Smyrna Tıp Dergisi ***Araştırma Makalesi***

Examining the Difference in LDL Size Due to Aging
Yaşlanmaya Bağlı LDL Boyutundaki Farklılığın İncelenmesi

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Summary
Objective: Low density lipoprotein (LDL) is a risk factor in the pathogenesis of atherosclerosis. Individuals with particularly small and dense LDL are at greater risk. In this study, it was aimed to investigate the changes in LDL size due to aging.
Material and Methods: A total of 187 adults (21-95 years) without any cardiovascular disease were enrolled in the study. LDL size was determined using the modified Krauss and Burke model.
Results: The mean LDL size was found smaller in elderly adults ($p<0.001$). It was found that the mean LDL size was statistically significantly smaller in the elderly males compared to the elderly females and young males (for both, $p<0.001$). In addition, the mean LDL size was negatively correlated with age ($r= -0.646$; $p<0.001$). No correlation was found between mean LDL size and other lipid parameters, such as cholesterol triglycerides
Conclusion: Findings of this study indicate that LDL size is smaller in elderly males compared to elderly females and young males. The decrease in LDL size due to aging, especially in males, proves that male adults are risky in terms of atherosclerosis.
Key words: Aging, atherosclerosis, lipoprotein size, low-density lipoprotein

Özet
Amaç: Düşük yoğunluklu lipoprotein (LDL), ateroskleroz patogenezinde bir risk faktörüdür. Özellikle küçük ve yoğun LDL'si olan bireyler daha fazla risk altındadır. Bu çalışmada yaşlanmaya bağlı olarak LDL boyutundaki değişikliklerin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Çalışmaya herhangi bir kardiyovasküler hastalığı olmayan toplam 187 yetişkin (21-95 yaş) alındı. LDL boyutu, modifiye Krauss ve Burke modeli kullanılarak belirlendi.
Bulgular: Ortalama LDL boyutu yaşlı erişkinlerde daha küçük bulundu ($p<0.001$). Yaşlı erkeklerde ortalama LDL boyutunun yaşlı kadınlara ve genç erkeklere göre istatistiksel olarak anlamlı derecede daha küçük olduğu bulundu (her ikisi $p<0.001$). Ek olarak, ortalama LDL boyutu yaşla negatif korelasyon gösterdi ($r= -0.646$; $p<0.001$). Ortalama LDL boyutu ile kolesterol trigliseritleri gibi diğer lipid parametreleri arasında bir ilişki bulunmadı.
Sonuç: Bu çalışmanın bulguları, yaşlı erkeklerde LDL boyutunun yaşlı kadınlara ve genç erkeklere göre daha küçük olduğunu göstermektedir. Özellikle erkeklerde yaşlanmaya bağlı olarak LDL boyutunun azalması, erkek erişkinlerin ateroskleroz açısından riskli olduğunu kanıtlamaktadır.
Anahtar Kelimeler: Yaşlanma, ateroskleroz, lipoprotein boyutu, düşük yoğunluklu lipoprotein,

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Introduction

Low density lipoproteins (LDL) are the primary cholesterol-carrying lipoproteins in plasma. The density of LDL ranges between 1,019-1,063 g/mL and contains 50% cholesterol, 25% protein, 20% phospholipid and 5% triglyceride. LDL particles also differ in size (1,2). Recent studies suggest that it is quality of LDL-not quantity-that exerts a significant impact on cardiovascular risk.

Apolipoprotein (apo) B, a 550-kD polypeptide comprises over 95% of LDL mass. Elevated plasma LDL levels of LDL cholesterol and apo B have been associated with premature coronary artery disease (CAD). In addition, hypertriglyceridemia with increased levels of

small-dense LDL is a characteristic feature of diabetic dyslipidemia (3).

LDL particles are classified into two phenotypes: the majority have a predominance of medium-to-larger sized LDL (pattern A) and there is a higher proportion of small-sized LDL (pattern B) (3). Some studies have argued that these two subclasses of LDL are inherited through a dominant mode as a single gene trait (4), while other studies have proposed that LDL subclasses are significantly influenced by environmental factors (5). The LDL pattern with a predominance of small and dense particles has been associated with the presence of myocardial infarction (MI) and coronary artery disease (CAD), but this relationship is not independent of triglycerides (6). Studies have shown that normocholesterolemic individuals with a high concentration of small and dense LDL particles are at increased risk for CAD. On the other hand, it has been reported that small LDL particles are harmful to vascular health because of the increased propensity to oxidation (7). The relationship between LDL size and diseases has been investigated, including Type II diabetes mellitus (3), dyslipidemia in adolescents (8), carotid atherosclerosis (9), obesity (10), kidney and liver transplants (11), and familial combined hyperlipidemia (12).

