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HDL3-C but not HDL2-C as a protective factor for cardiovascular diseases a nested case-control study in an Iranian population

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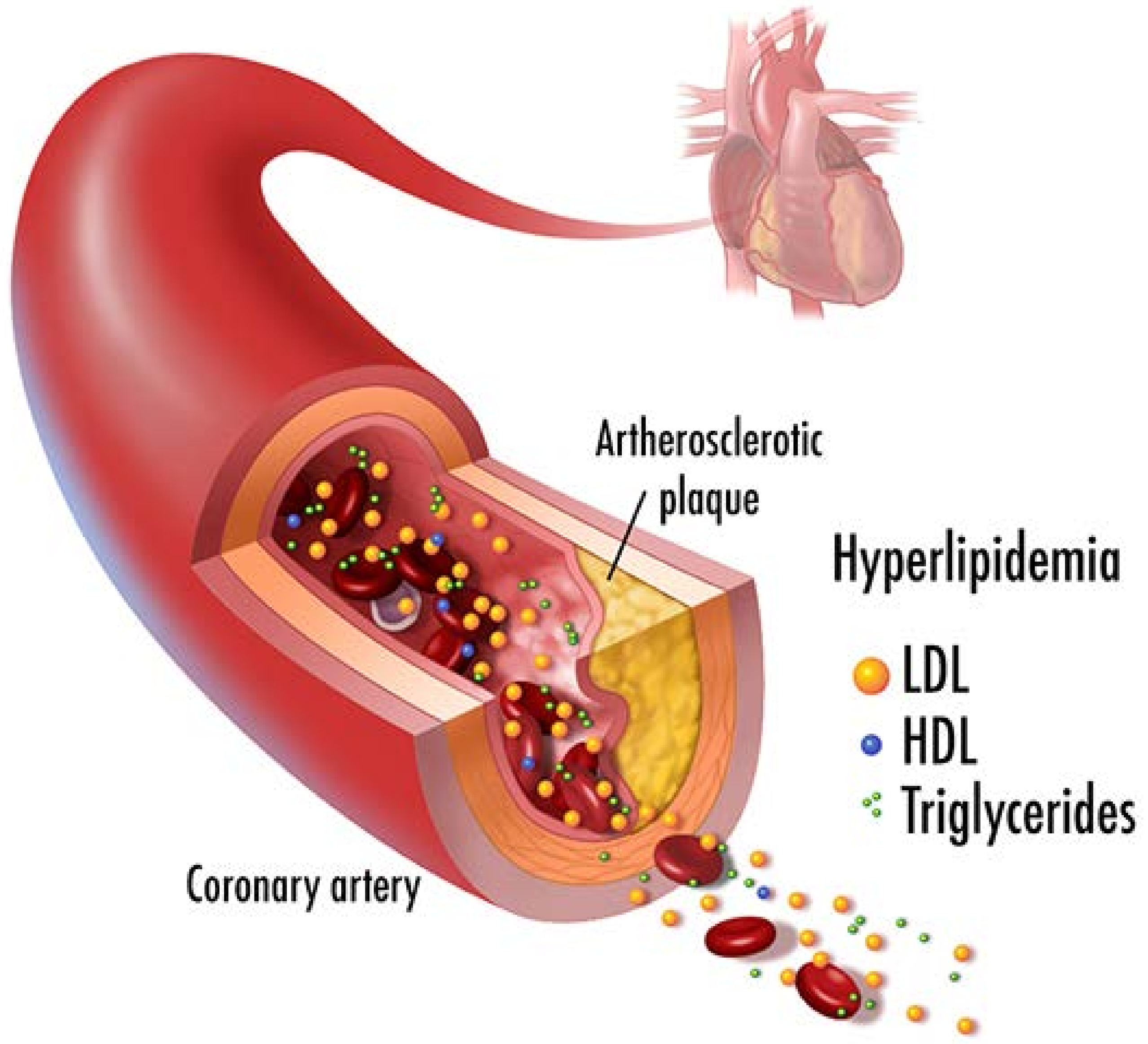
- Cardiovascular disease (CVD)
  - one of the most common public health concerns
  - responsible for approximately one-third of all mortality worldwide

- According to the report on the global burden of diseases (GBD) 2015
  - Iran is cited among the countries with the highest CVD rate

- *Roth GA, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol. 2017;70(1):1-25.*

