APPLICATION OF FRIEDEWALD'S LDL-CHOLESTEROL ESTIMATION FORMULA TO SERUM LIPIDS IN THE JAPANESE POPULATION

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When direct measurement of serum low density lipoprotein cholesterol (LDL-c) is not available, it can be estimated from total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) by using the formula proposed by Friedewald:

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + \text{TG}/k], \quad k = 5$$

This formula assumes the triglyceride/cholesterol ratio in VLDL to be 5:1. However, it is changeable with serum triglyceride levels, and averaged as 4 among Japanese with triglycerides less than 400 mg/dl. The formula provides a better fit for Japanese people, when $k = 4$, rather than original $k = 5$. In addition, a better estimation is gained, if k is rotated according to the triglyceride levels of individual subjects; i.e. 3 for those with triglycerides less than 150 mg/dl, 4 for those with triglycerides from 150 to 299 mg/dl, 5 for those with triglycerides from 300 to 400 mg/dl. The percent error of estimation is less than 5% when $k = 4$, and about 1% when variable k is employed for populations of about 1,000 subjects in number.

AMONG serum lipoproteins LDL plays a central role in cellular metabolism of cholesterol¹ and atherosclerosis in man and experimental animals². However, their direct determination requires ultracentrifugation which takes time for a small number of samples³.

In epidemiological studies, clinical trials, large scale surveys and multicenter collaborative studies where a large number of specimens are determined, often repeatedly and within a short period of time, it is of great practical use to have such an estimation formula as Friedewald's⁴:

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + \text{TG}/k], \\ k = 5 \dots \dots \dots (1)$$

Key Words:

Serum lipids in Japan
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This formula (for abbreviation see footnote) is based on the assumptions that i) total cholesterol distributes solely in three major lipoproteins of VLDL, LDL and HDL, i.e.;

$$\text{TC} = \text{VLDL-c} + \text{LDL-c} + \text{HDL-c}$$

ii) triglycerides are transported exclusively by VLDL, and iii) the ratio of triglyceride/cholesterol (TG/C) is 5:1 in VLDL.

On testing the applicability of this formula to serum lipids of Japanese, the validity of the last assumption is questioned, since serum triglyceride levels have been demonstrated to be lower in Japan⁵⁻⁷ than in the US where the formula is derived from subjects with a different nutritional backgrounds^{8,9}.

We have examined the ratio of TG/C ratio in VLDL, measured LDL-c concentrations in Japanese, not only for normal healthy subjects but for hyperlipidemic patients of both non-familial and familial origin, and evaluated the

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Abbreviation: TC: total cholesterol; C: cholesterol; TG: triglycerides; VLDL-c: very low density lipoprotein-cholesterol; LDL-c: low density lipoprotein-cholesterol; HDL-c: high density lipoprotein-cholesterol

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TABLE 1 THE BACKGROUND MAJOR LIPIDS OF THE SUBJECTS

	<i>n</i> (m:f)	Age (year)	Total Chole- sterol (mg/dl)	Trigly- cerides (mg/dl)	HDL- chole- sterol (mg/dl)
<i>VLDL-c/TG Ratio Determination</i>					
<i>Normal Subjects</i>	26 (18:8)	56 ± 15	180 ± 24	97 ± 31	55 ± 13
<i>Hyperlipidemia</i>	49 (26:23)	56 ± 14	284 ± 58	244 ± 330	44 ± 10
<i>TG < 150 mg/dl</i>	24 (10:14)	—	312 ± 39	88 ± 28	41 ± 10
<i>150–299 mg/dl</i>	17 (8:9)	—	268 ± 69	219 ± 40	46 ± 11
<i>300–749 mg/dl</i>	6 (6:0)	—	231 ± 32	447 ± 139	49 ± 6
<i>750 mg/dl –</i>	2 (2:0)	—	266 ± 31	1642 ± 601	—
<i>Familial Hyper-C.</i>	27 (12:15)	52 ± 14	313 ± 41	105 ± 56	40 ± 10
<i>TG < 150 mg/dl</i>	23 (11:12)	—	312 ± 41	88 ± 25	41 ± 10
<i>150 mg/dl –</i>	4 (1:3)	—	309 ± 54	205 ± 50	39 ± 9
<i>LDL-c Determination</i>					
<i>Total Subjects</i>	920 (470:450)	51 ± 23	212 ± 48	110 ± 147	54 ± 13
<i>Familial Hyper-C.</i>	53 (27:26)	53 ± 14	319 ± 60	149 ± 82	44 ± 12
<i>TG < 150 mg/dl</i>	30 (15:15)	51 ± 14	311 ± 44	88 ± 30	47 ± 9
<i>TG > 150 mg/dl</i>	23 (12:11)	55 ± 13	329 ± 76	228 ± 57	40 ± 14
<i>Hyper-C: Hypercholesterolemia</i>					

