

## **A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects**

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**Summary** Recently, the presence of small dense low density lipoprotein (LDL) has been postulated to be a stronger risk factor for coronary heart disease than large LDL. While small dense LDL has been associated with individual components of the insulin resistance syndrome such as hypertension, high triglyceride level, low high density (HDL) cholesterol, and diabetes mellitus, there has been little work exploring whether LDL size is decreased in subjects with multiple metabolic disorders. We examined the association of LDL size and pattern to specific insulin (which does not cross-react with proinsulin), proinsulin, increased triglyceride, decreased HDL, hypertension and impaired glucose tolerance in 488 non-diabetic subjects from the San Antonio Heart Study. LDL size was significantly related to specific insulin, proinsulin and the fasting proinsulin/insulin ratio. Small dense LDL was significantly associated with high triglyceride level, decreased HDL cholesterol, hypertension and impaired glucose tolerance. LDL size (Å) decreased in a stepwise fashion with increasing number of the metabolic disorders described above

(zero  $262.6 \pm 9.4$ ; one  $257.0 \pm 9.3$ ; two  $256.4 \pm 9.4$ ; three  $249.0 \pm 9.1$ ; and four  $244.9 \pm 9.0$ ). These results were similar in men and women and in non-Hispanic whites and Mexican Americans. The association between LDL size and the number of metabolic disorders remained statistically significant even after adjustment for obesity, body fat distribution, gender, ethnicity, proinsulin and insulin concentrations. Furthermore, decreases in LDL size are also significantly associated with both a selective beta-cell defect (as estimated by the fasting proinsulin/insulin ratio) and insulin resistance (as estimated by the fasting insulin concentrations) although the association was somewhat stronger for the latter. We conclude that small dense LDL may form part of the insulin resistance syndrome in non-diabetic subjects. [Diabetologia (1995) 38: 1328–1336]

**Key words** Insulin resistance syndrome, low density lipoprotein size, triglyceride, high-density lipoprotein, hypertension.

Increased concentration of low density lipoprotein (LDL) cholesterol is widely recognized as a risk factor for coronary heart disease [1, 2]. There is consid-

erable heterogeneity in the size and density of LDL particles [3, 4]. Austin et al. found that most individuals can be assigned to one of two LDL subclass patterns (A or B) [5]. Small dense LDL particles (pattern B) are thought to be more atherogenic than larger LDL particles, although this association may not be statistically independent of triglyceride concentrations [5–7].

Recently, several clinical and epidemiological studies have shown an association between insulin concentrations and various metabolic and physiologic abnormalities including glucose intolerance, dyslipidaemia (specifically, increased triglyceride lev-

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*Abbreviations:* BMI, Body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; IGT, impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus; WHR, waist-hip ratio.

el and decreased high density lipoprotein (HDL cholesterol) and hypertension [8–11]. These disorders are also associated with insulin resistance [12–16]. In two prospective studies, several metabolic disorders related to the insulin resistance syndrome have been found to cluster together [17, 18].

Small dense LDL has been associated with most individual components of the insulin resistance syndrome including hypertriglyceridaemia [4–7, 19–22], low HDL cholesterol [5, 19–22], hypertension [20, 21, 23], diabetes [20, 22, 24, 25] and hyperinsulinaemia and insulin resistance [19–22]. Few data, however, are available on whether small dense LDL is a characteristic of the cluster of risk factors associated with insulin resistance. Selby et al. [20] have shown that women with multiple metabolic disorders are more likely to have small dense LDL than women with a single disorder.

Many of the studies describing the association of insulin resistance and/or hyperinsulinaemia to cardiovascular risk factors have been limited by the fact that insulin was measured with an assay that cross-reacts with proinsulin. Temple et al. [26] have suggested that proinsulin and split 32–33 proinsulin comprise the majority of circulating immunoreactive insulin in subjects with NIDDM. Several other studies have also suggested that proinsulin is elevated disproportionately in subjects with NIDDM [27–33]. Both proinsulin and insulin are also elevated in subjects with impaired glucose tolerance (IGT) [30, 33, 34]. However, the results on the ratio of proinsulin to insulin in subjects with IGT have been contradictory. In one study, the ratio of proinsulin to insulin was not elevated [31], whereas in another report the proinsulin/insulin ratio was increased [34]. Proinsulin concentrations are correlated with dyslipidaemia and hypertension in diabetic [35] and non-diabetic subjects [36]. However, it is not known whether proinsulin concentrations, specific insulin concentrations or both are associated with alterations in LDL size.