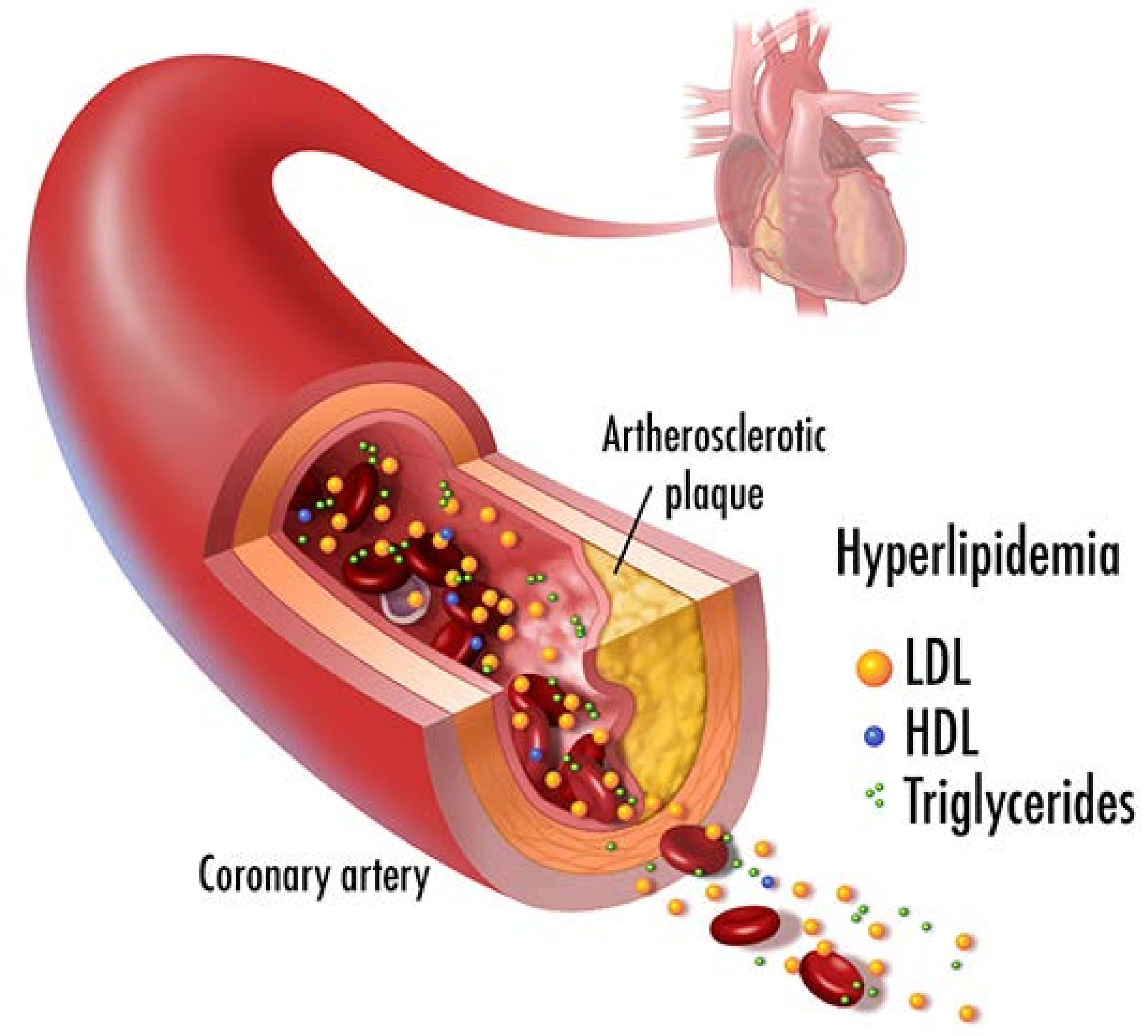


- Dyslipidemia
  - one of the most prevalent CVD risk factors in the Eastern Mediterranean Region (EMR).

- Low high-density lipoprotein cholesterol (HDL-C)
  - the most common lipid abnormality in the Iranian population (prevalence: > 73% in women and > 64% in men)

- HDL-C
  - according to clinical and epidemiological studies, HDL-C is inversely related to incident CVD events, particularly coronary heart disease (CHD).

- *Turk-Adawi K, et al. Cardiovascular disease in the Eastern Mediterranean region: epidemiology and risk factor burden. Nat Rev Cardiol. 2018;15(2):106-19.*
- *Aryan Z, et al. The prevalence, awareness, and treatment of lipid abnormalities in Iranian adults: Surveillance of risk factors of noncommunicable diseases in Iran 2016. Journal of clinical lipidology. 2018;12(6):1471-81. e4.*
- *Baghbani-Oskouei A, et al. F. Serum Lipids During 20 Years in the Tehran Lipid and Glucose Study: Prevalence, Trends and Impact on Non-Communicable Diseases. Int J Endocrinol Metab. 2018;16(4 Suppl):e84750.*



