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Direct assessment of plasma low density lipoprotein and high density lipoprotein cholesterol levels and coronary heart disease: results from the Framingham Offspring Study

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Abstract

BACKGROUND—We evaluated direct low density lipoprotein (LDL) cholesterol (C) and high density lipoprotein (HDL) cholesterol (C) versus standard methods using fasting plasma samples from participants in cycle 6 of the Framingham Offspring Study.

METHODS—Direct LDL-C and HDL-C measurements were performed on fasting plasma from male (1335 controls, 173 CHD cases) and female (1606 controls, 74 cases) participants, and compared with LDL-C, as calculated with the Friedewald formula, and HDL-C, as measured after dextran-$Mg2^{+}$ precipitation.

RESULTS—Values for direct LDL-C and HDL-C correlated well with standard methods **(both about r^2=0.94,** $p<0.001$) with similar absolute values. Biases of > 10% were present for 7.7% of samples for LDL-C, while for HDL-C this value was 8.5%. Despite higher use of cholesterol lowering medication in CHD cases, calculated or direct LDL-C values were still well above recommended values [< 2.6 mmol/L, (100 mg/dl)] in CHD cases, especially in females.

CONCLUSIONS—Direct assays for both LDL-C and HDL-C provide an acceptable guide for lipid treatment. In the Framingham Offspring Study participants, most CHD cases have LDL-C levels above the recommended target.

Keywords

cholesterol; LDL; HDL; coronary heart disease; Framingham Offspring Study

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INTRODUCTION

Coronary heart disease (CHD) remains a leading cause of death in the United States. Significant risk factors include gender, age, smoking, diabetes, hypertension, elevated low density lipoprotein (LDL) cholesterol (C) [>4.14mmol/L], and decreased high density lipoprotein (HDL)-C [<1.03mmol/L](1). Calculated LDL-C has been established by the Adult Treatment Panel of the National Cholesterol Education Program as the lipid target for CHD risk reduction; this parameter is commonly not measured but rather calculated by the Friedewald formula (1,2). In the Framingham Offspring Study, we have previously compared the LDL-C values calculated with this formula with LDL-C values obtained by ultracentrifugation, and our data indicated that calculated LDL-C values are quite similar to those obtained after ultracentrifugation, provided the subjects had fasted overnight and the plasma triglyceride levels were below 2.8 mmol/L (approximately 250 mg/dl) (3). Another issue is that, in the past, HDL-C measurement had required a manual precipitation step (4). Therefore there was a great need for the development of high-throughput, well standardized, automated assays for the direct measurement of both HDL-C, and LDL-C. Such assays have been developed and our goal was to compare values obtained with these assays with HDL-C values obtained after precipitation as well as LDL-C calculated with the Friedewald formula in plasma samples from male and female participants in cycle 6 of the Framingham Offspring Study. We also wished to determine whether subjects with CHD in this population were at the recommended goals for LDL-C established for such patients by the Adult Treatment Panel of the National Cholesterol Education Program of the National Institutes of Health (1).

MATERIALS AND METHODS

Subjects were participants in the Framingham Offspring Study (FOS), a long-term community-based prospective observational study of risk factors for CHD. These subjects are the offspring and their spouses of participants in the original Framingham Heart Study (FHS). During cycle 6 of FOS (1995–98) participants had a standardized medical history, physical examination, and fasting lipid measurements (5). Exclusion criteria were drug or alcohol abuse. Selection criteria for the CHD cases included a history of angina, myocardial infarction, or coronary insufficiency. We performed our analyses an all available plasma samples from male and female participants in cycle 6, which comprised 173 male CHD cases and 1335 male controls and 74 female cases and 1606 female controls. Total cholesterol and triglyceride levels were determined by standard enzymatic methods as previously described (5,6). HDL-C was isolated from the supernatant after dextran sulfate-Mg^{2+} precipitation (4). LDL-C was calculated using the Friedewald formula (2).

In this study, archived plasma samples, frozen at −80 °C and never thawed, were used for the direct measurement of LDL-C and HDL-C. Homogeneous direct assay kits for LDL-C and HDL-C were obtained from Kyowa Medex Co. (Tokyo, Japan) as previously described and run in an automated fashion on a Hitachi 911 analyzer (Hitachi Co., Tokyo Japan) (7,8). The direct HDL-C assay had intra-assay and inter-assay coefficients of variation of 0.82% and 0.76%, respectively. For the direct LDL-C assay, these values were 0.73% and 1.13%, respectively. Cholesterol, triglyceride, and HDL cholesterol measurements were standardized by the Lipid Standardization Program of the Centers for Disease Controls and Prevention (Atlanta, GA). All laboratory personnel were blinded with regard to identification of study subjects, and only numbered samples were used.