accuracy of the estimated LDL-c by comparing them with those measured. Consequently, we have gained a revised formula that provides a better fit for estimation of serum LDL-c of Japanese.

SUBJECTS AND METHODS

A total of 102 subjects was employed for determination of TG/C ratio in VLDL. Of these, 24 were normolipidemic (total cholesterol less than 220 mg/dl and triglycerides less than 150 mg/dl). There were 18 males and 8 females with an average age of 56 ± 15 (male 57 ± 15, female 55 ± 15). A second group of 49 were hyperlipidemic (male 28, female 21) of non-familial origin with an average age of 56 ± 14 (male 54 ± 11, female 58 ± 15) among which 24 were type IIa, 15 were type IIb and 10 were type IV. The third group of 27 were heterozygotes of familial hypercholesterolemia (male 12, female 15) with an average age of 52 ± 14 (male 52 ± 11, female 56 ± 15) who were diagnosed by the trias of total cholesterol above 300 mg/dl, presence of tendon xanthomas and positive family history.¹⁰

The subjects used for determination of VLDL-c and LDL-c concentrations were 920 normo- and hyperlipidemic subjects (male 470, female 450) with an average age of 56 ± 14 (male 51 ± 22,

female 51 ± 24). They were grouped by their triglyceride levels: 765 subjects (male 360, female 405) less than 150 mg/dl, 131 subjects (male 91, female 40) from 150 to 299 mg/dl, 21 subjects (male 17, female 4) from 300 to 749 mg/dl, and 3 subjects (male 2, female 1) above 750 mg/dl. The background major lipids of a total of 102 subjects for VLDL-TG/C ratio determination, and 920 subjects with normo- and hyperlipidemia of non-familial origin and 53 subjects with familial hypercholesterolemia for VLDL-c and LDL-c determination and comparison are listed in Table 1.

VLDL, LDL and HDL were isolated by ultracentrifugation using Lp-42 Ti rotor (Beckman, Palo Alto) at 42,000 rpm, 222,000 G, 4°C, for 4 hours.¹¹ Total cholesterol,¹² VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol¹³ and triglycerides¹⁴ were determined by enzymatic methods using an autoanalyser (ABA-100, Abbott, Irving).

Estimation was made for LDL-c using the Friedewald equation (1). Firstly by applying each 3, 4, 5 and 6 for k to 920 subjects with normo- and hyperlipidemias and 53 subjects with heterozygous familial hypercholesterolemia. The results were then compared among subgroups stratified by their triglyceride levels. Secondly by choosing 4 and 5 for k to all 920 subjects and

TABLE II THE TRIGLYCERIDE/CHOLESTEROL RATIO IN VLDL IN NORMAL AND HYPERLIPIDEMIC SUBJECTS OF NON-FAMILIAL AND FAMILIAL ORIGIN