- A complex association between HDL-C levels and CVD risk

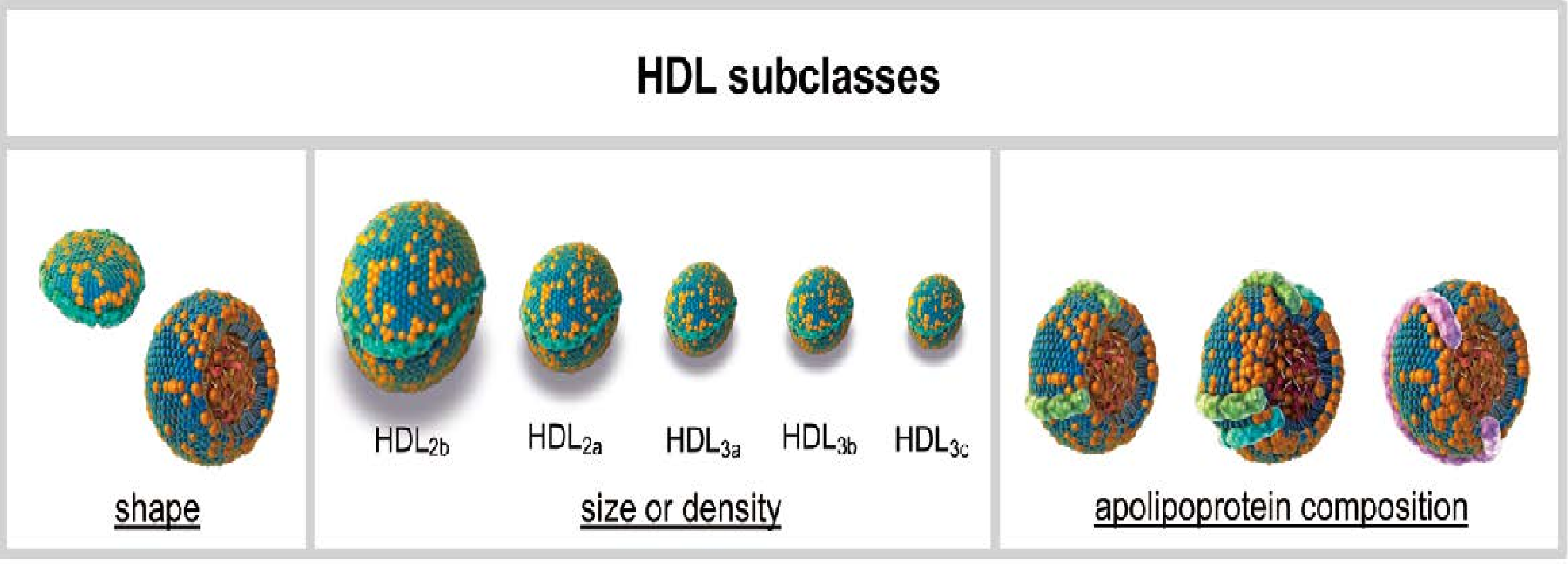
- Recent challenges about the long-established idea that:
  - higher HDL-C is consistently advantageous 
  - low HDL-C is always unfavorable 