We have examined for the first time the association between proinsulin, specific insulin (using an antibody that does not cross-react with proinsulin) and the fasting proinsulin/insulin ratio to LDL size. In this report, we also examine the association of LDL size and pattern to a number of metabolic disorders (hypertension, low HDL, hypertriglyceridaemia and impaired glucose tolerance) identified in prospective studies of the insulin resistance syndrome [17, 18] in men and women from a biethnic population. In previous studies, subjects who have multiple metabolic disorders have higher insulin concentrations [17] and are more insulin resistant [37] than subjects who develop only a single disorder. Since age, obesity and body fat distribution are associated with both insulin resistance and LDL size [20, 21] we also adjusted for these possible confounding variables. Since diabetes has been associated with a preponderance of small dense

LDL in a number of reports [20, 22, 24, 25] including our own population [24] we have examined these associations in non-diabetic subjects.

## Subjects and methods

The San Antonio Heart Study is a population-based study of diabetes and cardiovascular disease in Mexican Americans and non-Hispanic whites. Mexican Americans were defined as individuals whose ancestry and cultural traditions derived from a Mexican national origin [38]. Detailed descriptions of the 1979–1982 survey (phase I) and the 1984–1988 survey (phase II) as well as the 8-year follow-up have been previously published [39–41]. This study was approved by the Institutional Review Board for the University of Texas Health Science Center at San Antonio. All subjects gave informed consent. The results presented in this study are based on the first two (out of six) census tracts in the 7-year follow-up of the phase II cohort, which began in October 1990.

At the follow-up examination, blood specimens were obtained following a 12–14 h fast, and a second specimen was obtained 2 h after administration of a 75-g glucose equivalent load (Orangedex, Custom Laboratories, Baltimore, Md., USA). Plasma glucose concentrations were measured with an Abbott VIP Analyzer (Abbott Laboratories, North Chicago, Ill., USA). For subjects included in the present report we measured serum insulin concentrations by a commercial double antibody radioimmunoassay (RIA) (human insulin-specific RIA method, LINCO Research, St. Louis, Mo., USA) in which proinsulin cross-reactivity is less than 0.2%. The lower limit of sensitivity of the Linco assay was 14.4 pmol/l. The intra-assay coefficient of variation was 4.5% and the interassay coefficient of variation was less than 10% [42]. Proinsulin concentrations were measured in the laboratory of Dr. R. Bowsher (Lilly Laboratory for Clinical Research, Indianapolis, Ind., USA) by a nonequilibrium RIA method [36]. The polyclonal antibody used in this assay (168AB) recognizes a proinsulin-specific epitope formed by the intact A-chain-C-peptide junction. Fasting lipids and lipoproteins were measured using methods described previously [39]. The intra-assay coefficient of variation ranged from 6 to 21% using controls prepared at 5, 50 and 250 pmol/l.

Diabetes mellitus was diagnosed according to the criteria of the World Health Organization (WHO) (fasting plasma glucose  $\geq 7.8$  mmol/l (140 mg/dl) and/or 2-h plasma glucose value  $\geq 11.1$  mmol/l (200 mg/dl)) [43]. Subjects who did not meet these criteria but who were being treated with oral anti-diabetic agents or insulin were also considered to have diabetes. Impaired glucose tolerance was also diagnosed according to WHO criteria (fasting plasma glucose  $< 7.8$  mmol/l (140 mg/dl) and 2-h plasma glucose between 7.8 mmol/l (140 mg/dl) and 11.1 mmol/l (200 mg/dl)) [42]. Since the focus of this report is on the insulin resistance syndrome in non-diabetic subjects and since LDL size is related to diabetes [20, 22, 24, 25] we excluded the 125 subjects with diabetes.

Anthropometric measurements (height, weight, waist and hip circumferences) were obtained after participants had removed their shoes and upper garments and donned an examining gown [44]. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The ratio of waist-to-hip circumference (WHR) was used as a measure of body fat distribution.

