Serum Oxidized Low Density Lipoprotein (oxLDL) levels are increased in hyperlipidemic subjects but not associated with LDL size

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Background and Aim

It is well established that hyperlipidemia is associated with formation of smaller, denser LDL particles (sdLDL) in plasma. Many studies suggested that sdLDL particles have increased atherogenicity compared to larger buoyant subtypes (1). Several reasons were suggested in this regard: I) sdLDL particles have decreased receptor-mediated uptake by peripheral tissues due to their altered structure. II) sdLDL particles are easily trapped in the sub-endothelial space predisposed by injury to the arterial wall. *In vitro* studies suggest that small LDL particles are more prone to oxidation in the sub-endothelial space than the normal large buoyant LDL particles (2). Oxidation of LDL (oxLDL) is believed to be a key factor in atherosclerosis progression. Macrophages recognize OxLDL forming "foam cells" that develop into fatty streaks predisposing plaque formation initiating atherosclerosis (3) (**Figure 1**).
AIM: To examine if increased sdLDL in hyperlipidemia is associated with increased serum oxLDL levels by measuring oxLDL in hypertriglyceridemic and control subjects, and to determine the association serum oxLDL with different lipid profile measures.

Subjects and Methods

Forty seven serum samples (32 males and 15 females) were included in this study. Twenty nine were hyperlipidemic patients, and 18 healthy controls were included in this study. Serum triglycerides, total cholesterol, HDL, LDL, fasting glucose, apoB and apo-A1 were measured in all subjects using automated clinical chemistry analyzers. LDL size was measured by polyacrylamide gradient gel electrophoresis (PAGGE). A sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure oxidized LDL levels (Malondialdehyde-modified LDL). Statistical analysis was performed by SPSS (version 13.0 for Windows).

Results

Parameters	Control n = 18	Hyperlipidemic n = 29	P value
Glucose (mmol/L)	5.9 ± 0.7	6.6 ± 0.6	0.161
Total Chol. (mmol/L)	4.6 ± 0.2	5.7 ± 0.2	0.001
TG (mmol/L)	0.8 ± 0.1	3.0 ± 0.2	< 0.0001
HDL-C (mmol/L)	1.35 ± 0.06	1.02 ± 0.06	< 0.0001
LDL-C (mmol/L)	3.1 ± 0.2	3.8 ± 0.2	0.017
Apo A-1 (g/L)	1.30 ± 0.05	1.15 ± 0.04	0.033
ApoB (g/L)	0.89 ± 0.06	1.20 ± 0.05	< 0.0001
TG/HDL	0.66 ± 0.10	3.22 ± 0.33	< 0.0001
ApoB/ApoA1	0.70 ± 0.05	1.06 ± 0.04	< 0.0001
LDL/HDL	2.4 ± 0.2	3.8 ± 0.1	< 0.0001
ApoB/LDL-C	0.29 ± 0.01	0.32 ± 0.01	0.06
oxLDL levels (U/L)	122.4 ± 14.9	186.6 ± 11.8	0.002
LDL size (nm)	25.2 ± 0.6	22.4 ± 0.8	0.008
ApoB/LDL size (g/L.nm)	0.036 ± 0.002	0.057 ± 0.004	0.001

$P \leq 0.05$: Significant.
Data are expressed as mean ± Standard Error of the mean. LDL, low density lipoprotein; Total Chol, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; apo A-I, apolipoprotein A-I; apo B, Apolipoprotein B, oxLDL, oxidized low density lipoprotein.

Table 2: Correlations of oxLDL-C with different lipid parameters.

Parameters	Correlation Coefficient	P value
Glucose (mmol/L)	0.238	0.108
Chol. (mmol/L)	0.578	< 0.0001
TG (mmol/L)	0.279	0.058
HDL-C (mmol/L)	-0.100	0.502
LDL-C (mmol/L)	0.545	< 0.0001
Apo A-1 (g/L)	0.212	0.153
ApoB (g/L)	0.684	< 0.0001
TG/HDL	0.213	0.151
ApoB/ApoA	0.458	0.001
LDL-C/HDL-C	0.491	< 0.0001
ApoB/LDL	0.237	0.109
LDL size (nm)	-0.180	0.226
ApoB/LDL size (g/L.nm)	0.527	< 0.0001

$P \leq 0.05$, Significant.
LDL-C, low density lipoprotein cholesterol; Total Chol, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; apo A-I, apolipoprotein A-I; apo B, apolipoprotein B; oxLDL, oxidized low density lipoprotein.

Anthropometrics: Controls: Age 36.2 ± 2.4, BMI 23.6 ± 0.8, Hyperlipidemic: Age 45.3 ± 1.9, BMI 28.5 ± 0.7. Lipid and glucose parameters and mean differences are shown in (**Table 1**).

Correlation analysis: oxLDL showed no significant correlation with LDL size. Moreover, no significant correlation was seen with TG or glucose levels. However a strong association was found between oxLDL and apoB levels in serum (**Table 2**).