The world's general population is aging due to the decline in fertility and sustained decrease in mortality. The transition from a young to an elderly population has brought epidemiological changes with continuing growth in the prevalence of chronic diseases. Aging is considered the greatest risk factor of cardiovascular disease resulting from changes in the lipid profile of the elderly (13). In addition, gender-related differences in cardiovascular disease suggest that LDL size may also vary between the two sexes (14). However, age and gender-related differences in LDL size are rare in the literature (15). The objective of this study was to investigate the differences in LDL size due to aging.

Materials and Methods

Study Design and Patients

The study was initiated after the approval of Ordu University Faculty of Medicine Clinical Research Ethics Committee (11/03/2022; no: 2022-58). The study was conducted voluntarily, all participants were informed about the objectives of the study and gave signed informed consent before the blood sample collection. This study was conducted in accordance with the 1964 Declaration of Helsinki.

Blood samples were collected from adults who applied to the Ordu University Faculty of Medicine Clinical Research blood collection center. A total of 187 adults aged 21-95 years, who accepted to participate to the study and underwent biochemical analysis were included in the study. Adults who underwent biochemical analysis during the study period were included. Adults aged under 18 years, those with severe coronary, renal, hepatic, inflammatory disease, and those with missing data were excluded from the study. Adults' demographics such as age and gender, weight, height, and body mass index, LDL cholesterol, and triglyceride levels, comorbidities (diabetes, asthma, osteoporosis, heart failure, renal failure, rheumatism, dementia, hypercholesterolemia, glaucoma, coronary artery disease, cancer, hepatitis B, thyroid disease, thalassemia, hypertension, and others), status and amount of smoking, familial history (hypertension, CVD, diabetes mellitus, cirrhosis, thyroid disease, asthma, and others) and drugs used (oral antibiotics, fosamax, singulair, inhibace, and others) were recorded. Adults were divided into two groups as the adults aged <60 years and those aged ≥ 60 years. The adults were also divided into two groups according to gender. Data of the adults used in this study was obtained from the hospital electronic information system and patient files and were compared between the groups and possible correlations were investigated.

Serum Lipid Analysis

Blood samples were collected after an overnight fast and serum was prepared by low speed centrifugation at 4 ^{o}C. Serum aliquots were stored at -80 ^{o}C for LDL size measurement. Serum cholesterol and triglyceride concentrations were measured enzymatically by the methods described by Dia-Iyatron (Dia-Iyatron Co., Tokyo, Japan). The reference intervals were taken as 3.62–5.69 mmol/l for total cholesterol and 0.38–1.69 mmol/l for triglycerides.

Determination of LDL Size

LDL size was determined using the modified Krauss and Burke (7) model as described by

Tsukamoto et al. (16). Briefly, 10 mL of apoferritin (51 g of protein/l; Sigma, St.Louis, USA) was added into a mixture of a 10-mL serum sample, 40 ml of 310 g/L sucrose containing 0.6 g/L EDTA and 0.1 g/L bromophenol blue (BPB), and the mixture was kept in a freezer until analysis. Apoferritin and thyroglobulin (high-molecular-weight protein standards kit for electrophoresis (HMW) Pharmacia, Uppsala, Sweden) were used as the standards. The standard 25.7 and 27.0 nm LDL particles used in the analysis were estimated by the method using negative staining in electron microscopy (17). Electrophoresis was performed at 48 ºC with a 5-mL volume of specimen applied to each gel line. The voltage was adjusted to 20 V and 70 V for each 15 min and finally at 125 V for 24 h using Tris (0.089 mol/l)-boric acid (0.089 mol/l)-Na2EDTA (0.003 mol/l) buffer with pH 8.3. The gels were then removed and stained with 4 g/l Coomassie brilliant blue (CBBØR250; PAGE Blue 83; Daiichi Pure Chemicals) for 4 h, and after de-staining, the stained gels were scanned with an imaging scanner equipped with a charge-coupled device (CCD) sensor (Epson GT-7600S, Seiko-Epson Co.Ltd., Nagano, Japan) and stored as image files. For lipid staining, gradient gels were also stained with 10 g/l Sudan black B (Daiichi Pure Chemicals) in 600 g/l ethanol overnight and de-stained with 100 g/l acetic acids more for 4 h to restore the gel to its original length.