Systolic blood pressure (first phase) and diastolic blood pressure (fifth phase) were measured to the nearest even digit with a random zero sphygmomanometer (Hawksley-Gelman,

Lancing, Sussex, UK) [45]. Hypertension was defined as a systolic blood pressure greater or equal than 140 mmHg and/or a diastolic blood pressure greater or equal than 90 mmHg and/or current use of antihypertensive medication which corresponds to the mild hypertension category of the Joint National Committee on Detection, Education and Treatment of High Blood Pressure (JNC V) recommendations [46].

LDL size and subclass pattern were determined in plasma samples by the method of Krauss and Burke [4] in the laboratory of the Medlantic Research Institute, Washington, D. C., USA. Plasma samples for determination of LDL size were stored at  $-70^{\circ}\text{C}$  (without thawing) until the analyses for LDL size were done, an average of 3 months later. Gradient gels were obtained from Isolab, Inc. (Akron, Ohio, USA). Measurement of the particle sizes was calibrated using LDL sub-fractions whose molecular diameter had been determined by analytical ultracentrifugation (courtesy of Dr. R. Krauss, Donner Laboratories, Berkeley, Calif., USA). In almost all subjects, a predominant LDL peak could be determined. The size of the predominant peak was considered the individuals' LDL size. Subjects were classified into three groups on the basis of size and shape of the major peak. A major gradient peak of greater than  $257\text{\AA}$  with skewing toward smaller particles was classified as pattern A. Individuals with a predominant peak of less than  $253.5\text{\AA}$  were classified as pattern B and those subjects between  $253.5$  and  $257\text{\AA}$ , were classified as pattern I unless the size was very close to the cutpoints and the peak had the characteristic shape of either the A or B pattern. The mean sizes in ( $\text{\AA}$ ) of the A, I and B peaks were  $264.7 \pm 0.3$ ,  $255.3 \pm 0.4$ , and  $244.5 \pm 0.4$ , respectively. The interassay coefficient of variation for eight control pools ( $240$ – $263\text{\AA}$ ) ranged from 1.8 to 3.6% [19].

On the basis of previous prospective epidemiologic studies [17, 18], we considered four metabolic conditions to be most closely associated with the insulin resistance syndrome: IGT; hypertension; low HDL cholesterol; and high triglyceride concentrations. Triglyceride concentrations were dichotomized as above or below  $2.3\text{ mmol/l}$  ( $200\text{ mg/dl}$ ) as recently recommended by the National Cholesterol Education Program (NCEP) [47]. Also as recommended by the NCEP, HDLC was dichotomized as above or below  $0.9\text{ mmol/l}$  ( $35\text{ mg/dl}$ ) for men. For women, we used a higher cutoff-point  $1.2\text{ mmol/l}$  ( $45\text{ mg/dl}$ ) than recommended by the NCEP to increase the number of abnormal values. We used the WHO criteria [43] for the diagnosis of impaired glucose tolerance and the mild hypertension definition of the JNC V [45] as noted.

### Statistical analyses

Statistical techniques included Spearman correlation coefficients, partial correlation coefficients, parametric analyses of variance, multiple linear regression, multiple logistic regression and chi-squared tests. We confirmed that the distribution of LDL size of the predominant peak was normally distributed using normal probability plots. The skewness of the LDL size distribution was  $-0.16$  and the kurtosis was  $-0.71$  also suggesting an approximately normal distribution. Triglyceride concentrations were logarithmically transformed to reduce skewness and kurtosis. Statistical analyses were performed on the natural logarithms and the results were back-transformed into their natural units for presentation in the Tables. Interactions between ethnicity (or sex) and other variables (number of metabolic disorders, LDL size, insulin, etc.) were examined using both logistic regression and analyses of variance. There were no statistically significant interactions ( $p > 0.20$ ). Therefore,

the ethnic groups and both sexes were pooled in most analyses for greater statistical power and ease of presentation.

### Results

Table 1 shows the anthropometric, metabolic and haemodynamic characteristics of non-diabetic subjects by gender. The mean LDL size in women is  $258.3 \pm 0.6\text{\AA}$  and in men is  $256.0 \pm 0.7\text{\AA}$  ( $p = 0.029$ ).