STATISTICAL ANALYSIS

Descriptive statistics including means (standard deviations) and median [inter quartile range] for continuous variables and proportions for categorical variables were computed for all-study variables and all-study groups. The distribution of the variables is provided in the normal population, then by menopausal status in the Supplementary material, and finally in men and women with and without CHD. Groups were compared using two sample *t*-tests for continuous variables and Chi-square tests for categorical variables. In the prospective follow-up analysis over a mean of 7.5 years, CHD cases at baseline (cycle 6) were excluded. For this analysis, adjusted means and standard errors for plasma LDL-C and HDL-C levels in CHD and CHD-free subjects were calculated using analysis of covariance techniques with adjustments for lipid-lowering medications, age, smoking, hypertension, diabetes, BMI, as well as standard lipid parameters. Moreover, potential confounding factors were used as covariates. In addition, we used the generalized estimating equation approach, PROC GEN MED and SAS to account for correlated data caused by familial relations and matched design. For continuous measures which were highly skewed, we used log-transformed values for statistical analysis. To evaluate to association between the various lipid parameters and the odds of developing CHD we used logistic regression models with CHD status as a dependent variable and lipid parameters as independent variables.

RESULTS

In the entire population the correlation coefficient between HDL-C values obtained after precipitation and values obtained by the direct method was 0.97 ($p<0.0001$). The identical correlation coefficient was obtained ($r=0.97$, $p<0.0001$) between calculated LDL-C and direct LDL-C. Bias plots for HDL-C assessed with the direct method and with the precipitation method, and for LDL-C assessed with the direct and the Friedewald formula, are shown in the Supplementary Material (Figures 1–4). For subjects without CHD, diabetes, and not on medications known to affect cholesterol levels (n = 2082), HDL-C biases greater than 5%, 10%, and 15% were observed in 26.2%, 8.5%, and 1.6% of the population, respectively, while for LDL-C these values were 27.1%, 7.98%, and 2.0%, respectively.

In order to examine differences between assays further In supplementary figure 5 we have plotted % bias for HDL cholesterol (HDL Kyowa – HDL Dextran/ HDL Dextran) versus HDL cholesterol values (Dextran) for normal subjects. Here we see that there is a negative slope (−0.0192, $p<0.001$), indicating that the bias moves from slightly positive bias to a slightly negative bias at higher HDL C values (dextran). In supplementary figure 5 we have also plotted % bias for HDL cholesterol versus triglyceride values. Here again we see a negative slope (−0.0292, $p<0.001$), again indicating that the bias moves from a slightly positive bias to a negative bias as triglyceride levels increase. For abnormal subjects (with CHD, diabetes, or on lipid lowering medication) % bias plots for HDL cholesterol are shown in supplementary figure 6. The negative slope for % bias versus HDL cholesterol was much greater (−0.0665, $p<0.001$) in these subjects than in control subjects (−0.0192). Similarly for the % bias versus triglyceride levels the negative slope (−0.033, $p<0.001$) was somewhat greater than that observed in the controls (−0.0292).

In supplementary figure 7 we have plotted % bias (direct LDL – calculated LDL/calculated LDL versus calculated LDL cholesterol in normal subjects. Here there is downward slope with a positive bias at low calculated LDL cholesterol values and a negative bias at high calculated values (−0.0492, $p <0.001$) If the direct method is more accurate then this would imply some benefits of the direct method in improving accuracy. In supplementary figure 7 we have also plotted the % bias for LDL cholesterol versus triglyceride for normal subjects.

Here we see a very slight positive slope (0.00832, $p<0.001$). indicating that at higher triglyceride levels, there is a somewhat greater bias between the two methods. In supplemtary figure 8 we have plotted % bias for LDL cholesterol versus LDL cholesterol values (calculated) in abnormal subjects (CHD, diabetes, or on lipid lowering medication). Here we see an even greater negative slope in these subjects (−0.072, $p<0.001$). than in the normals (−0.0492). In supplementary figure 8 we have also plotted the % bias for LDL cholesterol versus triglyceride levels in the abnormal subjects. Here we also see a positive slope (0.0217, $p<0.001$), which is quite a bit greater than the slope observed in normal subjects (0.00832).