The stored electrophoresis images were transferred to Paint Shop Pro-7j graphic software (Jasc Software Inc., Tokyo, Japan) as a jpeg file and printed out on a clear white sheet for colour printing. Migration distances from the sample well (starting point) to apoferritin (a) and from the sample well to the apolipoprotein B contained in LDL molecules (b) were measured with Vernier callipers, and the relative migration distance (b/a) was calculated. The diameter of the LDL particles was estimated from the calibration curve plotted from the relative distances of the three calibrators, apoferritin, thyroglobulin, and standard LDL.

Statistical Analysis

Data obtained were analysed using SPSS version 23 (SPSS, Statistical Package for Social Sciences, IBM Inc., Armonk, NY, USD). The normality of the data was tested with the Kolmogorov-Smirnov method. The homogeneity of variance was examined with Levene's test. Non-normally distributed quantitative variables are expressed as the median and interquartile range (IQR), while categorical variables are given as frequency (n, %). Normally distributed numerical variables were compared between the groups with independent t-test. Skewed variables were compared with the Mann-Whitney U test. Kruskal-Wallis test was used for the variance analysis. The correlation between variables was explored by using the Spearman's correlation analysis. All p values less than 0.05 were considered statistically significant.

Results

A total of 187 adults who underwent biochemical analysis were enrolled in the study. Seventy-four (39.57%) were young and 113 (60.43%) were elderly adults. Out of the 187 adults, 120 (64.17%) were female and 67 (35.83%) were male. The mean age of all the participants was 62.6±22.10 years. The mean age in the elderly and young adults were 78.9±6.2 years and 37.6±6.2 years, respectively, while the mean age in female adults and male adults were 63.2±21.18 years and 61.5±23 years, respectively (Table 1).

Sixty-six (35.29%) adults had known comorbid diseases. The most common comorbidity was heart failure followed by diabetes mellitus. There was no statistically significant difference between the groups in terms of the laboratory values, including biochemical parameters and complete blood count analysis. No correlation was found between mean LDL size and other lipid parameters, such as cholesterol triglycerides. The mean LDL size, measured as 26.5±0.9 nm in the young adults and 24.9±1.1 nm in the elderly adults, was statistically significantly smaller in the elderly adults ($p<0.001$) (Figure 1). Moreover, it was negatively correlated with age ($r= -0.646$; $p<0.001$) (Figure 2).

The mean LDL size was also statistically significantly smaller in elderly male adults compared to elderly female adults ($p=0.001$) (Table 2, Figure 3).

When median values were used to evaluate LDL, statistically significant difference was found according to gender (Table 3). A box plot chart of LDL size according to age and gender is shown in figure 4.

Table 1. *Age distribution of the adults*

Gender		Mean ± SD	Median (min-max.)
	Young	37,5 ±6,4	37 (21 - 63)
Female	Elderly	78,6 ± 6,2	79 (66 - 95)
	Total	63,2 ± 21,8	73 (21 - 95)
	Young	37,8 ± 12,9	34 (21 - 64)
Male	Elderly	79,6 ± 6	80 (66 - 94)
	Total	61,5 ± 23	73 (21 - 94)
	Young	37,6 ± 12,3	34 (21 - 64)
Total	Elderly	78,9 ± 6,2	79 (66 - 95)
	Total	62,6 ± 22,2	73 (21 - 95)