Table 2 shows Pearson correlations between LDL size, insulin, proinsulin and cardiovascular risk factors. LDL size was significantly positively correlated with HDL cholesterol and inversely correlated with insulin, proinsulin, the fasting proinsulin/insulin ratio, triglyceride, total and LDL cholesterol, blood pressure and glucose levels. Insulin, proinsulin and the proinsulin/insulin ratio were also, in general, correlated with cardiovascular risk factors.

Table 3 shows partial correlation coefficients after adjustment for age, gender, BMI, WHR, glucose, triglyceride, and HDL cholesterol. Insulin, proinsulin and the fasting proinsulin/insulin ratio were all significantly inversely correlated with LDL size after adjustment for possible confounding variables.

We also considered whether the fasting proinsulin/insulin ratio (as a marker of selective beta-cell secondary failure) predicts LDL size independently of fasting insulin concentrations (as a marker of insulin resistance). To examine this issue we stratified non-diabetic subjects by both the median level of fasting insulin ( $110.2\text{ pmol/l}$ ) and the fasting proinsulin/insulin ratio ( $0.075$ ) (Fig. 1). Both fasting insulin concentrations ( $r = 0.002$ ) and the fasting proinsulin/insulin ratio ( $p = 0.008$ ) were inversely significantly related to LDL size but the association was stronger for fasting insulin. LDL size was lowest in hyperinsulinaemic subjects with a high fasting proinsulin/insulin ratio.

Figures 2 and 3 show LDL size and patterns by four selected metabolic disorders. Smaller, denser LDL was significantly related to increased triglyceride ( $p < 0.001$ ), decreased HDL cholesterol ( $p < 0.001$ ), hypertension ( $p = 0.031$ ) and IGT ( $p = 0.048$ ).

We next examined the association of the number of metabolic disorders to LDL size separately in men and women and also in Mexican Americans and non-Hispanic whites. LDL size was significantly related to the number of metabolic disorders after stratification by gender or ethnicity (data not shown). These results were similar in Mexican Americans and non-Hispanic whites separately, and in men and women separately. The gender  $\times$  number of metabolic disorders and the ethnicity  $\times$  number of metabolic disorder interaction terms were not statistically significant ( $p = 0.900$  and  $p = 0.451$ , respectively). We therefore combined the sexes and ethnic groups

**Table 1.** Anthropometric, metabolic and haemodynamic characteristics by gender in non-diabetic subjects

<i>n</i>	Women 284	Men 204	<i>p</i> -value
Age (years)	50.5 ± 0.8	49.7 ± 1.7	0.565
% Mexican American	68 %	54 %	0.015
Body mass index (kg/m <sup>2</sup> )	29.8 ± 0.5	28.9 ± 0.4	0.191
WHR	0.894 ± 0.01	0.966 ± 0.01	< 0.001
Total cholesterol (mmol/l)	5.77 ± 0.07	5.69 ± 0.07	0.566
LDL cholesterol (mmol/l)	3.72 ± 0.07	3.81 ± 0.08	0.436
HDL cholesterol (mmol/l)	1.20 ± 0.02	1.04 ± 0.02	< 0.001
Triglyceride (mmol/l)	1.82 ± 0.06	1.86 ± 0.08	0.696
Systolic blood pressure (mm Hg)	123.1 ± 1.2	127.8 ± 1.5	0.034
Diastolic blood pressure (mm Hg)	71.0 ± 0.7	76.7 ± 0.8	< 0.001
Fasting glucose (mmol/l)	4.96 ± 0.03	5.11 ± 0.04	0.017
2-h glucose (mmol/l)	6.70 ± 0.12	5.99 ± 0.14	0.001
Fasting insulin (pmol/l)	108.1 ± 4.5	115.2 ± 3.2	0.877
2-h insulin (pmol/l)	620 ± 35	370 ± 35	0.089
Fasting proinsulin (pmol/l)	8.0 ± 0.4	9.5 ± 0.3	0.200
2-h proinsulin (pmol/l)	40.5 ± 2.8	40.0 ± 1.9	0.672
Fasting proinsulin/insulin ratio	0.082 ± 0.002	0.074 ± 0.003	0.782
LDL size (Å)	258.3 ± 0.6	256.0 ± 0.7	0.029
LDL subclass pattern			
A	57 %	46 %	0.042
I	10 %	17 %	
B	33 %	37 %	
IGT (%)	30 %	15 %	0.002
Hypertriglyceridaemia (%)			
≥ 2.3 mmol (200 mg/dl)	20 %	27 %	0.823
Low HDL cholesterol (%)	34 %	50 %	0.003
men < 0.9 mmol/l (35 mg/dl); women < 1.2 mmol/l (45 mg/dl)			
% Hypertensive	25 %	30 %	0.316

Data are mean ± SEM  
(*n* = 488 subjects)

in subsequent analyses to simplify the presentation and improve statistical power.

