

Original Article

Low density lipoprotein subclasses in Asian and Caucasian adolescent boys

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South Asian adults are known to have very high rates of Coronary heart disease (CHD) and insulin resistance and, even as adolescents, may show higher risk factors for CHD. The aim of this study was to investigate the prevalence of small, dense low density lipoprotein (sdLDL) subclasses in a cohort of adolescent boys. The specific objective was to investigate the relationship between measures of fatness, ethnicity and LDL diameter in this cohort. Preformed native (non-denaturing) polyacrylamide 3-13% gradient gels and a multipurpose vertical electrophoresis system were used for the separation of LDL sub-fractions in a single school year cohort of boys aged 15-16 years (n=135). Latex beads and thyroglobulin standards were used to construct a calibration curve in order to calculate LDL particle diameters by regression (Total Lab Software v1.11). ANOVA was used to compare LDL size among different ethnic groups (SPSS and Stat View). The study sample was comprised of 45.2% Caucasians, 41.5% East Asians and 13.3% from the Indian subcontinent (South Asians). There was a non-significant trend for South Asians to have a lower LDL diameter than either Caucasians or East Asian boys which was independent of % total body fat (%TBF) and body mass index (BMI). This is the first adolescent cohort to examine sdLDL which included Caucasians, East and South Asians. It appears that the higher risk profile for CHD and diabetes noted in South Asian adults may be evident even during adolescence.

Key Words: adolescence, coronary heart disease, arteriosclerosis, small dense low density lipoproteins, LDL size, lipoprotein subfractions, non-denaturing gradient gel electrophoresis, Caucasian, Asian, Australia.

Introduction

Low density lipoproteins (LDL) are composed of heterogeneous particles differing in size, density and chemical composition. Two phenotypes, A (predominantly large buoyant particles) and B (predominantly small dense particles), have been characterized, and it has been demonstrated that the B phenotype (also called pattern B) may be a genetic marker for CHD.¹ Compared to large buoyant LDL particles, small dense LDL particles are associated with a more atherogenic type of lipoprotein profile², with increased plasma triglyceride, apolipoprotein (apo) B-100, and reduced levels of high density lipoprotein cholesterol and apo A-I.³ Even among children, racial and gender differences in lipids have been noted and may be attributable to environmental influences such as diet, exercise and obesity.^{4,6} The factors often associated with the decreased LDL size are male gender, increased triglyceride concentrations, and decreased HDL cholesterol concentrations.⁷ Levels of lipids and lipoproteins among children vary by sex, race/ethnicity, and are correlated with age, obesity, and other characteristics.⁸ However, most previous studies investigating the relationship between LDL particle size and CAD risk factors have been limited to adults, and little information is available in regard to adolescents.⁹

Based on studies in largely Caucasian American populations, the prevalence of pattern B is 10-15% in males under 20 years of age.³ One recent study of children aged 13-16 years found a prevalence of small dense LDL of 54% among 80 obese youths.⁹ Another study which investigated the relationship between insulin sensitivity, lipoprotein distribution, and LDL patterns in young adults found no participants with LDL phenotype pattern B.¹⁰

The aim of this study is to investigate the prevalence of LDL subclasses in a cohort of Australian adolescent boys from Caucasian, East Asian and South Asian background and to determine whether there are any differences by ethnicity in LDL subclasses when measures of fatness are controlled for.

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Table 1. Biochemical parameters of the cohort of adolescent boys. (Means \pm SD)