Figure 1. *Bar chart and box-plot of mean LDL size according to age groups*

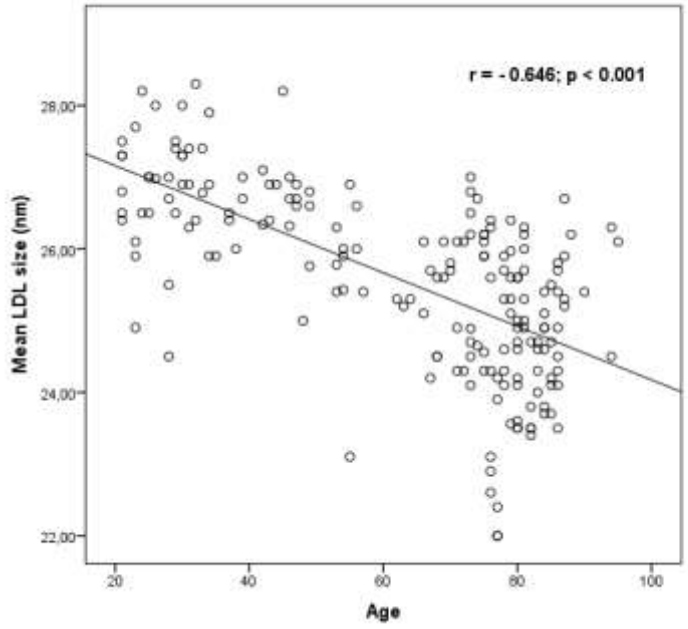


Figure 2. *Correlation between mean LDL size and age*

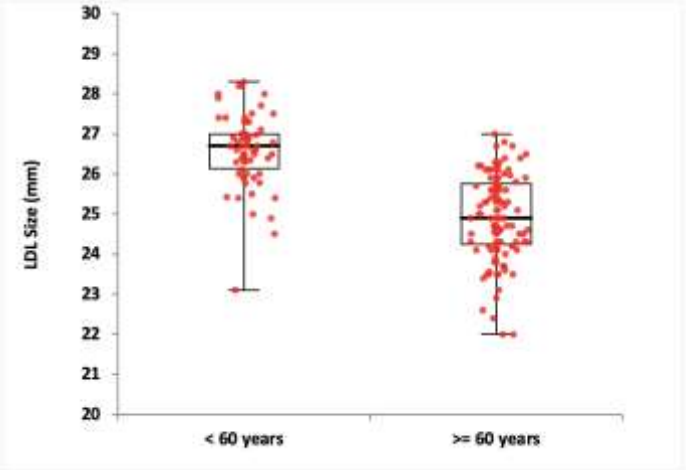


Table 2. *Comparison of elderly male and female adults in terms of LDL size*

	Elderly female	Elderly male	Test stat	p
LDL Size (nm)	25.2 ± 1	24.5 ± 1.1	3.277	0.001

Figure 3. *Box-plot of LDL size according to gender in elderly adults*

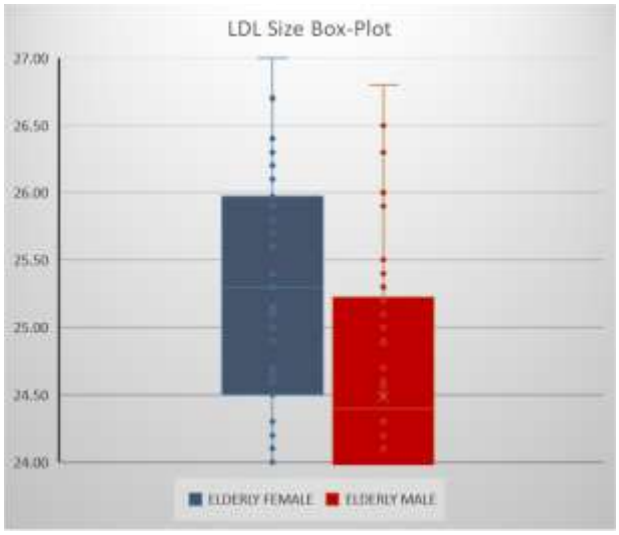
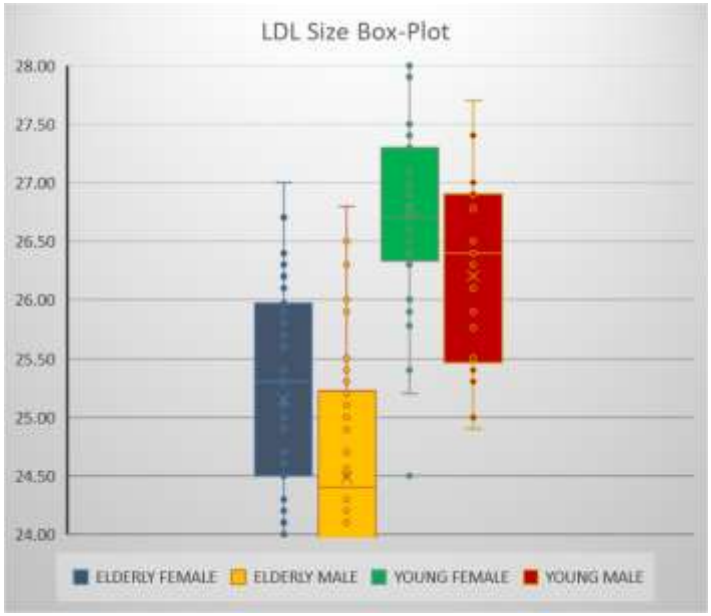