Figure 4 shows LDL size and proportion of pattern B by the number of metabolic disorders. LDL size decreased and the proportion of pattern B increased significantly with increasing number of metabolic disorders.

Table 4 shows the relationship stratified by the level of each metabolic disorder of LDL size to the number of remaining metabolic disorders (zero to three). In these analyses, LDL size remains significantly associated with the number of metabolic disorders even after stratification for high or low levels of triglyceride, HDL, IGT or hypertension.

Since age, obesity and body fat distribution may also be related to both LDL size and the number of metabolic disorders, we controlled for the effect of these possible confounding variables by multiple linear regression in non-diabetic subjects. In these analy-

ses, LDL size is the dependent variable. The number of metabolic disorders continued to be significantly related to LDL size even after adjustment for age, obesity, body fat distribution, gender and ethnicity (data not shown). For example, each additional metabolic disorder was associated with a 4.7 Å decrease in LDL size. Fasting insulin, the fasting proinsulin/insulin ratio and gender also predicted LDL size. When we repeated these analyses using multiple logistic regression models in which the presence of LDL subclass pattern B was the dependent variable we obtained very similar results (data not shown).

## Discussion

We have previously shown that in a smaller number of diabetic and non-diabetic subjects immunoreactive insulin concentrations are associated with a pre-

**Table 2.** Pearson correlation of LDL size with insulin and proinsulin and metabolic variables

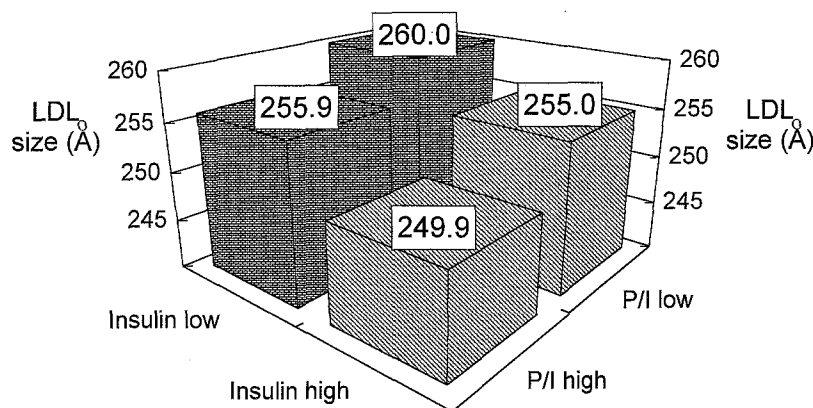
	LDL size	Insulin		Proinsulin		Fasting proinsulin/ insulin ratio
		Fasting	2-h	Fasting	2-h	
LDL size	–	–0.180 <sup>b</sup>	–0.197 <sup>b</sup>	–0.262 <sup>c</sup>	–0.239 <sup>b</sup>	–0.261 <sup>c</sup>
Triglyceride	–0.604 <sup>c</sup>	0.275 <sup>c</sup>	0.247 <sup>c</sup>	0.586 <sup>c</sup>	0.458 <sup>c</sup>	0.433 <sup>c</sup>
Total cholesterol	–0.204 <sup>b</sup>	–0.040	–0.021	0.161 <sup>c</sup>	0.089 <sup>a</sup>	0.187 <sup>b</sup>
HDL cholesterol	0.520 <sup>c</sup>	–0.222 <sup>b</sup>	–0.178 <sup>b</sup>	–0.137 <sup>a</sup>	–0.117 <sup>a</sup>	–0.102 <sup>a</sup>
LDL cholesterol	–0.121 <sup>a</sup>	–0.042	0.010	–0.035	–0.061	0.011
Systolic blood pressure	–0.149 <sup>b</sup>	0.170 <sup>c</sup>	0.180 <sup>c</sup>	0.175 <sup>c</sup>	0.121 <sup>b</sup>	0.139 <sup>b</sup>
Diastolic blood pressure	–0.109 <sup>a</sup>	0.130 <sup>b</sup>	0.140 <sup>b</sup>	0.142 <sup>b</sup>	0.037 <sup>a</sup>	0.037
Fasting glucose	–0.135 <sup>b</sup>	0.159 <sup>b</sup>	–0.131 <sup>b</sup>	0.306 <sup>c</sup>	0.158 <sup>b</sup>	0.266 <sup>c</sup>
2-h glucose	–0.150 <sup>b</sup>	0.256 <sup>c</sup>	0.006	0.378 <sup>c</sup>	0.214 <sup>c</sup>	0.249 <sup>c</sup>