Subjects	n	TG/C Ratio in VLDL
Normal Subjects	26	3.3 ± 1.2
Hyperlipidemia	49	3.5 ± 1.2
TG < 150 (mg/dl)	24	3.7 ± 1.9
150–229	17	4.3 ± 1.2
300–749	6	5.8 ± 1.1
750–	2	4.5 ± 1.4
Familial Hyper-C.	27	3.5 ± 1.4
TG < 150 (mg/dl)	23	3.4 ± 1.9
150–	4	4.2 ± 1.3
Total	102	3.5 ± 1.3

908 subjects with triglycerides less than 400 mg/dl, and 53 patients with familial hypercholesterolemia. And thirdly by switching 3, 4 or 5 for k in 920 subjects and 908 subjects with triglycerides less than 400 mg/dl according to their triglyceride levels of individual subjects, e.g., 3 for subjects with triglycerides less than 150 mg/dl, 4 for those with triglycerides from 150 to 299 mg/dl, and 5 for those with triglycerides above 300 mg/dl.

Comparison was made between estimated and measured LDL-c, and also between TG/k (k = 4, 5 and variable k with triglycerides) and measured VLDL-c. Net and percent errors, correlation coefficients, and regression equations were evaluated for the best fit k values. The scatter diagram of correlation was illustrated by a concentric ellipsoid. The statistical significance was assumed at the level of 5% (two-tailed test).

A multivariate analysis was also performed on 920 subjects to determine the relationship of total cholesterol, triglycerides and HDL-c to LDL-c, where LDL-c was set as the dependent variable and total cholesterol, triglycerides and HDL-c as independent variables, and their partial regression coefficients were compared. To facilitate estimation, nomograms were developed for calculation of TC-HDL-c and LDL-c.

RESULTS

The triglyceride/cholesterol ratio determined for VLDL is presented in Table II. In

normolipidemic subjects, it averaged 3.3 (standard deviations shown in Table II), while it was 3.7 in hyperlipidemia of non-familial origin with triglycerides less than 150 mg/dl, 4.3 in triglycerides 150–300 mg/dl, 5.8 in triglycerides 300–749 mg/dl, and 4.5 in triglycerides above 750 mg/dl. In hypercholesterolemia type IIa of familial origin with triglycerides less than 150 mg/dl, the ratio was 3.9, and in type IIb with triglycerides above 750 mg/dl, it was 4.2. These indicated that, both in non-familial and familial hyperlipidemias, the TG/C ratio in VLDL changed with triglyceride levels. These were: approximately 3, not only in normolipidemia but also in familial hypercholesterolemia type IIa with triglycerides less than 150 mg/dl; 4 in non-familial hyperlipidemias with triglyceridemia 150–299 mg/dl and in familial hypercholesterolemia type IIb with triglycerides above 150 mg/dl; and 5 in non-familial hyperlipidemia with triglycerides above 300 mg/dl.

LDL-c levels estimated by using k = 3, 4, 5, and 6 were compared with the measured LDL-c among subgroups as seen in Table III and IV. In normolipidemic subjects, k = 3 gave a mean of estimated LDL-c of 127 mg/dl vs a mean of measured LDL-c of 127 mg/dl (described only as the mean value), the net difference being 0.2 ± 20 mg/dl, percent error 1% (hereafter shown in parenthesis), $r = 0.8687$, and regression equation $y = 0.78x + 28.3$. Both the net and percent differences were smallest among the other estimations made by k = 4, 5, and 6. In type IIa familial hypercholesterolemia with triglycerides less than 150 mg/dl, k = 5 gave the smallest error where the estimated vs measured LDL-c was 234 mg/dl : 223 mg/dl, the difference being 11 ± 21 mg/dl (5%), $r = 0.8610$, and $y = 0.84x + 26.2$.