Table 3. *Comparison of LDL size between age group and gender*

	Elderly female (n=75)	Elderly male (n=38)	Young female (n=45)	Young male (n=29)	t	P[1]
LDL Size	25.3 (22-27)[a]	24.4 (22-26.8)[b]	26.7 (24.5-28.3)[c]	26.4 (23.1-27.7)[c]	88.393	<0.001

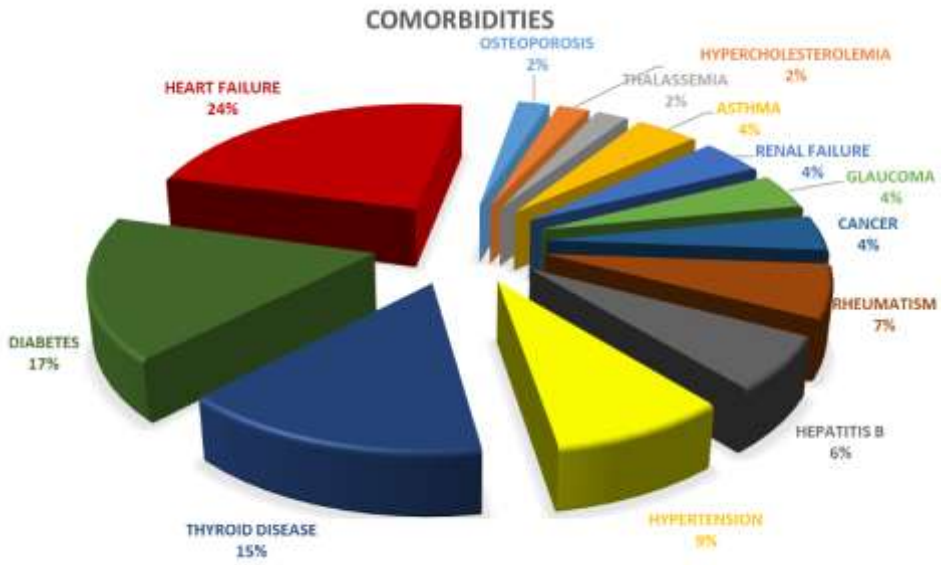
[a-b-c] : No statistically significant difference between the values with the same letters [1]Kruskal Wallis

Figure 4. *Box-plot chart of LDL size according to age and gender*



Chronic disease groups according to LDL elderly size were given in figure 5.

Figure 5. *LDL elderly size chronic disease groups*



Discussion

The objective of this study was to investigate the differences in LDL size due to ageing. In the study, mean LDL size between young and elderly adults was compared. LDL particles are heterogeneous in terms of size, density composition and physicochemical characteristics. It was found that LDL size was significantly smaller in elderly adults compared to young adults and in males compared to females.

Although several studies in the literature have associated LDL size with the development of atherosclerosis (7,18), some studies, however, do not confirm the independence of the association (9). This association may be explained by the atherogenicity of small dense LDL because of susceptibility to oxidation (8). Various reasons have been suggested for the atherogenicity of small dense LDL. One reason is that smaller, denser LDL particles are taken up more easily by arterial tissue than larger particles (19). In addition, smaller LDL particles may also reduce receptor-mediated uptake and increased proteoglycan binding (20). It has likewise been reported that small LDL is strongly associated with increased triglyceride and reduced HDL cholesterol as well as other traits of metabolic syndrome (21). Individuals with predominantly smaller LDL particles (pattern B) are at a higher risk of developing CVD than do those with predominantly larger LDL particles (pattern A) (22). Larger LDL sizes have been reported in the offspring of long-lived individuals than in age- and lifestyle-matched control subjects (23).