	Total group (n=135)	Caucasian (n=61)	East Asian (n=56)	South Asian (n=18)	P value
Total cholesterol (mmol/l)	4.0 \pm 0.8	3.9 \pm 0.7	4.1 \pm 0.9	4.1 \pm 0.6	0.387 ⁺
HDL-C (mmol/l)	1.3 \pm 0.4	1.3 \pm 0.4	1.4 \pm 0.4	1.3 \pm 0.3	0.286 ⁺
LDL-C (mmol/l)	2.3 \pm 0.8	2.2 \pm 0.7	2.3 \pm 1.0	2.4 \pm 0.6	0.636 ⁺
TC/HDL ratio	3.3 \pm 1.0	3.3 \pm 0.9	3.2 \pm 1.1	3.4 \pm 1.0	0.803 ⁺
Triglycerides (mmol/l)	0.9 \pm 0.2	0.9 \pm 0.3	0.9 \pm 0.1	0.9 \pm 0.2	0.835 ⁺
TG/HDL-C	0.7 \pm 0.3	0.8 \pm 0.4	0.7 \pm 0.3	0.7 \pm 0.3	0.500 ⁺
APO A-1 (mg/dl)	118.9 \pm 34.2	114.9 \pm 32.1	125.6 \pm 35.7	111.4 \pm 34.4	0.146 ⁺
Apo B (mg/dl)	77.3 \pm 26.4	72.6 \pm 24.4	82.5 \pm 29.5	76.9 \pm 20.4	0.128 ⁺
Apo A1/ B ratio	1.7 \pm 0.6	1.7 \pm 0.6	1.7 \pm 0.7	1.5 \pm 0.4	0.461 ⁺
Insulin (pmole/l)	71.6 \pm 43.6	93.6 \pm 85.5	69.3 \pm 29.9	93.6 \pm 85.5	0.491*
Insulin (log transformed)	4.1 \pm 0.5	4.1 \pm 0.5	4.2 \pm 0.4	4.3 \pm 0.6	0.234 ⁺

⁺ One Way ANOVA; * Kruskal Wallis test

Methods

Subjects

The LDL size determination was carried out on plasma collected from a single school year cohort of boys aged 15-16 years (n=135) who were recruited from a metropolitan boys high school for a cohort study, see Mehta S, Mahajan D, Steinbeck KS, Bermingham MA. Relationship between measures of fatness, lipids and ethnicity in a cohort of adolescent boys. *Annals of Nutrition & Metabolism*. 2002; 46:1.¹¹

Clinical data

Fasting blood samples had been analysed for glucose, insulin, total cholesterol, HDL cholesterol, triglyceride concentration, apolipoprotein A-I, apolipoprotein B-100 and low-density lipoprotein cholesterol. The anthropometric measurements of weight, height, waist and hip circumferences were used to calculate BMI (weight in kg/height in m²) and waist to hip ratio (WHR). Percent total body fat (%TBF) was estimated by bioelectric impedance analysis. Demographic and behavioural variables (age, country of origin, parent's medical history, eating habits, smoking, alcohol intake, and level of physical activity including vigorous and non vigorous and sedentary life style i.e. watching TV and computer or video games) were assessed by questionnaire. The classification of ethnic background was made from information provided in the questionnaire. Boys whose families originated either from India, Bangladesh or Sri Lanka, were grouped together as "South Asians". Those from China, Taiwan, Korea and the other countries of Indochina were grouped as "East Asians". Boys whose families originated from Europe were classified as Caucasians.

Biochemistry

Pre-formed native (non-denaturing) polyacrylamide 3-13% gradient gels and a multipurpose vertical electrophoresis system were used for the electrophoretic separation of LDL subfractions on plasma stored at -80°C and never thawed until just before use. After a short pre-electrophoresis of the gel, thyroglobulin and latex bead standards were located on to the gel with the standards

applied at either side of the samples. Samples and standards were mixed with BPB-sucrose solution and tracking dye respectively, before loading on to the gel. Electrophoresis was performed for a total of 2670 volt hours at increasing voltage in a cabinet controlled at 4°C. In order to determine LDL size, on completion of electrophoresis, the gel was cut into separate sections for standards and specimens. The portion containing standards was then stained for protein with Coomassie Brilliant Blue G in perchloric acid and destained with acetic acid.