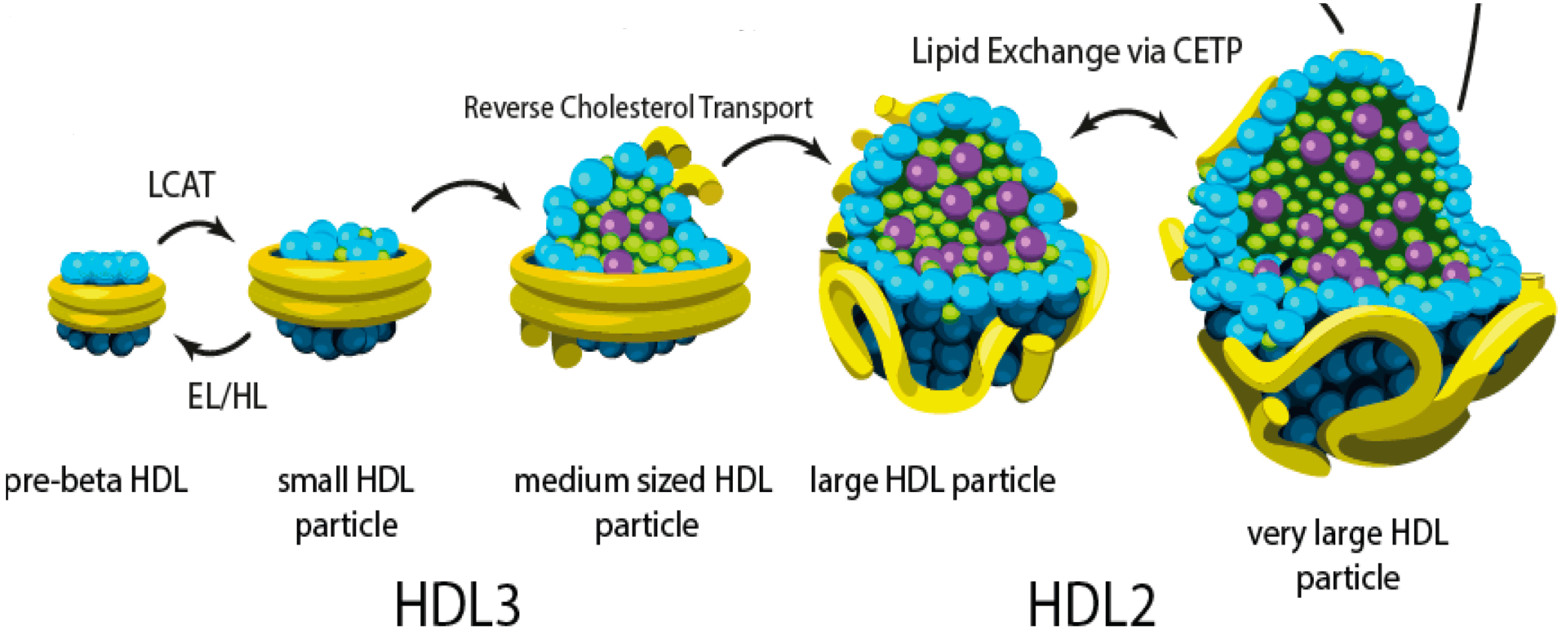
- In the established CHD, HDL-C level, has not consistently predicted the risk of major adverse cardiovascular events.

- *Voight BF,et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. The Lancet. 2012;380(9841):572-80.*
- *Salahuddin T, et al. Association of high-density lipoprotein particle concentration with cardiovascular risk following acute coronary syndrome: A case-cohort analysis of the dal-Outcomes trial. Am Heart J. 2020;221:60-6.*
- *Calabresi L, et al. High-density lipoprotein quantity or quality for cardiovascular prevention? Curr Pharm Des. 2010;16(13):1494-503.*

- Historically, using different techniques and procedures and considering HDL's physicochemical and functional properties have led to varying terms for defining HDL species.

- The two main subclasses are HDL2 and HDL3 based on their lipid-to-protein ratio, which correspondent to large and small HDL particles.





- The results of different studies regarding the associations between HDL-C subclasses, mostly HDL2- C and HDL3-C, with incident CVD were inconsistent.

- No study has been conducted on the associations between HDL-C subclasses and incident CVD in an Iranian population

- *Rosenson RS, et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clinical chemistry. 2011;57(3):392-410.*
- *Yu S, et al. High density lipoprotein subfractions and the risk of coronary heart disease: 9-years follow-up in the Caerphilly Study. Atherosclerosis. 2003;166(2):331-8.*
- *Martin SS, et al. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. Eur Heart J. 2015;36(1):22-30.*
- *Albers JJ, et al. Relationship of baseline HDL subclasses, small dense LDL and LDL triglyceride to cardiovascular events in the AIM-HIGH clinical trial. Atherosclerosis. 2016;251:454-9.*