Ageing is related to numerous physicochemical alterations in the body, including lipid profile. The ageing process is considered the most important risk factor of developing CVD because of the changes in the lipid profile of the elderly (24). It was thought that a better understanding of the differences in LDL size in the elderly and between genders could contribute to the management of CVD. However, little is known about LDL size in the elderly, and the number of such studies is quite limited, which in turn limits an exact comparison of the results between the studies. These shortcomings make the current study more important.

In this study, the mean LDL size was larger in young adults than in elderly adults. In addition, a smaller LDL size was found in elderly male adults, followed by elderly female young males, and then young female adults. Therefore, it was concluded that LDL size is smaller in elderly

adults compared to the young ones and in male adults compared to female ones.

Several studies in the literature have investigated LDL size in the elderly but with various aspects. For instance, Mykkänen et al. evaluated LDL size as a predictor of CVD in elderly males and females. After controlling for diabetes status in elderly males and females, they reported that LDL size was not a predictor of CVD (25). But the authors did not compare LDL size between sexes. In another study, Greene et al. investigated plasma LDL and HDL characteristics following a dietary intake of eggs and cholesterol-free egg substitute in the elderly population. They reported LDL size as 21.77±0.95 nm with eggs and 21.48±0.95 nm with egg substitute without significant difference between them. However, the diet effect in increasing LDL size was depicted in all participants (26). Studies have reported smaller LDL sizes in males compared to females (7,27). Lemieux et al. investigated LDL size in middle-aged and young males and found no significant difference in their mean LDL size: 25.2 in the young males (n=38) and 25.2 in middle-aged males (n=40) (28).

LDL size may also differ between various metabolic diseases, such as hypertension, diabetes mellitus and comorbid situations, including smoking, alcohol abuse and obesity. As we could not analyse our findings in this term, we did not look at a comparison with a few studies in the literature addressing these disorders and conditions separately because it was not the primary objective of this study. However, the lack of studies on LDL size between elderly and young people and male and female adults made it impossible to compare our findings. There is an urgent need for such studies, especially to determine cut-off value, sensitivity and specificity values for LDL size in the prediction of CVD.

Study Limitations

The major limitations of this study include its single-centre design and relatively low number of adults for such a study. Therefore, results cannot be generalised to the general population. Finally, LDL size based on the presence of various comorbidities was not investigated. Nonetheless, considering the lack of such studies in the literature, findings will be guiding for future studies to be performed on this issue.

Conclusion

Findings of the study indicate that LDL size is smaller in elderly males compared to elderly females and young males. These results may be useful in determining therapeutic strategies that target decreasing the proportion of smaller, denser LDL particles in various age groups and between sexes. However, further multicentre, randomised controlled studies that investigate LDL size in comorbidities are warranted.

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Data Availability: Data used in this study can be provided at the reasonable request

References

1. Ke LY, Engler DA, Lu J, Matsunami RK, Chan HC, Wang GJ et al. Chemical composition-oriented receptor selectivity of L5, a naturally occurring atherogenic low-density lipoprotein. Pure Appl Chem 2011;83(9):10.1351/PAC-CON-10-12-07.
2. Sánchez-Muniz FJ, Merinero MC, Rodríguez-Gil S, Ordovas JM, Ródenas S, Cuesta C. Dietary fat saturation affects apolipoprotein AII levels and HDL composition in postmenopausal women. J Nutr 2002;132(1):50-4.
3. Rizzo M, Rini GB, Berneis K. The clinical relevance of LDL size and subclasses modulation in patients with type-2 diabetes. Exp Clin Endocrinol Diabetes 2007;115(8):477-82.
4. Parhofer KG. The treatment of disorders of lipid metabolism. Dtsch Arztebl Int 2016;113(15):261-8.
5. Hua J, Malinski T. Variable effects of LDL subclasses of cholesterol on endothelial nitric oxide/peroxynitrite balance - The risks and clinical implications for cardiovascular disease. Int J Nanomedicine 2019;14:8973-87.
6. Pokharel Y, Tang Y, Bhardwaj B, Patel KK, Qintar M, O'Keefe JH et al. Association of low-density lipoprotein pattern with mortality after myocardial infarction: Insights from the TRIUMPH study. J Clin Lipidol 2017;11(6):1458-1470.e4.
7. Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on