<sup>a</sup>  $p < 0.05$ ; <sup>b</sup>  $p < 0.01$ ; <sup>c</sup>  $p < 0.001$  ( $n = 488$  subjects)

**Table 3.** Partial correlation coefficients of LDL size with insulin and proinsulin

	Insulin		Proinsulin		Fasting proinsulin/ insulin ratio
	Fasting	2-h	Fasting	2-h	
<i>Adjusted for age, gender, BMI and WHR</i>					
LDL size	–0.201 <sup>b</sup>	–0.156 <sup>a</sup>	–0.318 <sup>c</sup>	–0.221 <sup>b</sup>	–0.274 <sup>c</sup>
<i>Adjusted for age, gender, BMI, WHR, triglyceride, high density lipoprotein and fasting glucose</i>					
LDL size	–0.143 <sup>a</sup>	–0.105 <sup>a</sup>	–0.170 <sup>a</sup>	–0.110 <sup>a</sup>	–0.154 <sup>a</sup>

<sup>a</sup>  $p < 0.05$ ; <sup>b</sup>  $p < 0.01$ ; <sup>c</sup>  $p < 0.004$



**Fig. 1.** LDL size by fasting insulin (I) and the fasting proinsulin/insulin (P/I) ratio in non-diabetic subjects.  $p$ -values calculated by two-way analysis of variance (insulin  $p = 0.002$ ; proinsulin  $p = 0.008$ )

ponderance of smaller denser LDL particles [19]. We have now shown for the first time that LDL size is inversely associated with both specific insulin and proinsulin concentrations and the proinsulin/insulin ratio. This suggests that a more atherogenic LDL pattern may occur not only with insulin resistance but also in subjects with abnormal insulin secretion. However, the effect of insulin resistance (as estimated by fasting insulin concentrations) was somewhat stronger than the effect of abnormal beta-cell function (as judged by the fasting proinsulin/insulin ratio on LDL size (Fig. 1)). We have previously shown that increased proinsulin and specific insulin concentrations are associated with increased blood pressure, and increased triglyceride levels in a smaller number of subjects [36]. Furthermore, the fasting proinsulin/insulin ratio was associated with an in-

creased number of metabolic disorders in non-diabetic subjects [48].

We have shown in this report that small dense LDL, whether measured as LDL size, a continuous variable, or as the dichotomous B vs A LDL subclass pattern, is associated with multiple metabolic disorders. These associations were not dependent on obesity or on unfavourable body fat distribution, both of which exist in insulin-resistant subjects; furthermore, similar relations between LDL size and insulin resistance syndrome-related disorders occur in both men and women and in Mexican Americans and non-Hispanic whites. Our results confirm earlier work by Selby et al. [20] in the Kaiser Permanente Women Twins Study. In the current report as in a previous report [19], insulin concentrations were associated with LDL size. However, the number of metabolic disor-