Aim of the study

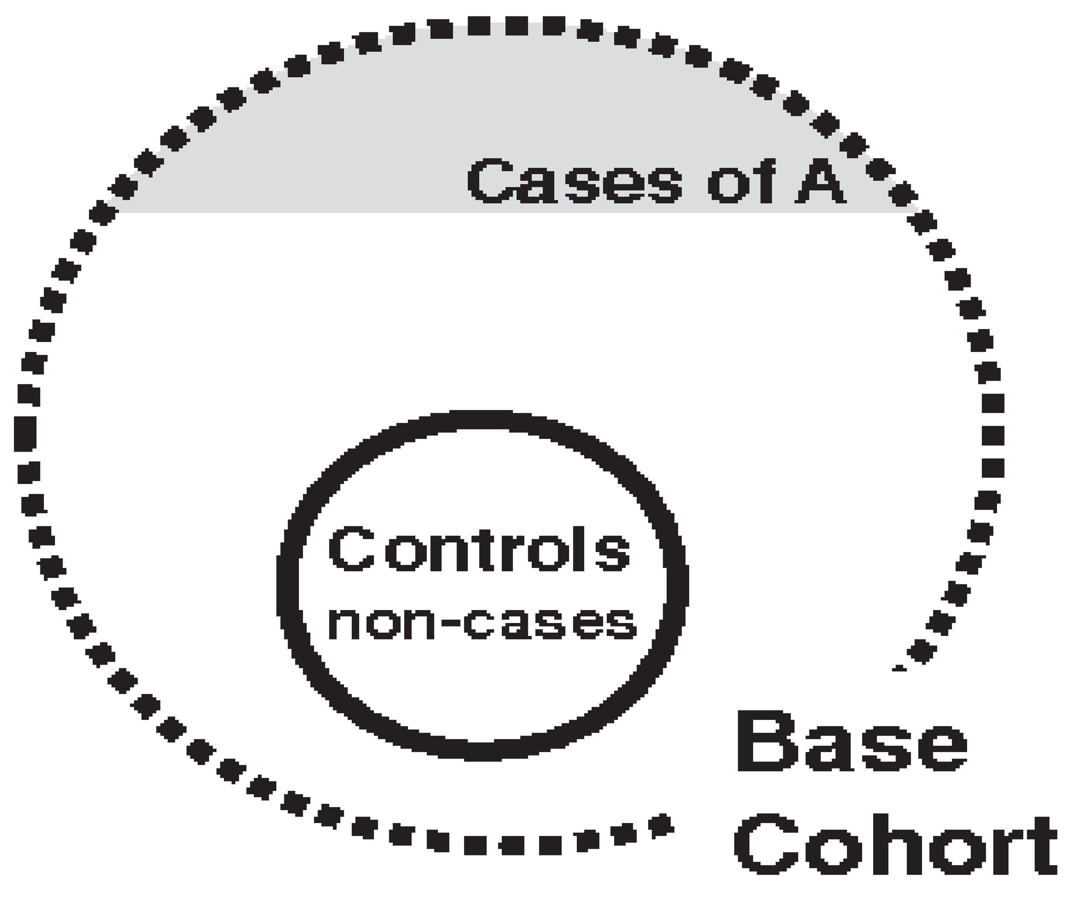
- To examine the associations of HDL2-C and HDL3-C with:
  - CVD
  - CHD

- within the framework of the Tehran Lipid and Glucose Study (TLGS)

  - the oldest cohort of the Middle East and North Africa (MENA) region

Methods

- Nested case-control design with a median follow-up of 11.6 years
- Study population
  - 740 randomly selected individuals aged ≥ 30 years [mean age 57.1 (11.3) years]
    - without CVD at the beginning of the study
    - 370 patients with incident CVD during follow-up
    - 370 age and sex-matched control subjects



- Laboratory measurements:
  - HCL-C measurement: direct enzymatic colorimetric assay
  - isolation of HDL-C subfractions: single-step precipitation method
    - using heparin/ manganese chloride (MnCl2)/ dextran sulfate (DS) reagent
  - cholesterol measurement: enzymatic colorimetric assay
- Statistical analysis:
  - multivariable conditional logistic regression in 3 models, with two approach to lipid parameters:
    - continuous
    - categorical

Table 1 Baseline characteristics of the study population: Tehran Lipid and Glucose Study

	Without CVD (Controls) N=370	With CVD (Cases) N=370	P value
Continuous Variables, Mean (SD)			
Age (year)	57.1 (11.3)	57.1 (11.3)	0.997
Systolic blood pressure (mmHg)	122.6 (17.9)	129.0 (20.5)	< 0.001
Diastolic blood pressure (mmHg)	76.3 (10.5)	79.1 (11.0)	< 0.001
Fasting plasma glucose (mmol/L)	5.76 (1.96)	6.26 (2.56)	0.003
2-hour post-challenge plasma glucose (mmol/L)	6.94 (3.39)	6.94 (3.14)	0.974
Body mass index (kg/mm^2)	28.6 (4.6)	28.7 (4.6)	0.651
Total cholesterol (mmol/L)	5.22 (1.01)	5.39 (1.07)	0.025
Ttiglycerides (mmol/L)	1.97 (1.27)	2.26 (1.60)	0.007
Non-HDL-C (mmol/L)	4.11 (0.93)	4.31 (0.99)	0.004
HDL-C (mmol/L)	1.11 (0.18)	1.08 (0.16)	0.027
HDL2-C (mmol/L)	0.34 (0.10)	0.35 (0.10)	0.059
HDL3-C (mmol/L)	0.77 (0.18)	0.73 (0.16)	0.001
HDL2-C/HDL3-C ratio	0.47 (0.20)	0.51 (0.21)	0.006
Categorical Variables, N (%)			
Gender			1.000
Male	202 (54.6)	202 (54.6)	
Female	168 (45.4)	168 (45.4)	
Family history of premature CVD, yes	89 (24.1)	114 (30.8)	0.039
Smoking			0.133
Never	294 (79.5)	271 (73.2)	
Past	33 (8.9)	45 (12.2)	
Current	43 (11.6)	54 (14.6)	
Hypertension, yes	100 (27.0)	140 (37.8)	0.002
Type 2 diabetes mellitus, yes	51 (13.8)	84 (22.7)	0.002
Low physical activity	226 (61.1)	233 (63.0)	0.596
Lipid lowering drugs, yes	29 (7.8)	31 (8.4)	0.788
Anti-diabetic medication	31 (8.4)	54 (14.6)	0.008
Anti- hypertension drugs	30 (8.1)	44 (11.9)	0.086

Values are presented as mean (standard deviation) for continuous variables and frequencies (%) for categorical variables.