The characteristics of male and female control subjects free of CHD, without diabetes, and not on cholesterol lowering medication, or hormonal replacement are shown in Table I (Supplementary Material). While men and women were of similar age, men had significantly higher body mass index, waist circumference, systolic and diastolic blood pressure, prevalence of hypertension, hypertension treatment, aspirin use, and alcohol intake than women. Men also had significantly lower total cholesterol and HDL-C values, and significantly higher triglyceride (TG), and total cholesterol/HDL-C ratios than females. HDL-C values were very similar whether measured following dextran precipitation or by the direct method. The same was true for calculated LDL-C versus direct LDL-C. These data indicate that the direct values provide adequate information on lipid status. The 75^{th} percentile for HDL-C in men was 1.38 mmol/L (53.4 mgdL), while for women this value was 1.71 mmol/L (66.2 mg/dL), and the 75^{th} percentile for LDL-C for men was 3.97 mmol/L (153.5 mg/dL), while for women this values was 3.98 mmol/L (154.0 mg/dL).

Information about premenopausal and postmenopausal women is shown in Table II (Supplementary Material). Postmenopausal women had significantly higher TC, TG, TC/HDL-C ratios, LDL-C, and non-HDL-C than did premenopausal women. However their HDL-C values were similar.

Data for men in the Framingham Offspring Study with and without CHD at cycle 6 are provided in Table 1. Men with CHD were significantly older, had higher DBP, were more likely to have a history of hypertension, to be on antihypertensive treatment or aspirin, to be taking beta-blockers and medication for diabetes and cholesterol, and to have diabetes than non-cases. Almost 47% of men with CHD and only about 11% of men without CHD were taking cholesterol-lowering medications. Men with CHD had significantly lower levels of both HDL-C and LDL-C, but had significantly higher triglyceride levels, and similar TC/HDL-C ratios as compared to controls. Mean HDL-C values obtained after precipitation and by the direct method were very similar. The same was the case for calculated and direct LDL-C. The mean direct LDL-C value in male CHD cases was approximately 18% higher than the goal LDL-C established for CHD patients of less than 2.6 mmol/L (100 mg/dL) by the Second Adult Treatment Panel of the National Cholesterol Education Program in 1994 (1). Of the 173 men with CHD at cycle 6, 92 were not on cholesterol-lowering medications. CHD men on cholesterol-lowering medications had significantly lower LDL-C levels than CHD not on cholesterol-lowering therapy (Friedewald LDL-C: 105±28 vs 125±29 mg/dl, respectively, $p<0.001$; and Kyowa LDL-C: 108±28 vs 129±30 mg/dl, respectively, $p<0.001$). HDL-C levels were similar in CHD men on or off cholesterol-lowering medications (NS).

Data for women with and without CHD is provided in Table 2. Women with CHD in the Framingham Offspring Study were significantly older, had a higher BMI, waist size, SBP, were more likely to have a history of hypertension, to be on antihypertensive treatment or aspirin, to be taking beta-blockers and medications for diabetes and cholesterol, and to have diabetes than non-cases. About 35% of women with CHD and only about 9% of women

without CHD were taking cholesterol-lowering medications. Women with CHD had significantly lower levels of HDL-C, but only when measured directly, than women without CHD. However, LDL-C levels, whether calculated or measured directly, were similar in CHD-free and CHD cases. Women with CHD had significantly higher triglyceride levels than controls. Mean HDL-C values obtained after precipitation and by the direct method were very similar, but the direct method was better in distinguishing cases from controls. Mean calculated and direct LDL-C values were also quite similar. The mean direct LDL-C value in female CHD cases was approximately 33% higher than the recommended goal of less than 2.6 mmol/L (100 mg/dL) established for CHD cases by the Second Adult Treatment Panel of the National Cholesterol Education Program in 1994. (1).

Since cycle 6 data was collected between 1995 and 1998 we had the opportunity to determine whether the measured parameters provided risk information above and beyond the standard parameters. After an average of 7.5 years of follow up, there were 117 new CHD cases in men and 60 new CHD cases in women. After excluding CHD cases at baseline, direct LDL-C and HDL-C provided the similar information as calculated LDL-C and HDL-C obtained after precipitation, respectively, indicating that these parameters can be used interchangeably.

