The Association of Apolipoprotein B and Low Density Lipoprotein with Cardiovascular Risk Factors in the Thai Population

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Background: *Although direct and calculated low density lipoprotein cholesterol (LDL-cholesterol) has been widely used as an important predictor for cardiovascular risk, many studies have shown that apolipoprotein B (apo B) may be a more important lipoprotein marker.*
Material and Method: *We performed a cross-sectional study on 191 volunteers who were Shinawatra employees during their annual physical check up. The following cardiovascular risk factors were recorded or measured: direct and calculated LDL, apo B, gender, age, total cholesterol, triglyceride, HDL-cholesterol (HDL), calculated LDL, fasting plasma glucose (FPG), systolic and diastolic blood pressure, smoking status, body mass index (BMI), waist circumference, and waist-hip ratio (WHR). Apo B, direct LDL and calculated LDL levels were tested for their associations with other potential cardiovascular risk factors.*
Results: *There were a total of 76 men and 115 women with an average age of 28.8 $\pm$ 5.4 years. Male gender, cigarette smoking, high cholesterol, high triglyceride, high fasting plasma glucose, hypertension, high WHR and high BMI were associated with increased apo B level. Only male gender and high cholesterol were associated with increased calculated and direct LDL level. The association of direct and calculated LDL-cholesterol level with cardiovascular risk factors appears to be similar.*
Conclusion: *We demonstrated that apo B level correlates more with other cardiovascular risk factors compared to direct and calculated LDL-cholesterol. The clinical relevance of this finding needs to be explored in large-scale studies.*

Keywords: *Apolipoprotein B, Low density lipoprotein, Cardiovascular risk factors*

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Although low density lipoprotein cholesterol (LDL-cholesterol) has been widely used as an important marker for cardiovascular risk[1,2], many studies have demonstrated that apolipoprotein B (apo B) level is a more powerful lipoprotein marker[3,4]. Apo B measurement has many advantages. It does not require any fasting blood sample. It is a direct measurement. This means that there is no need for calculation from multiple parameters, which may introduce errors. It is internationally standardized and accurate at all triglyceride levels[5]. One molecule of apo B is present in each of the atherogenic lipoprotein particles; therefore, the total apo B value indicates the total number of potentially atherogenic lipoproteins. Apo B is also essential for the binding process of LDL particles to their receptors. An excess of apo B containing particles is a main trigger in the atherogenic process[5,6]. Apolipo-

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protein-related mortality risk (AMORIS) study enrolled more than 100,000 subjects for the screening program and follow up for cardiovascular events. In this study, an increased apo B level and a decreased apo A1 level are the best predictors for cardiovascular events[7].

The objectives of our study were to determine the association between apo B level, and direct and calculated LDL levels with the conventional cardiovascular risk factors in the Thai population.

Material and Method

Study population

We studied 191 consecutive subjects from Shinawatra employees during their annual physical check up. We collected demographic data, medical history, weight, height, waist, hip, blood pressure, and lab analysis including fasting plasma glucose (FPG), cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-cholesterol), direct and calculated LDL-cholesterol and apo B levels.

Standing height was measured with the subject barefoot, back square against the wall and eyes looking straight ahead. Weight was measured in undergarments using a balanced scale to the nearest 200 grams. The scale was standardized to 0 before each use. Waist and hip circumference were performed to the nearest 0.1 cm using a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 grams (Ohaus tape). The waist circumference was taken over the unclothed abdomen at the smallest diameter between the costal margin and the iliac crest. The tape measure was kept horizontal and just tight enough to allow the little finger to be inserted between the tape and the subject's skin. The hip circumference was taken at the level of greater trochanters (usually the widest diameter around the buttocks). Waist-hip ratio (WHR) was then calculated.

Blood pressure was measured using a standard mercury sphygmomanometer twice on the right arm, and the exact values were recorded to the nearest 2 mmHg. A third measurement was performed if there was at least 10 mmHg difference between the first two readings and the average of the two closest values was used for the analysis. The correlation coefficients between the two measurements of systolic and diastolic blood pressure were 0.94 and 0.89 respectively.