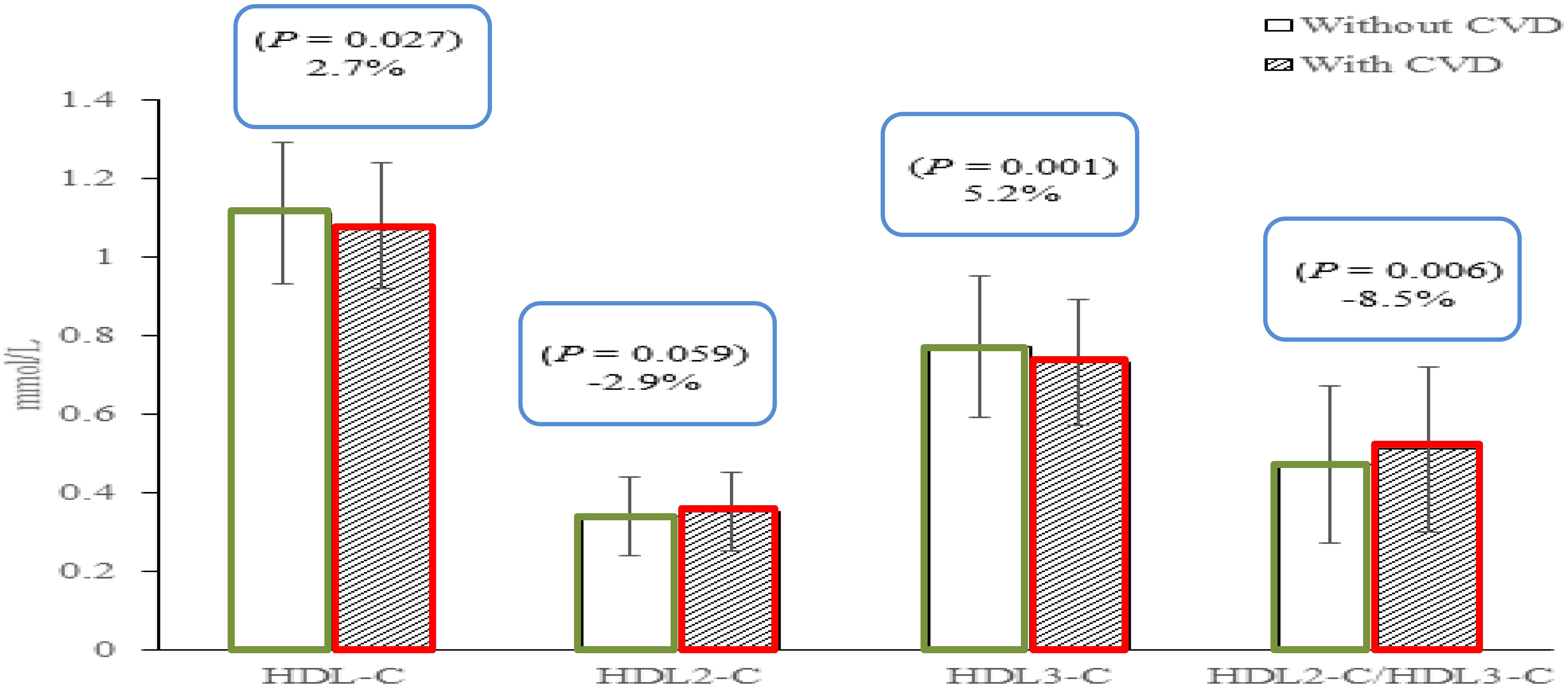
Participants with incident CVD:

↑ SBP, DBP, FPG
↑ family history of premature CVD
↑ Hypertension
↑ T2DM
↑ use anti-diabetic medication.
↑ TC
↑ TG
↑ non-HDL-C
↑ HDL2-C/HDL3-C

↓ HDL-C
↓ HDL3-C

HDL3-C accounted for 69.4% and 67.6% of HDL-C in the control and case groups, respectively.