The two parts of the gel were reassembled and scanned on a Total Lab Image scanner (Model Nr: Power Look III). The gel-images were scanned in 8-bit grayscale and analysed with the Total Lab Software v1.11 (Amersham Pharmacia Biotech AB, Sweden), which was used to determine the height of the peaks (in pixel). Latex beads and thyroglobulin standards were used to construct a calibration curve in order to calculate the LDL particle diameters by linear regression. The analysed gels were permanently preserved in sealed plastic bags containing bacteriostatic preservative. Pattern B was defined as a peak particle diameter (PPD) of $\frac{3}{4}$ 25.5 nm and pattern A as more than 25.5 nm. These values are used for adult data and may be inappropriate for children. A slightly higher cut off $\frac{3}{4}$ 26.0 nm for pattern B could be used.

Statistical analysis

All variables were first examined for normality. Variables with skewed distribution were log transformed before analysis, which achieved a normal distribution. Descriptive statistics (means, SD) were computed for all anthropometric, biochemical and behavioural variables. Variability between the groups was tested by Levene's test. One-way-analysis of variance (ANOVA) was used to compare the difference in continuous variables between tertiles of LDL size (using tertiles as grouping variables). The Kruskal-Wallis-Procedure (Non-parametric test for K-independent samples) was used for non-normal distributed data. Furthermore, the Post-Hoc test (Scheffe) was used to examine whether there were any differences between the ethnic groups among the tertiles of LDL size concerning the test variables. The data was also analysed

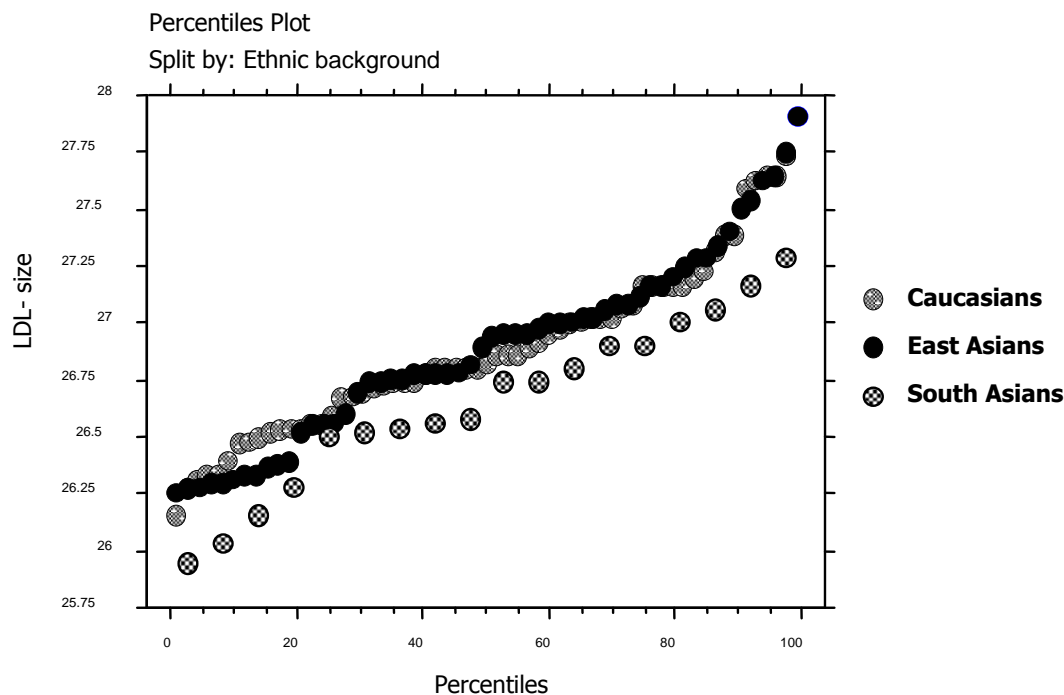


Figure 1. LDL size (nm) between 3 different ethnic groups
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by independent samples t-test using LDL-size as a grouping variable. Chi-squared test and Mann Whitney U test were used for non-continuous variables to compare the LDL-size between the 3 ethnic groups. Pearson's correlation analysis was performed to investigate the association between anthropometric, biochemical, behavioural variables and LDL-size. Risk was estimated by the calculation of odds ratios of categorical variables, and adjusted for confounding by logistic regression analysis. Two ethnic groups were used as categorical variables (group1: Caucasians and East Asians, group 2: South Asians). Caucasians and East Asians were combined for analysis as they had the same LDL size while South Asians tended to have a smaller LDL-size than either Caucasians or East Asians. In all statistical procedures, significance was reported at 95% confidence interval (CI) or probability value (P value) of less than 0.05. The statistical software packages Statview for Windows version 5.0 (SAS Institute Inc.), SPSS (Statistical Packages for Social Sciences) for Windows student version (SPSS Inc.) and SPSS for Macintosh version 10.0 (SAS Institute Inc.), were employed for the foregoing procedures.