Blood samples were taken after 10-12 hours fasting and were processed within 4 hours. The serum was stored at -70°C for further analysis. The laboratory performance including FPG, serum lipids (cholesterol, triglycerides, and HDL-cholesterol) was performed by Hitachi 717 and 917 automation systems. Calculated LDL-cholesterol level was derived by the Friedewald equation [total cholesterol - HDL-cholesterol - (triglycerides/5)][2]. Direct LDL-cholesterol level was measured by the enzymatic colorimetric method and apo B was measured by the immunoturbidimetric method. The coefficients of variation between run and within run were performed everyday and were less than 5%. The external quality control was performed every 4 weeks by joining the quality assurance program from Roche Diagnostics. The coefficients of variation for serum cholesterol, triglycerides and HDL-cholesterol were 2.29%, 3.09% and 3.45% respectively. The completeness of the data was in the range of 99.0-99.9%.

Statistical analysis

Continuous variables were described as mean $\pm$ standard deviation and categorical variables were described as frequencies and percentages. A comparison of continuous variables was made by the unpaired t-test and comparison of categorical variables was made by a chi-square test. In all tests, the criterion for statistical significance was two-sided $p \leq 0.05$. Association between the apo B and LDL-cholesterol level was assessed by the use of an unpaired t-test for comparing apo B direct and calculated LDL-cholesterol levels with the following grouping parameters: age ($<$ and $\geq$ 27 years), gender, smoking status, cholesterol level ($<$ and $\geq$ 200 mg/dl), triglyceride level ($<$ and $\geq$ 200 mg/dl), fasting plasma glucose ($<$ and $\geq$ 110 mg/dl), systemic hypertension (history of hypertension or systolic BP $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg), waist-hip ratio ($<$ and $\geq$ 0.75 for female, 0.85 for male), and body mass index ($<$ and $\geq$ 25 kg/m^2).

Results

Seventy six men and 115 women with an average age of 28.8 $\pm$ 5.4 years (median 27 years) enrolled in this study. Measuring parameters of the whole group are shown in Table 1. Grouping parameters are displayed in Table 2. Since there was only one subject with low HDL level, we did not analyze HDL as a grouping parameter.

Analysis of the association of apo B, with calculated and direct LDL-cholesterol was performed by comparing the levels of these profiles between groups of each cardiovascular risk factor including gender (Fig. 1A), age (Fig. 1B), smoking status (Fig. 1C), cholesterol level (Fig. 2A), triglyceride level (Fig. 2B), fasting plasma glucose level (Fig. 2C), systemic hypertension (Fig. 3A), WHR (Fig. 3B), and BMI (Fig. 3C).

Table 1. Measuring parameters

Parameters	Values (mean ± SD)
Apo B (mg/dl)	90.8 ± 20.2
Direct LDL (mg/dl)	116.4 ± 34.7
Calculated LDL (mg/dl)	120.5 ± 29.7
Weight (kg)	55.5 ± 11.4
Height (cm)	160.9 ± 8.4
BMI (kg/m^2)	21.3 ± 3.4
Waist circumference (cm)	70.2 ± 9.8
Hip circumference (cm)	90.0 ± 7.2
Waist-hip ratio	0.78 ± 0.07
Cholesterol (mg/dl)	199.9 ± 32.6
Triglyceride (mg/dl)	86.8 ± 59.6
HDL-cholesterol (mg/dl)	62.0 ± 14.5
Fasting plasma glucose (mg/dl)	88.9 ± 10.9
Systolic blood pressure (mmHg)	114.1 ± 12.3
Diastolic blood pressure (mmHg)	76.0 ± 9.2

Table 2. Grouping parameters

Parameters	Number of subjects (%)
Male gender	76 (39.8%)
Age (≥ 27 years)	92 (48.2%)
Current smoker	31 (16.2%)
High cholesterol level (≥ 200 mg/dl)	91 (47.6%)
High triglyceride level (≥ 200 mg/dl)	4 (2.1%)
High fasting plasma glucose (≥ 110 mg/dl)	8 (4.2%)
Systemic hypertension	15 (7.9%)
High waist-hip ratio (≥ 7.5 in female, 0.85 in male)	73 (38.2%)
High body mass index (≥ 25 kg/m^2)	26 (13.6%)

For gender and cholesterol levels, there were significant differences between group for apo B, direct and calculated LDL-cholesterol levels with apo B as the most significant p value. For age, significant differences between groups were demonstrated for apo B and calculated LDL-cholesterol at a comparable p value. For other cardiovascular risk factors, i.e., smoking, triglyceride level, fasting plasma glucose, hypertension, WHR and BMI, a significant difference was demonstrated only for apo B level but not for direct and calculated LDL-cholesterol levels.