Comparison of mean (standard deviation) of HDL-C, HDL2-C, HDL3-C, and HDL2-C/HDL3-C at baseline between CVD (−) and (+) subjects



Percent values are the relative differences.
HDL-C, high-density lipoprotein cholesterol

HDL3-C accounted for:

- 69.4% of HDL-C in the control group
- 67.6% of HDL-C in the case group

Table 2 ORs (95% CIs) from the multivariable analysis of CVD and CHD across HDL-C subclasses

	Crude model		Model 1		Model 2		Model 3	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
CVD								
HDL-C	0.97 (0.94-0.99)	0.016	0.97 (0.95-1.00)	0.023	0.95 (0.92-0.98)	< 0.001	**0.95 (0.92-0.98)**	**< 0.001**
HDL2-C	1.03 (0.99-1.07)	0.066	1.03 (0.99-1.07)				0.98 (0.94-1.04)	0.555
HDL3-C	0.95 (0.93-0.97)	< 0.001	0.96 (0.93-0.98)	0.001	0.95 (0.93-0.98)	0.001	**0.95 (0.93-0.98)**	**0.001**
HDL2-C/HDL3-C	3.14 (1.44-6.85)	0.004	2.70 (1.21-6.05)	0.015	1.79 (0.76-4.19)	0.182	1.67 (0.68-4.10)	0.266
CHD								
HDL-C	0.96 (0.93-0.99)	0.010	0.96 (0.94-0.99)	0.011	0.94 (0.91-0.97)	< 0.001	**0.94 (0.91-0.97)**	**< 0.001**
HDL2-C	1.04 (1.00-1.09)	0.045	1.05 (1.00-1.10)				0.99 (0.93-1.05)	0.780
HDL3-C	0.94 (0.91-0.97)	< 0.001	0.94 (0.91-0.97)	< 0.001	0.94 (0.91-0.97)	< 0.001	**0.94 (0.91-0.97)**	**< 0.001**
HDL2-C/HDL3-C	4.27 (1.74-10.47)	0.002	4.09 (1.61-10.37)	0.003	2.62 (0.97-7.09)	0.058	2.44 (0.86-6.94)	0.095

5 % ↓ risk of CVD

6 % ↓ risk of CHD

ORs (95% CI) for per 1 unit increase in each lipid measure

Model 1: adjusted for body mass index, current smoking, hypertension, type 2 diabetes mellitus, lipid lowering drugs, family history of cardiovascular diseases and low physical activity

Model 2: model 1 + non-HDL-C

Model 3: model 2 + triglycerides

OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; CHD, coronary heart disease; HDL-C, high density lipoprotein cholesterol

- **CVD**
  - ✓ in all models
  - ✓ HDL-C and HDL3-C were associated with lower risk

- **CHD**
  - ✓ in all models
  - ✓ HDL-C and HDL3-C were associated with lower risk

- HDL2-C & HDL2-C/HDL3-C
  - ✓ Further adjustment for non-HDL-C, attenuated the associations of these parameters with incident CVD or CHD.

Table 3 Association of HDL-C subclasses with the incidence of CVD: Tehran Lipid and Glucose Study

	Quartile 2		Quartile 3		Quartile 4		P for trend
HDL-C (mmol/L)	**0.98 ≤ HDL-C < 1.08**		**1.08 ≤ HDL-C < 1.20**		**1.20 ≤ HDL-C**		
Model 2	0.58 (0.36-0.93)	0.023	0.52 (0.32-0.84)	0.008	**0.43 (0.25-0.72)**	**0.002**	**0.002**
Model 3	0.58 (0.36-0.94)	0.027	0.52 (0.32-0.86)	0.010	**0.43 (0.25-0.74)**	**0.002**	**0.003**

	Quartile 4	
HDL3-C (mmol/L)	**0.85 ≤ HDL3-C**	
Crude	0.47 (0.29-0.75)	0.002
Model 1	0.50 (0.31-0.81)	0.005
Model 2	0.47 (0.29-0.77)	0.003
Model 3	**0.46 (0.27-0.78)**	**0.004**

Q4 of HDL-C (≥ 46.4 mg/dL)
57 % ↓ risk of CVD

Q4 of HDL3-C
54 % ↓ risk of CVD

	Quartile 2		Quartile 3		Quartile 4		P for trend
HDL2-C/HDL3-C	**0.34 ≤ HDL2-C/HDL3-C < 0.46**		**0.46 ≤ HDL2-C/HDL3-C < 0.60**		**0.60 ≤ HDL2-C/HDL3-C**		
Crude	1.35 (0.74-2.49)	0.320	1.65 (0.88-3.09)	0.111	2.17 (1.17-4.01)	**0.013**	**0.003**
Model 1	1.20 (0.77-1.86)	0.420	1.33 (0.85-2.09)	0.209	1.74 (1.14-2.65)	**0.010**	**0.009**
Model 2	1.12 (0.72-1.75)	0.612	1.15 (0.72-1.83)	0.571	1.40 (0.89-2.21)	0.142	0.149
Model 3	1.12 (0.72-1.75)	0.625	1.13 (0.70-1.81)	0.610	1.36 (0.84-2.20)	0.207	0.225