atherogenic dyslipidemia. Am J Clin Nutr 2006; 83:1025-31.
8. Azadbakht L, Mirmiran P, Hedayati M, Esmaillzadeh A, Shiva N, Azizi F. Particle size of LDL is affected by the National Cholesterol Education Program (NCEP) step II diet in dyslipidaemic adolescents. Br J Nutr 2007;98(1):134-9.
9. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC et al. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007;192(1):211-7.
10. Klempel MC, Kroeger CM, Bhutani S, Trepanowski JF, Varady KA. Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. Nutr J 2012;11:98.
11. Siirtola A, Antikainen M, Ala-Houhala M, Koivisto AM, Solakivi T, Virtanen SM et al. Insulin resistance, LDL particle size, and LDL susceptibility to oxidation in pediatric kidney and liver recipients. Kidney Int 2005;67(5):2046-55.
12. Sirtori CR, Lovati MR, Manzoni C, Monetti M, Pazzucconi F, Gatti E. Soy and cholesterol reduction: clinical experience. J Nutr 1995;125(3 Suppl):598S-605S.
13. Danesio de Souza J, Queiroz Ribeiro A, Oliveira Martinho K, Franco FS, Martins MV, Rodrigues MG et al. Lipid profile and associated factors among elderly people, attended at the family health strategy, VICOSA/MG. Nutr Hosp 2015;32(2):771-8.
14. Mosca L, Barrett-Connor E, Wenger NK. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. Circulation 2011;124(19):2145-54.
15. Russo G, Pintaudi B, Giorda C, Lucisano G, Antonio N, Cristofaro MR et al. Age- and gender-related differences in LDL-cholesterol management in outpatients with Type 2 Diabetes Mellitus. Int J Endocrinol 2015;2015:957105.
16. Tsukamoto H, Takei I, Ishii K, Watanabe K. Simplified method for the diameter sizing of serum low-density lipoprotein using polyacrylamide gradient gel electrophoresis. Clin Chem Lab Med 2004;42(9):1009-12.
17. Kondo A, Muranaka Y, Ohta I, Kanno T. Dynamic reaction in a homogeneous HDL-cholesterol assay visualized by electron microscopy. Clin Chem 1999;45:1974-80.
18. Kojima M, Kanno H, Yamazaki Y, Koyama S, Kanazawa S, Arisaka O. Association of low-density lipoprotein particle size distribution and cardiovascular risk factors in children. Acta Paediatr 2005;94(3):281-6.
19. Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. Atherosclerosis 1996;123:43-56.
20. Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. J Lipid Res 1998; 39:1263–73.
21. Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001;286(12):1468-74.
22. Sacks FM, Campos H. Clinical review 163: cardiovascular endocrinology: low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab 2003;88:4525–32.
23. Postmus I, Deelen J, Sedaghat S, Trompet S,Craen AJM, Heijmans BT et al. LDL cholesterol still a problem in old age? A Mendelian randomization study. Int J Epidemiol. 2015;44(2):604-12.
24. Ferré RJ, Morelló-Castro, G. Validation of the otoacoustic emissions in presbyacusis. Acta Otorrinolaringol Esp 2003;54(3):177-82.
25. Mykkänen L, Kuusisto J, Haffner SM, Laakso M, Austin MA. LDL size and risk of coronary heart disease in elderly men and women. Arterioscler Thromb Vasc Biol 1999;19(11):2742-8.
26. Greene CM, Waters D, Clark RM, Contois JH, Fernandez ML. Plasma LDL and HDL characteristics and carotenoid content are positively influenced by egg consumption in an elderly population. Nutr Metab (Lond). 2006;3:6.
27. Schaffer AE, D'Alessio DA, Guyton JR. Extreme elevations of low-density lipoprotein cholesterol with very low carbohydrate, high fat diets. J Clin Lipidol 2021;15(3):525-6.
28. Lemieux I, Pascot A, Tchernof A, Bergeron J, Prud'Homme D, Bouchard C et al. Visceral adipose tissue and low-density lipoprotein particle size in middle-aged versus young men. Metabolism 1999;48(10):1322-7.

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