In hyperlipidemia with triglycerides 150–299 mg/dl, both k = 4 and 5 yielded a reasonable estimation within an error of less than 5%. When k = 4 was used, estimated LDL-c averaged as 129 mg/dl vs measured LDL-c as 136 mg/dl. The difference was -7 ± 29 mg/dl (–4%), $r = 0.7276$, and $y = 0.68x + 479$. If we chose k = 5, estimated vs measured LDL-c was 139 mg/dl : 136 mg/dl, the difference being 3 ± 28 mg/dl (4%), $r = 0.7337$, and $y = 0.69x + 40.1$. In type IIb familial hypercholesterolemia with triglycerides above 150 mg/dl, k = 4 gave a better estimation. The estimated vs measured LDL-c was 232 mg/dl : 224 mg/dl, the error being 8 ± 29 mg/dl (4%), $r = 0.9260$ and $y = 0.84x + 20.9$.

In hyperlipidemias with triglycerides 300–

TABLE III COMPARISON BETWEEN ESTIMATED AND MEASURED LDL-c FOR THE NON-FAMILIAL HYPERLIPIDEMIC SUBJECTS WITH DIFFERENT SERUM TRIGLYCERIDE LEVELS

Trigly- ceride		<i>k</i>			
		<i>k</i> = 3	<i>k</i> = 4	<i>k</i> = 5	<i>k</i> = 6
below 150 mg/dl	<i>n</i>	765	765	765	765
	<i>e</i> -LDL	127 ± 41	134 ± 41	138 ± 42	140 ± 42
	<i>m</i> -LDL	127 ± 37	127 ± 37	127 ± 37	127 ± 37
	$\bar{\Delta}$	0.2 ± 20	7 ± 20	11 ± 20	14 ± 20
	% $\bar{\Delta}$	1 ± 17	6 ± 17	10 ± 17	12 ± 17
	<i>r</i>	0.8687	0.8773	0.8808	0.8824
	<i>y</i>	0.78 <i>x</i> + 28.28	0.78 <i>x</i> + 22.34	0.78 <i>x</i> + 29.95	0.78 <i>x</i> + 17.19
150 - 299 mg/dl	<i>n</i>	131	131	131	131
	<i>e</i> -LDL	112 ± 40	129 ± 40	139 ± 40	146 ± 40
	<i>m</i> -LDL	136 ± 37	136 ± 37	136 ± 37	136 ± 37
	$\bar{\Delta}$	-23 ± 29	-7 ± 29	3 ± 28	10 ± 28
	% $\bar{\Delta}$	-16 ± 23	-4 ± 22	4 ± 22	10 ± 23
	<i>r</i>	0.7141	0.7276	0.7337	0.7369
	<i>y</i>	0.66 <i>x</i> + 61.28	0.68 <i>x</i> + 47.91	0.69 <i>x</i> + 40.09	0.69 <i>x</i> + 35.03
300 - 749 mg/dl	<i>n</i>	21	21	21	21
	<i>e</i> -LDL	68 ± 47	102 ± 43	122 ± 41	136 ± 40
	<i>m</i> -LDL	120 ± 37	120 ± 37	120 ± 37	120 ± 37
	$\bar{\Delta}$	-52 ± 33	-18 ± 29	2 ± 27	15 ± 26
	% $\bar{\Delta}$	-47 ± 35	-16 ± 27	3 ± 26	16 ± 27
	<i>r</i>	0.7064	0.7469	0.7654	0.7739
	<i>y</i>	0.56 <i>x</i> + 82.31	0.64 <i>x</i> + 94.97	0.69 <i>x</i> + 36.62	0.71 <i>x</i> + 24.17
above 750 mg/dl	<i>n</i>	3	3	3	3
	<i>e</i> -LDL	-340 ± 454	-182 ± 312	-87 ± 227	-24 ± 170
	<i>m</i> -LDL	129 ± 39	124 ± 39	124 ± 39	124 ± 39
	$\bar{\Delta}$	-464 ± 439	-306 ± 297	-211 ± 213	-148 ± 157
	% $\bar{\Delta}$	-414 ± 422	-272 ± 286	-187 ± 204	-130 ± 150
	<i>r</i>	0.4277	0.4335	0.4405	0.4490
	<i>y</i>	0.04 <i>x</i> + 136.34	0.05 <i>x</i> + 133.74	0.07 <i>x</i> + 130.53	0.10 <i>x</i> + 126.45

e-LDL: estimated LDL (mg/dl), *m*-LDL: measured LDL (mg/dl), $\bar{\Delta}$: mean difference between estimated and measured LDL (mg/dl), % $\bar{\Delta}$: percent mean difference (%), *r*: regression coefficient, *y*: regression equation

749 mg/dl, *k* = 5 gave the smallest mean difference of 2 ± 27 mg/dl, (3%), estimated vs measured LDL-c being 122 mg/dl : 120 mg/dl, *r* = 0.7654, and *y* = 0.69 *x* + 36.6.