DISCUSSION

In this population in which samples were collected between 1995 and 1998, assays for direct LDL-C and HDL-C measurement obtained from Kyowa Medex performed very well as compared to calculated LDL-C and HDL-C measured after dextran sulfate-Mg^{2+} precipitation, with very high correlation coefficients, within- and between-run coefficients of variations of less than 2.0%, and very similar absolute values. Moreover, these direct assays can be used on frozen plasma stored at −80 °C. Of note in this population is that despite four-fold higher use of cholesterol-lowering medication in male and female CHD cases versus controls, less than half of cases were receiving such medication. In addition, male cases had significantly lower HDL-C levels than controls regardless of the method, while for female CHD cases HDL-C levels were significantly lower only when assessed with the direct method. Most importantly, at the time these subjects were sampled, the recommendation of the National Cholesterol Education Program was that all CHD cases should be treated to achieve a target LDL cholesterol below 2.6 mmol/L(100 mg/dL). Despite these recommendations, their mean LDL cholesterol values were 2.99 mmol/L (116 mg/dl) in men using calculated values and 3.07 mmol/L (119 mg/dl) using direct values, both significantly lower than values in controls which were 3.32 mmol/L (128 mg/dl) and 3.37 mmol/L (130 mg/dL), respectively. Using direct values, the mean LDL-C was still about 18% higher than the target LDL-C for CHD patients of < 2.6 mmol/L (100 mg/dl) which were recommended in 1994 (1).

In women, the mean LDL-C value in cases was 3.33 mmol/L (129 mg/dL) for calculated values and 3.46 mmol/L (134mg/dL) for direct values; these values were very similar to controls, and more than 0.78 mmol/L (30 mg/dL) or 33% higher than the recommended target LDL-C value for CHD cases of 2.6 mmol/L or 100 mg/dl. Overall, our data indicate that the **direct** assays for both LDL-C and HDL-C provide an **acceptable** guide for lipid treatment, and that in this population there is still substantial residual CHD risk and under-treatment of LDL-C values, with a very high percentage of CHD cases not being at the recommended LDL-C targets, especially in women.

Our data indicate that the **direct** HDL-C assay provides very similar data to that obtained after dextran precipitation. This finding is important since **direc**t HDL-C assays are now very widely used and have not been evaluated in large scale population studies of CHD risk.

We have similar data for the direct LDL-C assay. In this regard we agree with the recommendations of Nauck et al (9) that direct assessment of LDL-C is not always required unless the subject has a triglyceride level above 4.5 mmol/L (400 mg/dL). Moreover, the direct assays of LDL-C provides far greater precision than calculated LDL-C based on the Friedewald formula.

Direct assays of LDL-C and HDL-C have been evaluated by other investigators. Bairaktari et al and Okada et al have reviewed the various direct LDL-C assays that are available (10,11). De Ferranti et al. have reported that the direct LDL-C assay known as N-geneous LDL-C obtained from Equal Diagnostics (Exton, PA, USA) correlated very highly with values obtained by ultracentrifugation and could be used in children evaluated in the non-fasting state (12). Bayer et al carried out a multicenter evaluation of direct LDL-C assays obtained from four Japanese companies (Daiichi, Denka-Seiken, Kyowa, and Wako) (13). These authors report that all assays provided far greater precision than calculated values as compared to ultracentrifugation (beta quantification), and all correlated highly with one another (13). All methods appeared reliable at triglycerides values of up to 8 mmol/L (13). The authors concluded that these methods provided an obvious technological advance over calculated LDL-C (13). It has been reported that the presence of paraproteinemia as observed in multiple myeloma patients can markedly affect the results obtained for LDL-C and HDL-C based on analyses with the N-geneous LDL-C and HDL-C assays (Equal Diagnostics, exton, PA, USA) (14). These types of assays may also provide erroneous results in patients with cholestatic jaundice as compared to results obtained by high performance liquid chromatography (15).