Results

The study sample was comprised of 45.2% Caucasians, 41.5% East Asians and 13.3% from the Indian sub-continent (South Asians). The particle diameter for LDL (LDLPPD) was determined on one hundred and thirty-five ($n=135$) adolescent boys. The mean age of the subjects was 15.7 ± 0.45 years (range 14-16). The basic anthropometric, behavioural and biochemical profiles (Table 1) have already been determined and published.¹¹

These results showed that mean BMI for the total group was 21.3 ± 3.2 kg/m² with no significant difference by ethnicity while mean WHR was significantly higher in the South Asian group (0.83 ± 0.50) compared with both the Caucasians and East Asians, 0.80 ± 0.50 ($P=0.03$) and 0.79 ± 0.58 ($P=0.006$), respectively; however, mean waist (74.2 ± 8.7 cm), was not significantly different. The mean %TBF for the total group was 17.3 ± 7.1 , with corresponding values of 16.8 ± 7.9 , 17.3 ± 6.2 , and 19.3 ± 7.0 for the Caucasians, East Asians and South Asians, respectively. Although South Asians showed a trend to-wards a higher value of %TBF compared to the Caucasian and East Asian group these differences did not reach statistical significance.

It is common to define pattern B phenotype in adults as a peak particle diameter of <25.5 nm, and pattern A as >25.5 nm. The values of LDL particle diameter were normally distributed and the mean LDL size of the study group was 26.9 ± 0.4 nm, hence no participant had LDL phenotype B according to the above classification. To further investigate the distribution of lipoprotein subclasses (sdLDL) the values of LDL diameter were stratified by tertiles at the 33rd and 67th percentile bands, which were used as cut off points as the number of subjects could not be evenly divided among the tertiles. LDL particle diameters, of the total group and stratified by ethnicity are shown in Table 2.

There was a non-significant trend for South Asians to have a lower LDL size, than both Caucasians and East Asians, respectively ($P=0.065$). When the tertiles of LDL diameter were examined, it was found that 50% ($n=9$) of South Asians were in the lowest tertile of measurement (25.95 - 26.69 nm) compared to 29.5% ($n=18$) and 28.6%

Table 2. LDL diameter of adolescent boys stratified by ethnicity (mean \pm SD).

	Total group (n=135)	Caucasian (n=61)	East Asian (n=56)	South Asian (n=18)	P value [†]
LDL-size (nm)	26.9 \pm 0.4	26.9 \pm 0.4	26.9 \pm 0.4	26.7 \pm 0.4	0.065

[†] One Way ANOVA

(n=16) of Caucasians and East Asians respectively. As can be seen from the percentile plot shown in figure 1 South Asians tended to have a smaller LDL size distribution than either Caucasians or East Asians at every percentile band.

Boys in the lowest tertile (25.95-26.69nm) of LDL peak particle diameter (LDLPPD) had significantly lower HDL (high density lipoprotein cholesterol), compared to boys in the highest tertile of measurement, (1.2 vs. 1.4 mmol/l, ($P=0.008$). The triglyceride/HDL ratio, sometimes used as an index of atherogenicity, was significantly higher in boys of tertile 3, than in boys of tertile 1 (0.8 CI 0.69-0.97 vs. 0.6 CI 0.58-0.68, $P=0.012$). Furthermore, the apolipoprotein A-1 concentration was significantly ($P=0.029$) lower in the lowest compared to the highest tertile of the LDL range, (110.0 mg/dl CI 100.85-119.27 vs. 129.0 mg/dl CI 117.31-140.61).