In the group having triglycerides above 750 mg/dl, none of *k* = 3, 4, 5, 6 gave a satisfactory estimation. All yielded negative LDL-c because of the large triglyceride values.

From these data, the most suitable *k* was 3 for normolipidemia and type IIa familial hyper-

cholesterolemia with triglycerides less than 150 mg/dl, while it was either 4 or 5 for non-familial hyperlipidemias with triglycerides 150-299 mg/dl, 4 in type IIb familial hypercholesterolemia with triglycerides above 150 mg/dl, and 5 for hyperlipidemias with triglycerides 300-749 mg/dl.

As both *k* = 4 and 5 provided a reasonable estimation for hyperlipidemia with triglycerides from 150 through 749 mg/dl, we compared the estimated and measured LDL-c by applying each

TABLE IV COMPARISON BETWEEN ESTIMATED AND MEASURED LDL-c FOR FAMILIAL HYPERCHOLESTEROLEMIA TYPE IIa AND IIb

Familial Hypercholesterolemia		<i>k</i>		
		3	4	5
Type IIa (TG < 150 mg/dl)	<i>n</i>	30	30	30
	<i>e</i> -LDL-c	234 ± 42	242 ± 42	246 ± 42
	<i>m</i> -LDL-c	223 ± 40	223 ± 40	223 ± 40
	$\bar{\Delta}$	11 ± 21	18 ± 22	22 ± 22
	% $\bar{\Delta}$	5 ± 10	9 ± 11	11 ± 11
	γ	0.8610	0.8610	0.8594
	$y = ax + b$	0.84 x + 26.2	0.83 x + 23.2	0.82 x + 22.3
Type IIb (TG ≤ 150 mg/dl)	<i>n</i>	23	23	23
	<i>e</i> -LDL-c	213 ± 77	232 ± 77	244 ± 77
	<i>m</i> -LDL-c	224 ± 68	224 ± 68	224 ± 68
	$\bar{\Delta}$	-11 ± 30	8 ± 29	19 ± 29
	% $\bar{\Delta}$	-6 ± 12	4 ± 12	8 ± 13
	γ	0.9254	0.9260	0.9254
	$y = ax + b$	0.83 x + 48.8	0.83 x + 32.5	0.84 x + 20.9

For abbreviation see the footnote in Table III

$k = 4$ and 5 uniformly to all 920 subjects, and also by switching either one of 3, 4, and 5 to 920 subjects according to their triglyceride levels as described previously. The results are presented in Table V and Fig. 1. When $k = 4$ was applied, the estimated and measured LDL-c came out as 132 mg/dl : 128 mg/dl. The difference was 4 ± 23 mg/dl (4%), $r = 0.8326$, and $y = 0.73x + 31.4$. When $k = 5$, the estimated vs measured LDL-c was 137 mg/dl : 128 mg/dl, the difference being 9 ± 22 mg/dl (8%), $r = 0.8468$, and $y = 0.75x + 24.8$. When we adopted variable k according to triglyceride levels, the estimated vs measured LDL-c was 127 mg/dl : 128 mg/dl, the difference being -1 ± 22 mg/dl (-0.1%), $r = 0.8403$, and $y = 0.75x + 22.2$. Also applied were $k = 4$, 5 and variable k to 53 familial hypercholesterolemia as in the bottom of Table 5. Taken together, the net and percent difference became smaller in the order of variable k , $k = 4$ and $k = 5$, while correlation coefficients tended to be slightly greater in the order of $k = 5$, variable k and $k = 4$.

