels viz 35 energy % by Olefsky⁶ in 10 healthy and 20 hypertriglyceridaemic adults resulted in essentially no change in plasma TC levels (Fig 1).

Furthermore, Kashyap *et al*¹² observed insignificant plasma TC responses in adults who consumed 100 g of either saturated or unsaturated fat, which also indicated that the type of dietary fat did not influence the postprandial plasma TC response.

Although the classical regression equation of Hegsted¹³ predicted a rise in plasma TC of about 5 mg/dl for every 100 mg cholesterol increase in intake, it is pertinent to note that the multiple feeding trials from which this equation was derived were conducted over 4 weeks. Compared to the predicted value, the mean rise in plasma TC reported in a recent review of 68 published clinical studies was only 2.3 mg/dl¹⁴. Thus, the small effect of dietary cholesterol on plasma TC, and the fact that the half-life of the cholesterol in plasma lipoproteins is about 38 days¹⁵, largely explains the finding that a cholesterol-rich meal did not significantly affect the postprandial plasma TC concentration¹⁶.

Consistent with the above findings on the postprandial plasma TC response, in many cases during 12 hours, it has been recommended that for the purpose of blood cholesterol screening, random (non-fasting) specimens may be used^{17,18,19,20}.

Biological Variation in Plasma TC

Transient changes in plasma TC concentrations can occur as a consequence of compositional changes that accompany the metabolism of lipoproteins. The magnitude of this biological variation differs appreciably among individuals and is influenced by environmental and behavioural factors^{21,22,23,24}.

Considerable changes in plasma TC have been observed within a few hours in individuals; fluctuations as high as 40% compared to initial values have been recorded²¹. More drastic changes (usually increases) in plasma TC were seen in individuals subjected to stress²².

However, under normal circumstances, the variation in plasma TC in most individuals is much less dramatic. The average change in plasma TC over 48 hours has been reported to be about 8% of the baseline value²⁵, while diurnal CV for plasma TC (fasting plus random specimens) averaged 2.5%¹¹.

The total intraindividual CV (CV_t) comprises the intraindividual biological CV (CV_b) and the analytical CV (CV_a), with the following relationship¹¹:

$$CV_t^2 = CV_b^2 + CV_a^2$$

The CV_t can be estimated by acquiring multiple specimens from the same individual over a long period²⁴. In this manner, it was reported that the CV_t for plasma TC increased with the sampling interval viz 4.8% for within-month variation and 6.1% for within-year variation.

From the data set reported by Cooper *et al*²⁴, it can be estimated that the CV_b and CV_t in an average person are 6.0% and 6.9% respectively, which agreed with a biological variation of 5% to 6% reported by Warnick and Albers²⁶.

Intraindividual CV_t with the Single Cholesterol Assay

Although the assay of additional specimens from the same person, and to a lesser extent replicate measurements of the same specimen, can reduce CV_t ²⁴, very often in blood cholesterol screening only a single specimen and a single cholesterol assay are possible because of logistic and budgetary constraints. Due to the substantial biological fluctuations in plasma TC and higher analytical CVs that may be associated under conditions during actual on-site screening, the validity of the single cholesterol assay in blood cholesterol screening has been raised.

Current guidelines recommend that, ideally, an assay system should have a level of precision consistent with a CV of 3% while overall accuracy or bias should be within $\pm 3\%$ of the true or reference value. However, as a short-term interim analytical performance target, a CV of 5% and a bias of $\pm 5\%$ are acceptable^{27,28}.

Although good analytical systems can achieve CVs of less than 2% under the conventional laboratory setting, the 'user-friendly' compact chemistry analysers (which seem attractive for use in blood cholesterol screening because they facilitate completion of diagnosis and treatment decision during a single patient/subject visit) were reported to achieve CVs for cholesterol analysis ranging from 1.7% to 5.6% with an estimated mean of about 3% during screening programmes²⁸. However, in laboratory and field evaluation of these desktop instruments, CVs as high as 5.5% to 9.4% have been reported²⁹.

Considering an average CV_b of 6.0% from the data set of Cooper *et al*²⁴, and a modest CV_a of 5.0% during on-site screening, the CV_t for TC based on a single assay and a single specimen would approximate 7.8%. This estimate agrees with the CV_t of 8.0% reported for paired analyses performed on fasting specimens from 7,055 subjects³⁰.

In view of the substantial CV_b , there is consensus that the TC value obtained in the screening exercise should be confirmed with an analytical system having a CV_a of 3% or less under the conventional laboratory setting during the follow-up visit(s) of the patient before initiating diet or other therapy.

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