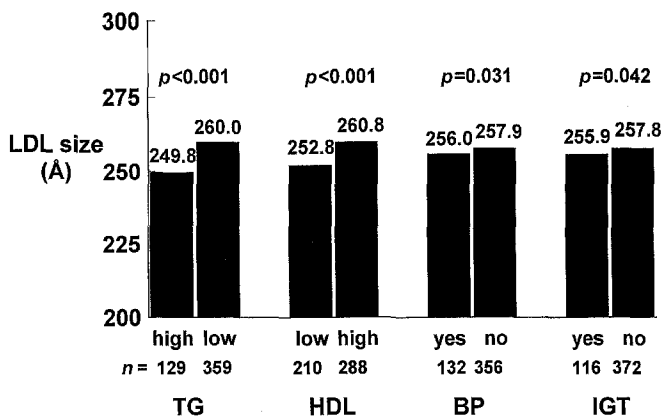


Fig. 2. LDL size by: triglyceride; HDL cholesterol; blood pressure; and impaired glucose tolerance in non-diabetic subjects

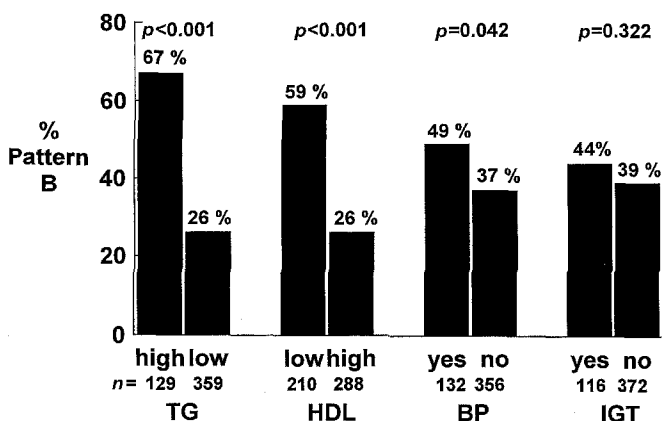


Fig. 3. The percent LDL subclass pattern B by: triglyceride; HDL cholesterol; blood pressure; and impaired glucose tolerance in non-diabetic subjects

ders was significantly related to LDL size even after controlling for insulin concentrations. Since the correlation between fasting insulin concentrations and insulin resistance is only moderate ( $r = -0.6$ ) [49, 50], it is possible that controlling for insulin resistance directly might statistically account for the entire association between the insulin resistance syndrome and LDL size.

In this report, as in previous studies, LDL size was lower in subjects with higher triglyceride [4-7, 19-22] and low HDL cholesterol concentrations [5, 19-22]. Subjects with hypertriglyceridaemia and decreased HDL cholesterol also had a significantly higher prevalence of LDL subclass B.

Subjects with hypertension also had significantly smaller LDL size and a higher prevalence of LDL subclass pattern B than normotensive subjects. Selby et al. [20] found a significant association between hypertension and LDL subclass pattern B, but not LDL size. LDL size has also been found to be reduced in subjects with familial dyslipidaemic hypertension [51] and in subjects taking beta blockers [23]. Exclusion of the subjects on pharmacological therapy

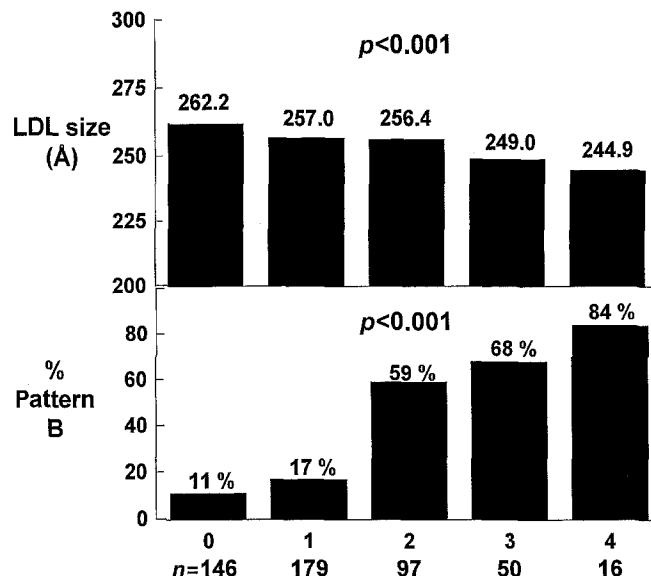


Fig. 4. LDL size and subclass pattern by the number of metabolic disorders in non-diabetic subjects

for hypertension did not affect the association between LDL size and the number of metabolic disorders (data not shown). In the current report, the association between hypertension and LDL size or pattern was not statistically significant after adjusting for the number of other metabolic disorders. This suggests that the smaller denser LDL observed in the current report is most likely due to the higher triglyceride and lower HDL cholesterol observed in hypertensive subjects.