It must also be said that there are some limitations to our study in that we used frozen plasma stored at −80 degrees C (never thawed) for the new online direct assays for LDL and HDL cholesterol. Moreover there were biases between these assays. For all subjects HDL-C biases between assays of greater than 10% were observed in 8.5% of the population. With regard to % bias for HDL cholesterol (HDL Kyowa – HDL Dextran/ HDL Dextran) versus HDL cholesterol values (Dextran) significant negative slopes of around 2% were seen in normals and around 7% in subjects with heart disease diabetes or on lipid lowering medication. The % bias for HDL cholesterol versus triglyceride values indicated similar negative slopes of around 3% in both normals and abnormal. These overall data suggest that the actual level of HDL cholesterol and triglyceride can affect the results obtained with newer direct method versus the older dextran method. The data do suggest that the newer HDL C assay yields somewhat lower values than the older method at higher HDL C values and at higher triglycerides levels. This latter observation is especially the case for abnormal subjects especially at higher HDL C values based on the dextran method. These type of issues are important because the newer methods for measuring HDL cholesterol have become very widely adopted and in the case of the assay studied, this is the one widely marked by Roche Diagnostics worldwide. Nevertheless it is reassuring the correlation between these assays is 0.97, and in general very similar values are obtained.

With regard to direct LDL C one would assume that such an assay would provide more accurate results than calculated LDL C based on the Friedewald formula originally developed by Friedewald, Levy, and Fredrickson at the National Institutes of Health. However our data clearly indicates that in most circumstances a direct assessment of LDL C is not necessary. Values with a greater than 10% bias for LDL C between the direct and the calculated LDL C were only observed in 8.0% of the population. When % bias for these measures (direct LDL – calculated LDL/calculated LDL) was examined versus calculated LDL cholesterol values there was a negative slope of about 5% in normals and about 7% in abnormal subjects indicating a different bias at low values than at high values. If the direct method is more accurate then this would imply some benefits of the direct method in

improving accuracy. When % bias for LDL C assays was examined versus triglyceride there was only a very slight positive slope of around 1% in normals, while in abnormal subjects it was around 2%. The data would suggest that at higher triglyceride values somewhat higher LDL C values are obtained by the direct method than by the Friedewald formula.

In conclusion, our overall data indicate that **direct** assays for both LDL-C and HDL-C provide an adequate assessment of plasma levels, and that in this population there is still substantial residual risk in CHD cases, with most CHD cases not at recommended target LDL-C goals, especially in women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	Body Mass Index
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
TC	Total cholesterol
TG	Triglyceride
TC/HDL-C	Total cholesterol/High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
CHD	Coronary Heart Disease
SD	standard deviation

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Table 1

Characteristics and Plasma Lipid Parameters in Men with and without CHD

	Men		
Variable	**Non CHD (n=1335)**	**CHD (n=173)**	**p value for differences**
Age, years	58.0 (9.7)	65.3 (7.9)	<0.0001
BMI, kg/m^2	28.5 (4.4)	28.7 (4.3)	0.6249
BMI >30, %	30.1	31.8	0.6542 *
Waist, cm	101.3 (10.9)	102.4 (10.9)	0.1922
Waist<102, %	42.6	46.2	0.3669 *
SBP, mmHg	129.6 (17.1)	129.4 (17.6)	0.8633
DBP, mmHg	77.7 (9.3)	73.4 (9.5)	<0.0001
Hypertension, %	41.9	66.5	<0.0001 *
Hypertensive treatment, %	27.7	58.5	<0.0001 *
Takes aspirin regularly, %	31.1	78.0	<0.0001 *
$^{\infty}$Diabetes mellitus, %	10.1	26.6	<0.0001 *
Oral glycemic treatment, %	4.4	12.2	<0.0001 *
On Insulin, %	1.4	4.6	0.0029 *
On Beta Blockers, %	9.4	54.9	<0.0001 *
Cholesterol lowering treatment, %	11.4	46.8	<0.0001 *
Cigarette smokers, %	14.5	12.7	0.5190 *
>One drink alcohol / week, %	54.5	46.8	0.0563 *
TC, mg/dl (mmol/L)	200±35,(5.17±0.9)	186±39, (4.81±1.0)	<0.0001
TG, [mg/dl] [mmol/L]	118 [83–166] 1.32 [0.93–1.86]	127 [93–187] 1.42 [1.04–2.09]	0.0357
HDL-C$^{(a)}$, mg/dl (mmol/L)	44±12, (1.14±0.3)	40±12, (1.04±0.3)	<0.0001
HDL-C$^{(b)}$, mg/dl (mmol/L)	45±12, (1.17±0.3)	41±12,(1.05±0.3)	<0.0001
TC/HDL-C$^{(a)}$ ratio	4.66 [3.78 –5.64]	4.72 [3.85 –5.47]	0.6531
TC/HDL-C$^{(b)}$ ratio	4.57 [3.68–5.58]	4.59 [3.77–5.58]	0.2740
Non HDL-C$^{(a)}$, mg/dl (mmol/L)	156±35, (4.02±0.9)	145±35, (3.75±0.9)	0.0002
Non HDL-C$^{(b)}$, mg/dl (mmol/L)	155±35, (4.00±0.9)	145±35, (3.76±0.9)	0.0013
Calculated LDL-C$^{(c)}$,mg/dl (mmol/L)	128±31, (3.32±0.8)	116±31, (2.99±0.8)	<0.0001
Direct LDL-C$^{(d)}$, mg/dl (mmol/L)	130±31, (3.37±0.8)	119±31, (3.07±0.8)	<0.0001

Data are means±SD or median [inter quartile range] unless otherwise indicated.