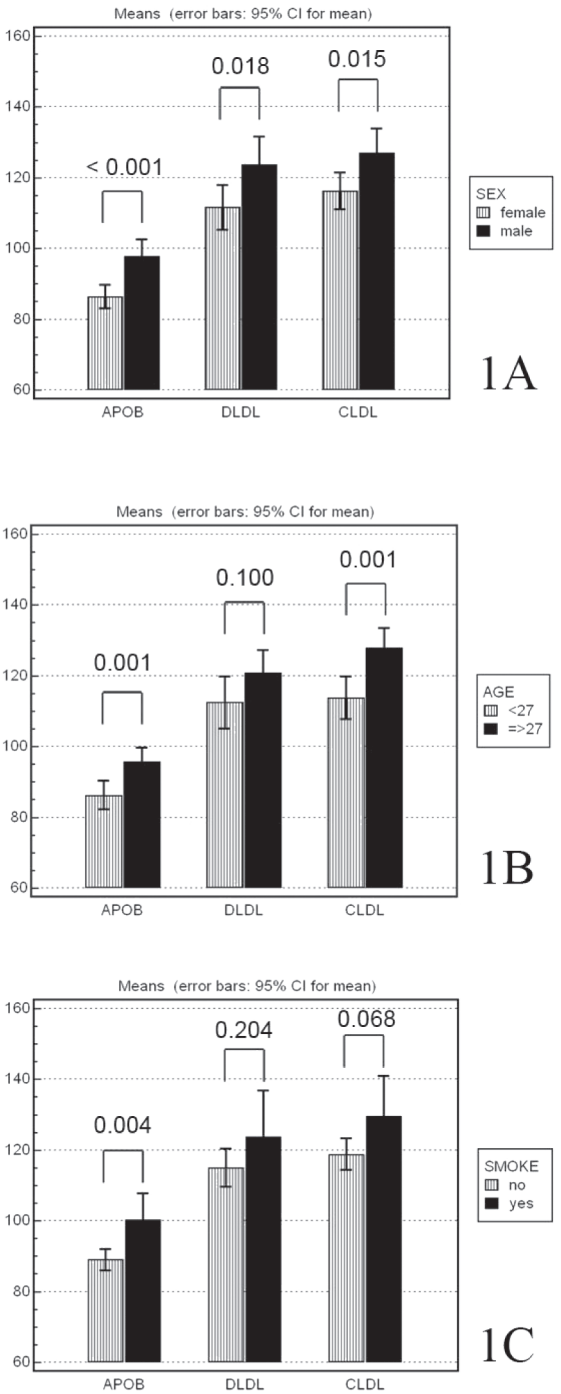
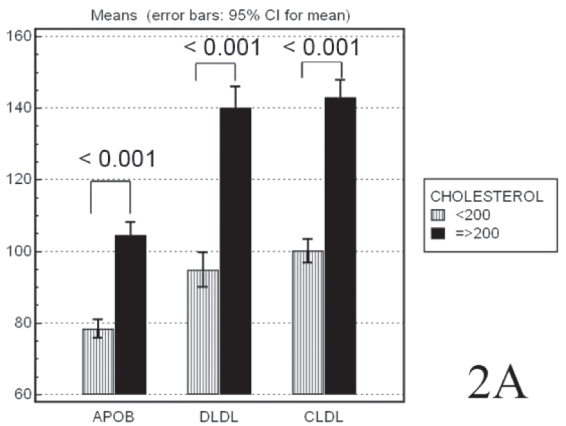
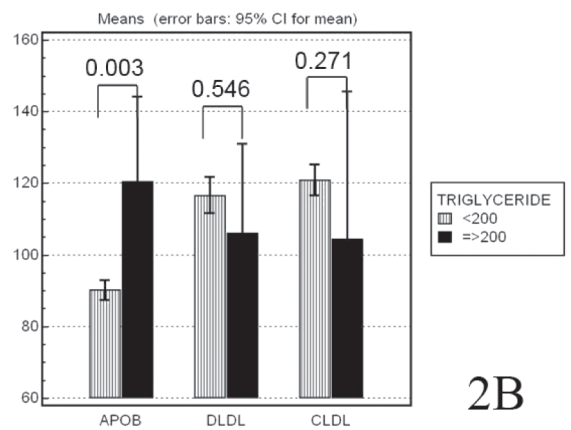