Although a number of studies has shown that subjects with NIDDM have smaller denser LDL than non-diabetic subjects [20, 22, 24, 25], few data are available on the effect of IGT on LDL size. In the current report, we observed smaller LDL size in subjects with IGT compared to subjects with normal glucose tolerance (Fig. 2). Selby et al. [20] have shown that in women with IGT LDL size was intermediate between those with normal glucose tolerance and diabetes. Recently, Austin et al. [52] have shown a preponderance of small dense LDL prior to the onset of NIDDM in elderly Finnish subjects.

The associations of LDL size with hypertriglyceridaemia and low HDL cholesterol was much stronger than its association with hypertension or IGT. However, the association between LDL size and the number of multiple metabolic disorders was not only dependent on the triglyceride and HDL concentrations. LDL size significantly decreased in a stepwise fashion with the increasing number of metabolic disorders (Fig. 4). Thus, the impact of having three or four metabolic defects on LDL composition was larger than having only two disorders (most likely to be high triglyceride level and low HDL cholesterol).

Subjects with the insulin resistance syndrome have higher triglyceride and lower HDL cholesterol levels

**Table 4.** LDL size and the proportion of pattern B<sup>a</sup> according to the number of metabolic disorders stratified by levels of selected metabolic disorders in non-diabetic subjects

	Number of metabolic disorders				<i>p</i> -value	
	0	1	2	3		
<i>Triglyceride</i>					TG	NOMD
high ( <i>n</i> = 129)	254.4	249.6	248.5	244.9	< 0.001	< 0.001
(% pattern B)*	(65 %)	(79 %)	(85 %)	(89 %)	(< 0.001)	(0.002)
low ( <i>n</i> = 359)	262.6	257.5	260.9	252.7		
(% pattern B)*	(16 %)	(39 %)	(54 %)	(67 %)		
<i>HDL cholesterol</i>					HDL	NOMD
low ( <i>n</i> = 210)	255.1	254.9	248.1	244.9	< 0.001	< 0.001
(% pattern B)*	(51 %)	(45 %)	(75 %)	(89 %)	(< 0.001)	(< 0.002)
high ( <i>n</i> = 288)	262.6	260.5	258.9	254.4		
(% pattern B)*	(16 %)	(38 %)	(58 %)	(60 %)		
<i>Hypertension</i>					↑ BP	NOMD
yes ( <i>n</i> = 132)	258.3	256.5	250.0	244.9	0.444	< 0.001
(% pattern B)*	(16 %)	(36 %)	(80 %)	(80 %)	(0.872)	(< 0.001)
no ( <i>n</i> = 356)	262.6	256.6	252.7	247.1		
(% pattern B)*	(16 %)	(46 %)	(65 %)	(86 %)		
<i>IGT</i>					IGT	NOMD
yes ( <i>n</i> = 110)	261.8	258.4	247.6	244.9	0.182	< 0.001
(% pattern B)*	(21 %)	(46 %)	(79 %)	(89 %)	(0.363)	(< 0.001)
no ( <i>n</i> = 372)	263.5	255.9	253.4	247.8		
(% pattern B)*	(16 %)	(36 %)	(59 %)	(60 %)		

Data are mean ± SEM (*n* = 488 subjects)

NOMD, Number of metabolic disorders.

\* Subjects with pattern I excluded from these analyses

<sup>a</sup> Maximum number of metabolic disorders is three since one disorder is being stratified

[8–12] which may contribute to increased risk for coronary heart disease. However, they have relatively normal LDL cholesterol levels. We have shown in this report that a preponderance of small dense LDL is also strongly associated with the metabolic disorders which characterize the insulin resistance syndrome, suggesting that small dense LDL may explain part of the increased risk of atherosclerosis in this disorder. Furthermore, decreases in LDL size are also significantly associated with both a selective beta-cell defect (as estimated by the fasting proinsulin/insulin ratio) and insulin resistance (as estimated by the fasting insulin concentrations) although the association was somewhat stronger for the latter. We conclude that small dense LDL may form part of the insulin resistance syndrome.

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