* p value obtained by Chi-Square test;

(a) high density lipoprotein cholesterol measured by dextran precipitation method.

(b) high density lipoprotein cholesterol measured by direct assay.

(c) Low density lipoprotein cholesterol calculated according to the Friedewald formula.

[(d)] Low density lipoprotein cholesterol measured by direct assay.

Table 2

Characteristics and Plasma Lipid Parameters in Women with and without CHD

		Women	
Variable	**Non CHD (n= 1606)**	**CHD (n= 74)**	**p value for differences**
Age, Years	58.1 (9.6)	66.1 (8.0)	<0.0001
BMI, kg/m^2	27.3 (5.7)	29.1 (5.4)	0.0082
BMI >30, %	25.8	35.1	0.0757 *
Waist, cm	93.9 (14.8)	101.1(13.3)	<0.0001
Waist>102, %	26.3	39.7	0.0110 *
SBP, mmHg	126.5 (19.6)	140.4 (24.6)	<0.0001
DBP, mmHg	73.9 (9.1)	73.6 (11.6)	0.8502
Hypertension, %	35.8	83.8	<0.0001 *
Hypertensive treatment, %	22.9	73.0	<0.0001 *
Takes aspirin regularly, %	19.8	62.2	<0.0001 *
$^{\infty}$Diabetes mellitus, %	6.9	25.0	<0.0001 *
Oral glycemic treatment, %	2.5	10.8	<0.0001 *
On Insulin, %	1.2	9.5	<0.0001 *
On Beta-blockers, %	8.8	47.3	<0.0001 *
Cholesterol lowering treatment, %	8.8	35.1	<0.0001 *
On Estrogen therapy, %	26.3	23.0	0.5307 *
Post menopausal, %	76.5	94.6	0.0003 *
Cigarette smokers, %	15.5	18.9	0.4322 *
>One drink alcohol / week, %	30.5	21.6	0.1035 *
TC, mg/dl (mmol/L)	212±39, (5.48±1.0)	216±39, (5.58±1.0)	0.3887
TG, [mg/dl] [mmol/L]	111 [78–163] 1.24 [0.87–1.83]	136 [95–182] 1.52 [1.06–2.04]	0.0133
HDL-C$^{(a)}$, mg/dl (mmol/L)	58±16, (1.49±0.4)	54±16, (1.40±0.4)	0.0725
HDL-C$^{(b)}$, mg/dl (mmol/L)	57±16, (1.48±0.4)	52±16, (1.35±0.4)	0.0087
TC/HDL-C$^{(a)}$ ratio	3.71 [2.99 –4.64]	4.09 [3.47–4.70]	0.0508
TC/HDL-C$^{(b)}$ ratio	3.74 [2.96 –4.71]	4.06 [3.47–5.31]	0.0114
Non HDL-C$^{(a)}$, mg/dl (mmol/L)	154±39, (3.98±1.0)	162±39,(4.18±1.0)	0.1177
Non HDL-C$^{(b)}$, mg/dl (mmol/L)	155±43, (4.00±1.1)	164±39, (4.23±1.0)	0.0656
Calculated LDL-C$^{(c)}$, mg/dl (mmol/L)	128±35, (3.31±0.9)	129±31, (3.33±0.8)	0.9241
Direct LDL-C$^{(d)}$,mg/dl (mmol/L)	132±35, (3.42±0.9)	134±31, (3.46±0.8)	0.7155

Data are means±SD or median [inter quartile range] unless otherwise indicated.

* p value obtained by Chi-Square test;

(a) high density lipoprotein cholesterol measured by dextran precipitation method.

(b) high density lipoprotein cholesterol measured by direct assay.

(c) Low density lipoprotein cholesterol calculated according to the Friedewald formula.

(d) Low density lipoprotein cholesterol measured by direct assay.