Since large triglyceride values yielded negative LDL-c, we limited application only to those with triglycerides less than 400 mg/dl as Friedewald originally did, and made estimations for 908 subjects whose triglycerides were below 400 mg/dl. Then, as in the middle of Table V, $k = 4$ yielded the estimated vs measured LDL-c as 133 mg/dl : 128 mg/dl. The difference was 5 ± 22

mg/dl (5%), $r = 0.8487$, and $y = 0.76x + 26.8$. When $k = 5$, the estimated vs measured was 138 mg/dl : 128 mg/dl, the difference being 10 ± 24 mg/dl (9%) $r = 0.8577$ and $y = 0.77x + 21.9$. When variable k was applied, the estimated vs measured LDL-c was 127 mg/dl : 128 mg/dl. The difference was 0.1 ± 18 mg/dl (-0.6%), $r = 0.8466$ and $y = 0.76x + 30.7$. The net and percent difference was again smaller in the order of variable k , $k = 4$ and $k = 5$, while correlation coefficients tended to be slightly larger in the opposite order.

Comparison was further made for TG/ k ($k = 4$, 5 and variable) with measured VLDL-c. The results are shown in Table VI. When $k = 4$, the mean of TG/4 was 28 mg/dl, while the measured VLDL-c averaged as 32 mg/dl, the difference being -4 ± 18 mg/dl (13%), $r = 0.5590$ and $y = 0.37x + 21.3$. When $k = 5$, the mean of TG/5 vs measured VLDL-c was 22 mg/dl : 32 mg/dl, the difference being -10 ± 16 mg/dl (31%), $r = 0.5590$ and $y = 0.47x + 21.3$. When k was varied with triglyceride levels, the mean of both TG/ k and measured VLDL-c were 32 mg/dl, the difference being 0.1 ± 12 mg/dl (0.1%), $r = 0.5632$ and $y = 0.53x + 14.4$.

A multivariate analysis was performed on 920 subjects, where LDL-c was adopted as the criterion variable, and total cholesterol, triglycerides and HDL-c as explanatory variables. It gave

TABLE V COMPARISON BETWEEN MEASURED AND ESTIMATED LDL-c BY USING $k = 4, 5$ AND VARIABLE k IN THE SUBJECTS WITH HYPERLIPIDEMIA OF NON-FAMILIAL AND FAMILIAL ORIGIN

Subjects		k		
		4	5	Variable 3~6
Total Subjects	n	920	920	920
	e -LDL-c	132 ± 42	137 ± 42	127 ± 41
	m -LDL-c	128 ± 37	128 ± 37	128 ± 37
	$\bar{\Delta}$	4 ± 23	9 ± 22	-1 ± 22
	$\% \bar{\Delta}$	4 ± 20	8 ± 19	-0.1 ± 19
	γ	0.8326	0.8468	0.8403
	$y = ax + b$	$0.73x + 31.4$	$0.75x + 24.8$	$0.75x + 22.2$
Triglycerides below 400 mg/dl	n	908	908	908
	e -LDL-c	133 ± 41	138 ± 41	127 ± 41
	m -LDL-c	128 ± 37	128 ± 37	128 ± 37
	$\bar{\Delta}$	5 ± 22	10 ± 24	0.1 ± 18
	$\% \bar{\Delta}$	5 ± 18	9 ± 18	-0.6 ± 22
	γ	0.8487	0.8577	0.8466
	$y = ax + b$	$0.76x + 26.8$	$0.77x + 21.9$	$0.76x + 30.7$
Familial Hypercholesterolemia	n	53	53	53
	e -LDL-c	238 ± 59	245 ± 58	234 ± 58
	m -LDL-c	224 ± 54	224 ± 54	224 ± 54
	$\bar{\Delta}$	14 ± 25	21 ± 25	10 ± 25
	$\% \bar{\Delta}$	7 ± 12	10 ± 12	5 ± 12
	γ	0.9026	0.9049	0.9031
	$y = ax + b$	$0.82x + 28.8$	$0.83x + 20.6$	$0.83x + 29.5$