Stepwise multiple regression demonstrated that the main predictor of oxLDL levels in serum was apoB levels among all measured lipid parameters that exhibited a significant association with oxLDL levels (Figure 1). 46.8 % variation in oxLDL was determined by ApoB levels (determined by R^2). The regression coefficient was 163.23, indicating that an increase in ApoB levels by 1 g/L is expected to increase oxLDL levels by 163.23 U/L (**Table 3, Figure 2**). Other parameters that showed significant correlation with oxLDL (Chol, LDL-C, ApoB/ApoA, LDL/HDL, ApoB/LDL size) were all excluded from the regression model as non-significant predictors.

Table 3: Stepwise Multiple Regression Model for Predicting oxLDL.

Predictor	B	SE (B)	β
Constant	-14.27	29.021	NS
ApoB (g/L)	163.23	25.936	0.684**
R^2		0.468	

**p<0.0001. NS: non significant

B, Regression Coefficient; SE (B), Standard error of regression coefficient; β, standardized coefficient; R^2, % of total variation; apo B, apolipoprotein B.

References:

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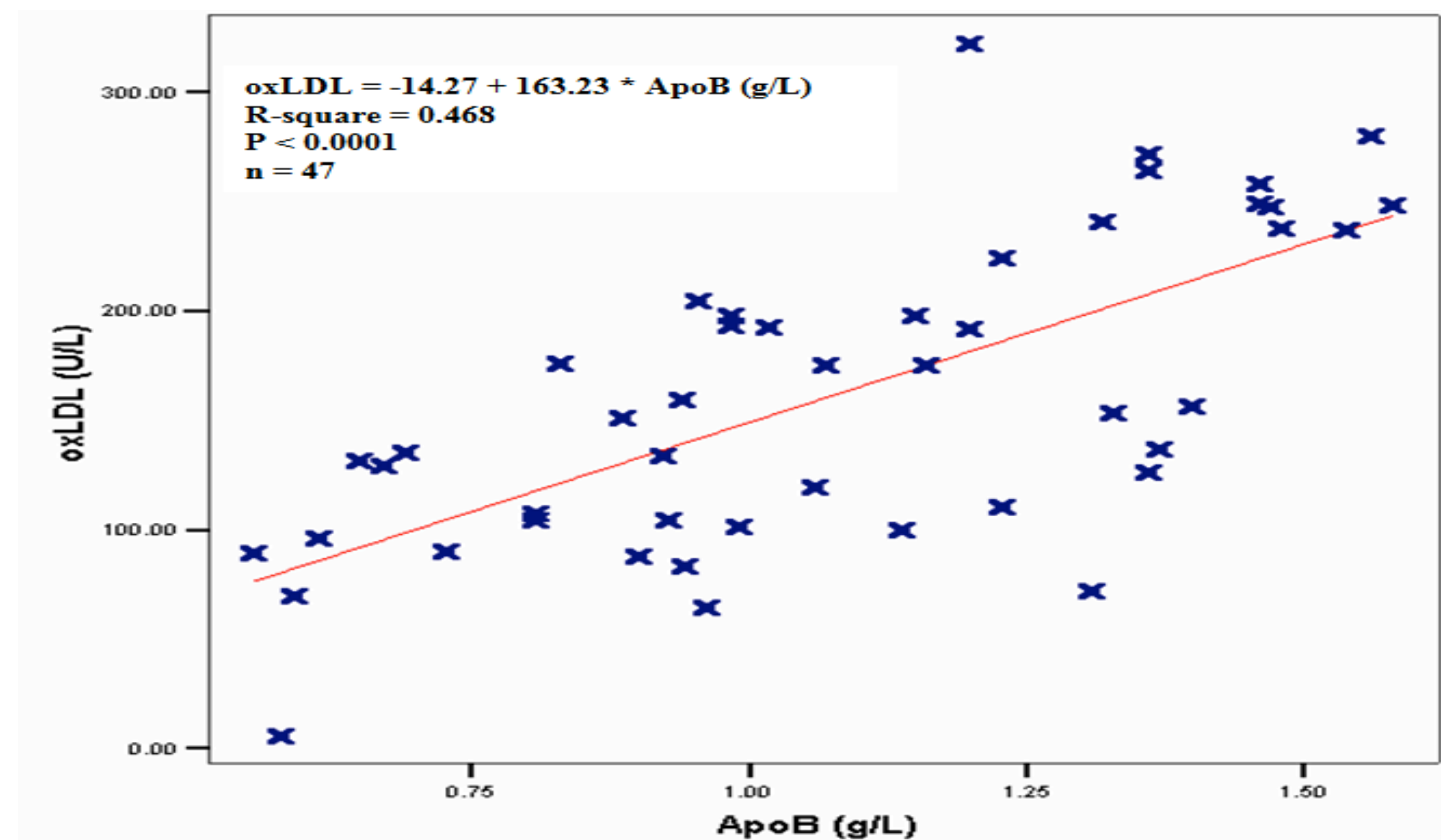
oxLDL = -14.27 + 163.23 * ApoB (g/L)
R-square = 0.468
P < 0.0001
n = 47

Figure 2: Correlation of ApoB with OxLDL serum levels

Summary and conclusion

This study showed that mean LDL size was smaller, and ox-LDL levels were significantly higher in the hypertriglycemic group compared with the normotriglycemic control group. However, there was no significant correlation of LDL size or lipid parameters with oxLDL levels in plasma. Interestingly, apoB showed the strongest association with oxLDL excluding the rest of the metabolic factors, as predictors of oxLDL.