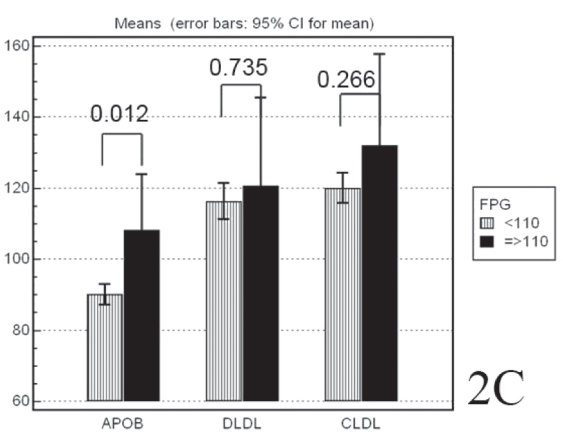
Fig. 1 Comparisons of apo B, direct and calculated LDL levels among grouping parameters of gender, age and smoking status (APOB = apolipoprotein B, DLDL = direct LDL, CLDL = calculated LDL)

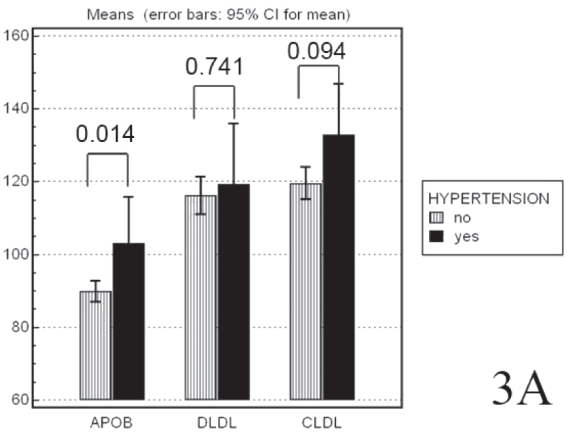
Discussion

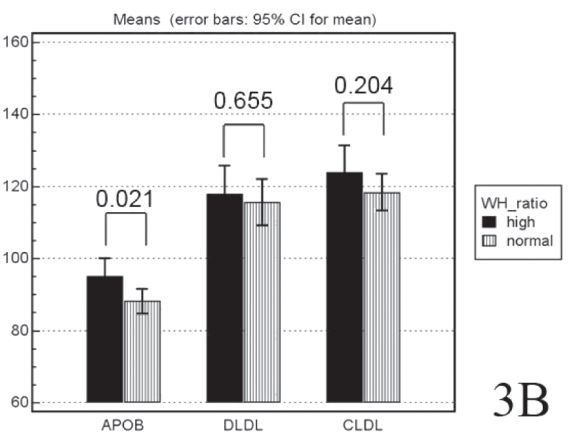
The results of this study showed that, in a selected group of the Thai population, apo B level was more related to cardiovascular risk factors than direct











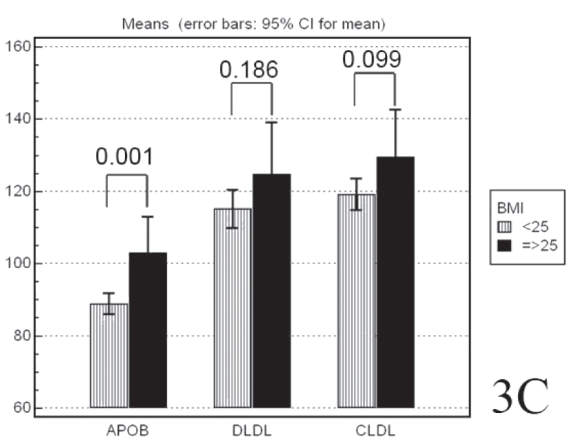


Fig. 2 Comparisons of apo B, direct and calculated LDL levels among grouping parameters of cholesterol, triglyceride and fasting plasma glucose (APOB = apolipoprotein B, DLDL = direct LDL, CLDL = calculated LDL)

Fig. 3 Comparisons of apo B, direct and calculated LDL levels among grouping parameters of systemic hypertension, waist-hip ratio and body mass index (APOB = apolipoprotein B, DLDL = direct LDL, CLDL = calculated LDL)

and calculated LDL-cholesterol level.