For abbreviation see the footnote in Table III

Fig.1. Correlation between estimated and measured LDL-c when $k = 4, 5$ and variable k

a multiple regression equation:

$$\text{LDL-c} = 0.958 (\text{TC}) - [0.647 (\text{HDL-c}) + 0.067 (\text{TG})] + 8.803$$

and multiple correlation coefficient 0.8580. Partial regression coefficients for explanatory variables were greater in the order of TC, HDL-c and triglycerides.

DISCUSSION

We have investigated the applicability of Friedewald's LDL-c estimation formula (1) to serum lipids and lipoproteins of Japanese, and reached the conclusion that $k = 4$, rather than Friedewald's original $k = 5$, is better suited for estimation of Japanese serum LDL-c. If we rotate k from 3 to 5 according to triglyceride levels of individual subjects, then the estimated LDL-c are closer to the measured LDL-c.

In conjunction with these conclusions,

TABLE VI COMPARISON BETWEEN TG/k AND MEASURED VLDL-c IN NORMAL AND HYPERLIPIDEMIC SUBJECTS

	<i>k</i>		
	4	5	Variable 3~6
<i>n</i>	920	920	920
TG/ <i>k</i>	28 ± 37	22 ± 29	32 ± 26
<i>m</i> -VLDL-c	32 ± 25	32 ± 25	32 ± 25
$\bar{\Delta}$	-4 ± 18	-10 ± 16	0.1 ± 15
% $\bar{\Delta}$	13 ± 15	31 ± 13	0.1 ± 12
γ	0.5590	0.5590	0.5632
$y = ax + b$	0.37x + 21.3	0.47x + 21.3	0.53x + 14.4

For the abbreviation see the footnote in Table III

Fig.2. Criteria for accuracy of comparison of two different methods

discussion is warranted on the following three points; i) rationale for estimation of LDL-c by TG/*k* ratio, ii) criteria for accuracy of LDL-c estimation, and iii) applicability of estimated LDL-c in clinical and field studies.

1. Estimation of LDL-c by TG/*k* Ratio

The first rationale for making the estimation of LDL-c possible by using TG/*k* is the correlation

between TG/*k* ratio and measured VLDL-c.

Friedewald's formula (1) assumes that the TG/C ratio is 5:1 in VLDL, thus TG/5 is equal to VLDL-c. This ratio however has been derived from subjects whose nutritional background is quite different from that of the majority of Japanese people. When serum triglycerides are elevated, it is known that triglyceride content is

Fig. 3. Nomogram for calculation of [TC-HDL-c]

raised in VLDL¹⁵ and their size increased.¹⁶

We have therefore checked the TG/C ratio in VLDL from 102 subjects with normo- and hyperlipidemias of both non-familial and familial origin, and found that VLDL-TG/C ratio is changeable with triglyceride levels, irrespective of non-familial or familial hyperlipidemias. The change in the ratio, however, is not random, but corresponds to serum triglyceride levels, probably within structural allowance of VLDL. There is thus a correlation between TG/k and measured VLDL-c, rendering the following calculation possible;

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + \text{VLDL-c}],$$

$$\text{VLDL-c} = \text{TG}/k$$

The second reason is the weight of factor TG/k in estimation. When we inspect the effect of k on the estimated LDL-c, only errors (D, difference between the estimated and measured LDL-c) have appreciable change with k, while the standard deviation (SD), correlation coefficient (r) and the slope (a) of regression line $y = ax + b$ remain relatively unchanged (Table III and IV). This suggests that a is mainly under the influence of total cholesterol, while D and SD under HDL-c and TG/k. However, as indicated by the multiple regression analysis, the partial regression coefficient of TG/k is as low as about 1/10 of that of total cholesterol and HDL-c. This relationship minimizes the variations due to TG/k ratio, and allows us to estimate LDL-c by using k = 4 or

Fig. 4. Nomogram for LDL-c estimation by the formula: $\text{LDL-c} = \text{TC} - [\text{HDL-c} + \text{TG}/k]$

variable k method.