As mentioned earlier¹¹ mean BMI, %TBF and waist were not different among the ethnic groups, but WHR was significantly higher in South Asians, who also had significantly higher %TBF than Caucasians when adjusted for BMI. At the mean BMI of the cohort, South Asians had an average of 4.5% more body fat than Caucasians and the evidence suggests this fat is centrally distributed. The distribution of sdLDL by ethnicity was examined by ANCOVA using BMI, %TBF and WHR, as covariates and no relationship between them was found. Using the logistic regression analysis (odds ratio and 95% CI) and 26.69 nm as cut off point, the relative risk of different ethnic groups having a smaller LDL size was calculated. South Asians have a 2.4 higher risk of having an LDL size less than 26.6 nm compared with Caucasians and East Asians. ($P=0.082$).

Discussion

The evidence that adult disease may have its origins in childhood and adolescence is solid.¹²⁻¹⁴ People with ancestry in the countries of the Indian subcontinent (South Asians), comprising more than one fifth of the global population, are highly susceptible to insulin resistance and CHD. This is the first adolescent cohort to include Caucasians, East and South Asians. Half the South Asian boys were in the lowest tertile of LDL diameter compared to 30% the other two ethnic groups; there was also a trend for South Asians to have a lower mean LDL diameter than either Caucasian or East Asian boys. This was not statistically significant (0.06) but the numbers of South Asian subjects were small and the diameter of the LDL for South Asians was lower at every percentile band

Levels of lipids and lipoproteins among children vary by sex, race/ethnicity, and are correlated with age, obesity, and other characteristics.⁸ Data currently available on LDL diameter in adolescents are extremely limited.

Based on studies in largely Caucasian American populations, the prevalence of pattern B is 10-15% in males under 20 years of age.³ One recent study of children aged 13-16 years found a prevalence of small dense LDL of 54% among 80 obese youths. In this study, the small, dense LDL phenotype group (SDDL) had significantly higher weight, waist circumference and VAT (visceral adipose tissue) than the large, buoyant LDL (LBDL) phenotype group. Taken together, Owen *et al.*, found peak particle diameter (PPD) to be significantly correlated with visceral adipose tissue, TG and TG/HDL ratio.⁹ These observations are not directly comparable with our study as all these subjects were obese. A study of healthy Japanese children, age range 7-13 years, did not report mean values, but found a prevalence of 9.3% pattern B with no gender differences.¹⁵ The Bogalusa Heart study¹⁶ which examined a subsample of 449 young adults aged 20-37 years showed no race differences between the LDL subclass pattern, but males had relatively less LDL1 (large, buoyant) and more LDL3 (small, dense) than females. This confirms the finding of Freedman *et al.*,⁸ and other studies^{17,18} which show that compared with girls, boys had a smaller mean LDL particle size; this highlights the fact that even in adolescence, boys are at higher risk. In the Bogalusa Heart study the LDL subclass pattern was associated significantly with BMI, waist circumference, triglycerides, HDL2 cholesterol, insulin, and glucose, with levels increasing from LDL1 pattern to LDL3 pattern. In our study, no significant association was found between LDL size and any indices of obesity (including percent body fat, BMI and WHR), however this is almost certainly due to the smaller numbers in our study where most boys were not obese or metabolically abnormal. In contrast, the study by Owen *et al.*, of LDL particle size in obese children (n=41), found PPD to be significantly correlated with visceral adipose tissue (measured by magnetic resonance imaging (MRI)). In the current study, adolescent boys of South Asian origin have been shown to have a significantly higher mean WHR and a trend towards higher %TBF compared to their Caucasian and East Asian counterparts. Furthermore, South Asians had a greater %TBF for the same BMI. These findings point to higher abdominal obesity among South Asian boys compared with Caucasians and East Asians. Studies over the last decade have shown increased triglyceride and decreased HDL to be a consistent pattern in South Asians in general, and in Asian Indians in particular.¹⁹⁻²¹ These findings were not confirmed in the current South Asian cohort, which did not find significant differences in HDL-C among the tertiles of LDL size. This finding could be due to the much smaller sample (n=18) of South Asians compared to Caucasians and East Asians. Interestingly, Caucasians in the lower tertile of

LDL diameter tended to have a significantly lower concentration of HDL-C, significantly higher TG/HDL ratio and showed a trend towards a smaller concentration of apolipoprotein A-I. This finding agrees with the results of Arisaka *et al.*, where children with small dense LDL subclass showed decreased HDL-C and apo A-I concentrations.¹⁵ As this study showed, South Asian were more than twice as likely to have a smaller LDL size than the other two ethnic groups.