These results suggest that the higher "number" of LDL particles, as reflected by apoB levels (*one apoB molecule per LDL particle*), exceed size, or cholesterol content in determining increased oxLDL levels in serum probably as a result of increased "number' of small LDL particles entering the sub-endothelial space and their ability to escape in their oxidized form into the serum. Although size may be a determinant of oxidation vulnerability, it does not appear as a predicting factor in this study. It may be also that apoB protein is more prone to malonaldeyde induced oxidation compared to apoA1 and other lipoprotein constituents.

These results support the notion that the number of particles, rather than cholesterol content , contribute to increased atherosclerosis risk probably due to increased availability of apoB molecules for oxidation (4).
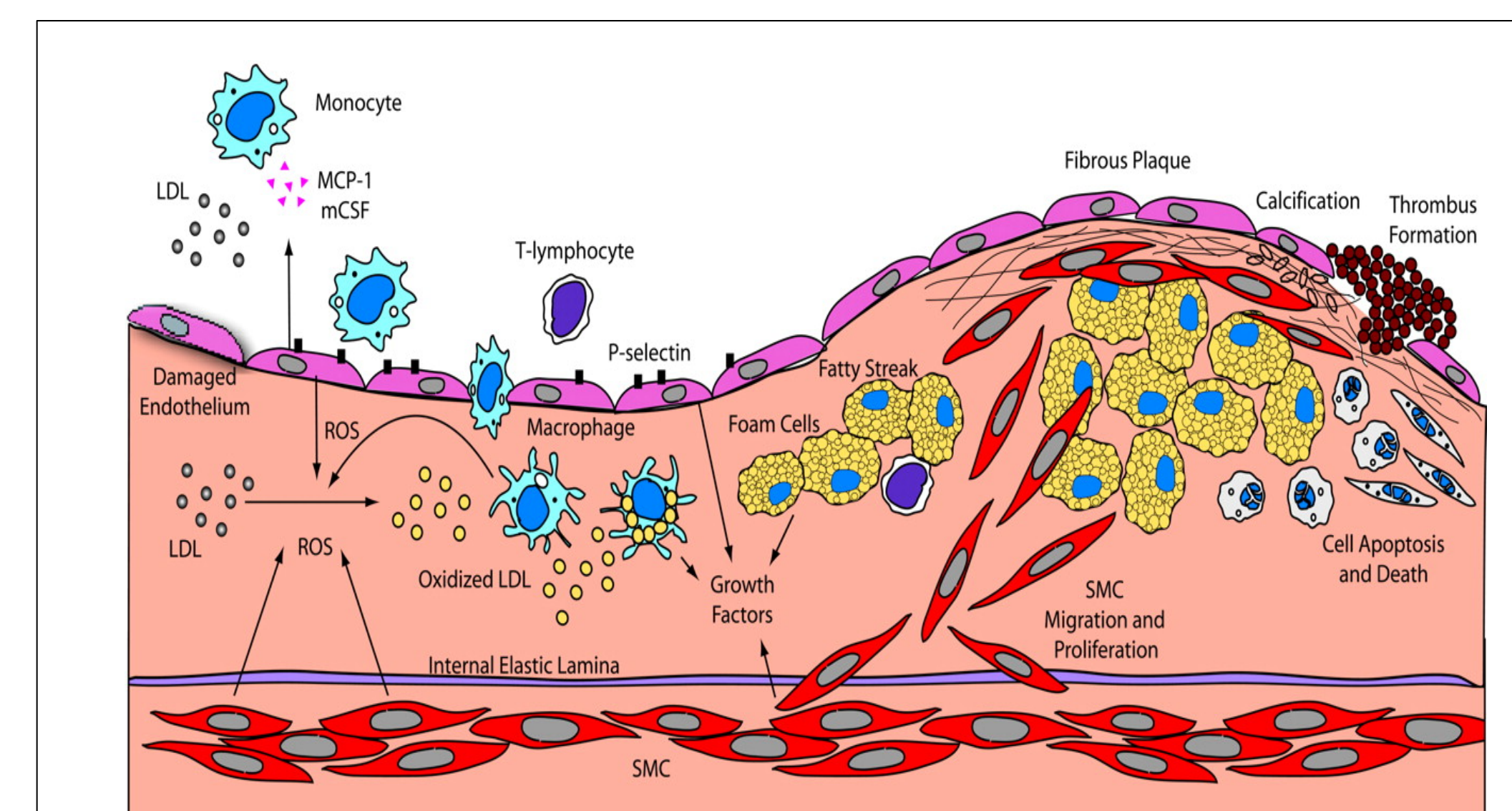

Figure 1: Formation of foam cells as a result of engulfing oxidized LDL particles