2. Criteria for Accuracy of LDL-c Estimation

Comparing the estimated LDL-c with the measured values, accuracy of the estimation is determined by the combination of D, SD, r and a. There are 6 combinations of them to define accuracy as shown in Fig. 2: (i) SD is small, r high, $D \approx 0$ and $a \approx 1$, (ii) SD is small, r high, $D \neq 0$ but $a \approx 1$, (iii) SD and r are large, $D \neq 0$, but $a \approx 1$, (iv) SD and r are large, but $D \neq 0$, and $a \neq 1$, (v) SD is large, r low, but $D \approx 0$ and $a \approx 1$, and (vi) SD is large, r low, $D \neq 0$ but $a \approx 1$.

Of these possibilities, accuracy is judged to be highest in the descending order of (i) to (vi), since in (i) both dispersion (SD and r) and deviation (D and a) of the estimated values are small. In contrast, they scatter widely and deviate markedly in (vi).

We have chosen the value of 4 as suitable k

because i) application of $k = 4$ to 908 subjects with triglycerides less than 400 mg/dl yielded small D of 5 mg/dl with moderate SD of 22 mg/dl, high r of 0.8489 and $a = 0.73$ (Table V) which corresponds to the above (ii), ii) the average TG/C ratio in VLDL of 102 subjects fell within the range of 3.5 ± 1.3 (Table II), and iii) TG/ k ($k = 4$) correlated best with the measured VLDL-c (Table VI). Thus by using $k = 4$, we have gained the estimated LDL-c averaging as 132 mg/dl vs the measured as 128 mg/dl.

If we rotate k for each individual from 3 to 6, as previously mentioned, we can gain a more accurate estimation: D is as minimal as 0.1 mg/dl and SD as 22 mg/dl, r as high as 0.8466 and $a = 0.76$, which matches to the above (i). Correlation is also high between TG/ k and measured VLDL-c, whereby the mean of estimated LDL-c is 127 mg/dl vs that of measured 128 mg/dl.

In light of the accuracy criteria described above, the estimated LDL-c using $k = 4$ is satisfactory with an average percent error within 5%. Likewise, the estimation by applying variable k is better, with an average percent error of about 1%, when we have a sample size of about 1,000 in number.

3. Applicability of Estimation of LDL-c

In clinical studies where a specific diagnosis has to be established and abnormalities in serum lipoproteins to be related to pathogenesis of a specific disease, LDL and other lipoproteins must be isolated by ultracentrifugation, their quantity measured, and their qualitative changes demonstrated by electrophoresis. In metabolic research where production, conversion, degradation and interaction of lipoproteins with cells or enzymes are investigated, biochemical analysis for serum lipoproteins including LDL is, of course, required.

However, in epidemiological studies, the number of subjects is usually too large to have serum lipoproteins individually analyzed. Yet it is still necessary to have knowledge of LDL, since it is considered to be the most important risk factor in coronary heart disease.¹⁷ In such cases if total cholesterol, triglycerides and HDL-c are known, LDL-c can be estimated.

For clinical trials on drugs, multicenter collaborative studies on patients, wide-scale surveys on nutrition and coronary risk factors, and long-term follow-up investigations of mortality due to atherosclerotic disease, the situation is the same, and the utility of estimation for LDL-c is quite valuable.

The variable k method, not fixed with 4 or 5, may further be applied to other populations than Japanese by employing a fitting k in correspondence to triglyceride levels of individual subjects.

To increase practical convenience, we have developed nomograms for the estimation of LDL-c either by using $k = 4$ or variable k from total cholesterol, triglycerides and HDL-c, as shown in Fig. 3 and 4. This will facilitate the use of estimation formulae in various fields.

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