In this study, the cut off point for sdLDL was obtained by examining all the data. Similarly, Rainwater *et al.*,²² used 26.2 nm as a cut off point for the investigation of LDL diameter, a point which is somewhat higher than the 25.5 nm which is usually used to separate pattern A from pattern B. In our study, the 33rd and 67th percentiles were used as cut off values, which was deemed to be more appropriate for a distribution of sdLDL in the adolescent cohort. In addition, 26.69 nm was used as a cut off point when the relative risk of different ethnic groups having a smaller LDL size was calculated. Austin *et al.*, has proposed that the inheritance of LDL subclass phenotypes is controlled by a single major genetic locus.²³ In addition to familial studies, evaluation of the LPL gene has also shown evidence for linkage to LDL subclass.²⁴ Although these studies mainly involved Caucasians, they point to a strong genetic influence on the occurrence of LDL sub-class. Thus, it is reasonable to assume that the genetic component has contributed towards the trend of South Asian adolescent boys to have a lower LDL diameter than either Caucasians or East Asians boys which was independent of % TBF and BMI.

In conclusion, our study shows that the higher risk profile for CHD and diabetes noted in South Asian adults may be evident even during adolescence. In this study, which was the first adolescent cohort to include Caucasians, East and South Asians, it was found that the risk of South Asians having smaller LDL diameter is more than doubled compared to Caucasian and East Asian boys. Although the magnitude of the increased risk is high, it is necessary to confirm our findings in a larger sample size. Our understanding of the risk factors involved in the excess of CHD found in Asian Indians is not complete, but this study has highlighted some possible contributions of important metabolic and genetic risk factors which may be influential even in childhood and adolescence and may eventually lead to intervention which will focus on young age groups and take account of ethnic differences.

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Original Article

Low density lipoprotein subclasses in Asian and Caucasian adolescent boys

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亞洲及高加索青春期男孩其低密度脂蛋白之次分類

已知有極高比例的南亞成年人罹患冠心病(CHD)及胰島素阻抗，甚至當他們在青少年時可能已顯示有較高的CHD危險因子。本研究目的為探討一個男性青少年世代中的小而密低密度脂蛋白(sdLDL)的次分類盛行情形。具體的目標是去探討這個世代中的男性青少年之肥胖測量值、種族及LDL直徑間的相關。單一學校年級世代的15-16歲 男孩(135名)的LDL次片段分離，使用原始的(未變性) 聚丙烯醯胺 3-13% 的梯度膠體及多目垂直電泳系統法。為了用迴歸去計算 LDL 粒子直徑，以乳膠粒子與甲狀腺球蛋白抗體的標準去建立標準曲線 (Total Lab Software v1.11)。使用 ANOVA 去比較不同種族之間的LDL大小(SPSS and Stat View)。這個研究樣本中 涵蓋 45.2% 高加索人、41.5% 東亞人及 13.3% 來自印度次大陸(南亞)的研究對象。南亞人比起高加索人及東亞男孩，在控制身體總脂肪(%TBF)及身體質量指數(BMI)後，其LDL直徑較低，雖然並不顯著。本研究是第一個評估包含高加索人、東亞及南亞人的sdLDL的研究。本研究結果指出南亞成年人有較高罹患CHD及糖尿病危險性的狀況，可能在其青春期時就已經看得出來。

關鍵字：青春期、冠心病、動脈粥狀腫、小而密低密度脂蛋白、LDL大小、脂蛋白次片段、未變性梯度膠體電泳、高加索人、亞洲人、澳洲。