Guidelines for the management of patients with documented coronary artery disease recommended an LDL-cholesterol goal of less than 100 mg/dl[1]. A greater reduction of LDL-cholesterol level to less than 70 mg/dl may also be beneficial in a very high risk group[2]. Although many guidelines provide recommendations for lipid management based on LDL-cholesterol level, there has been increasing evidence of the importance of apo B level[5]. Many studies have shown that apo B level is a stronger predictor for cardiovascular events than the LDL-cholesterol level[3,4,7,8]. Apo B levels of less than 90, 110, and 130 mg/dl have been recommended for those with documented coronary artery disease, at least 2 risk factors and one or no risk factor respectively[9]. The INTERHEART study recently published the outcome of the largest case control study for cardiovascular risk factors. They enrolled 15,000 patients with acute myocardial infarction in 52 countries and the same number for controls[10]. In this study, Apo B/apo A1 ratio was the strongest risk factor for cardiovascular disease. Recent studies have shown that apo B level is a better predictor of cardiovascular risk compared to other lipid profiles. It can predict cardiovascular events regardless of the level[11] and size[12] of LDL-cholesterol. It has been shown that a significant number of patients with LDL-cholesterol under target level still have elevated apo B levels. In contrast, when apo B level is under control LDL-cholesterol and non-HDL-cholesterol levels are usually below Adult Treatment Panel III targets[2]. Apo B level can also be applied irrespective of triglyceride level[13]. Results from our study support the importance of apo B in the Thai population since it is strongly related to various cardiovascular risk factors.

The risk of acute myocardial infarction can also be predicted very well by apo B/apo A1 ratio in subjects with normal LDL-cholesterol levels[7]. Among patients who were on lipid lowering treatment, only apo B level and apo B/apo A1 ratio, but not LDL-cholesterol level, were good predictors for major cardiac events[14]. Data on the evaluation of LDL-cholesterol, non-HDL-cholesterol and apo B level after lipid lowering treatment in 1,889 patients with dyslipidemia showed that despite a good control of LDL and non-HDL-cholesterol level, apo B level was considered as a good control in only 50%[15]. Another study showed that, depending on triglyceride level, 50-80% of patients who reached an LDL-cholesterol goal after treatment did not reach an apo B target[16]. Apo B had also been shown to be much more related to flow-mediated endothelial dysfunction, carotid intima-media thickness[17], coronary and extra-coronary calcium[18], and insulin resistance[19]. Compared to LDL-cholesterol, apo B and non-HDL-cholesterol are better predictors for coronary artery disease in men[20]. Plasma concentration of atherogenic lipoprotein particles measured by apo B is more predictive in the development of coronary artery disease than the cholesterol carried by these particles measured by non-HDL-cholesterol. Our findings also support this role of apo B since we demonstrated that apo B is more related to factors that are involved in various metabolic abnormalities such as triglyceride, fasting plasma glucose, hypertension, WHR and BMI than LDL-cholesterol level.

In conclusion, the apo B level is more significantly related to conventional cardiovascular risk factors than the direct and calculated LDL level in a selected group of the Thai population. Whether apo B level has any impact on the patient's management needs further study.

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ความสัมพันธ์ของระดับ apolipoprotein B และไขมันชนิด low density lipoprotein กับปัจจัยเสี่ยงของโรคหัวใจและหลอดเลือด

รุ่งโรจน์ กฤตยพงษ์, ชุณหเกษม โชตินัยวัตรกุล, จารุวรรณ คังคะเกตุ, เกียรติชัย ภูริปัญโญ, นิธิ มหานนท์

ตามที่หลายรายงานแสดงว่าระดับของ apolipoprotein B (apo B) มีความสำคัญในการทำนายความเสี่ยงทางด้านหัวใจและหลอดเลือดไม่น้อยกว่าระดับของไขมันชนิด low density lipoprotein (LDL) คณะผู้วิจัยจึงวิเคราะห์ความสัมพันธ์ของระดับ Apo B และไขมันชนิด LDL กับปัจจัยเสี่ยงต่าง ๆ ของโรคหัวใจและหลอดเลือด ได้แก่ เพศ, อายุ, การสูบบุหรี่, ระดับของ cholesterol, triglyceride, ระดับน้ำตาลในเลือด, ความดันโลหิตสูง, waist-hip ratio และ body mass index ในกลุ่มพนักงานบริษัทชินวัตร จำนวน 191 ราย ผลการศึกษา พบว่าระดับของ apo B มีความสัมพันธ์กับปัจจัยเสี่ยงต่าง ๆ ของโรคหัวใจและหลอดเลือดมากกว่าระดับของ LDL