- **CVD as outcome:**

- **HDL-C**
  - ✓ was associated with incident CVD in both models 2 and model 3 among all the quartiles.
- **HDL2-C**
  - ✓ No significant relationship between the increasing value of HDL2-C and incident CVD even in the crude model
- **HDL3-C**
  - ✓ The highest quartile of HDL3-C was associated with a 54% decreased risk of incident CVD.

HDL-C (mmol/L)	Quartile 2 0.98 ≤ HDL-C < 1.08		Quartile 3 1.08 ≤ HDL-C < 1.19		Quartile 4 1.19 ≤ HDL-C		P for trend
Crude	0.53 (0.32-0.87)	0.12	0.56 (0.34-0.92)	0.023	0.52 (0.31-0.87)	0.014	0.025
Model 1	0.51 (0.30-0.85)	0.011	0.51 (0.30-0.86)	0.011	0.52 (0.30-0.89)	0.016	0.027
Model 2	0.37 (0.21-0.65)	0.001	0.36 (0.20-0.64)	<0.001	0.32 (0.17-0.58)	< 0.001	0.001
Model 3	0.37 (0.21-0.66)	0.001	0.36 (0.20-0.65)	0.001	**0.32 (0.17-0.59)**	**< 0.001**	**0.001**

HDL3-C (mmol/L)	Quartile 2 0.61 ≤ HDL3-C < 0.73		Quartile 3 0.73 ≤ HDL3-C < 0.84		Quartile 4 0.60 ≤ HDL2-C/HDL3-C		P for trend
Model 2	0.57 (0.34-0.94)	0.027	0.53 (0.31-0.90)	0.018	0.34 (0.19-0.60)	**< 0.001**	**< 0.001**
Model 3	0.55 (0.33-0.93)	0.026	0.51 (0.30-0.89)	0.017	**0.32 (0.18-0.59)**	**< 0.001**	**0.001**

HDL2-C/HDL3- C	Quartile 4 0.60 ≤ HDL2-C/HDL3-C	
Crude	2.22 (1.09-4.52)	0.026
Model 1	2.52 (1.44-4.39)	0.001
Model 2	1.98 (1.09-3.60)	0.026
Model 3	**1.92 (1.02-3.61)**	**0.043**

0.60 ≤ HDL2-C/HDL3-C		
2.22 (1.09-4.52)	0.026	0.001
2.52 (1.44-4.39)	0.001	0.002
1.98 (1.09-3.60)	0.026	0.042
1.92 (1.02-3.61)	0.043	**0.072**

).35).
d lowering drugs, family history of cardiovascular diseases
dence interval

- **CHD as outcome:**

Q4 of HDL-C
(≥ 46.0 mg/dL)
68 % ↓ risk of CHD

✓ HDL2-C
✓ No significant relationship between the increasing value of HDL2-C and incident CHD even in the crude model

Q4 of HDL3-C
68 % ↓ risk of CHD

- **HDL2-C/HDL3-C**

✓ associated with a higher risk of

Q4 of HDL2-C/HDL3-C
92 % ↑ risk of CHD

92% higher risk of CHD.

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Some of the potential mechanisms

- ✓ HDL3 has more potent cholesterol efflux capacity compared to other HDL subclasses.
- ✓ HDL3- C has more anti-oxidative, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties.
- ✓ HDL3 is not only quantitatively protein-enriched but equally contains a much higher number of distinct, functional proteins than HDL2.
- ✓ Difference in the content of sphingosine-1-phosphate (S1P) & sphingomyelin (SM).
- ✓ The increased S1P/SM molar ratio in HDL3 is reported to be strongly and positively correlated with the anti-apoptotic and anti-oxidative activities of HDL subclasses.

- *Camont L, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol. 2013;33(12):2715-23.*
- *Du XM, et al. HDL particle size is a critical determinant of ABCA1-mediated macrophage cellular cholesterol export. Circ Res. 2015;116(7):1133-42.*
- *Kontush A, et al. Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. Arterioscler Thromb Vasc Biol. 2007;27(8):1843-9..*

Limitations

I. Measurement of HDL-C subclasses using a non-reference method, however:
   - ✓ this is the case in some similar epidemiological studies
   - ✓ excellent correlation of the single-precipitation method compared with the reference method (ultracentrifugation)

✓ Using a single baseline values HDL-C and its subclasses

- *Stampfer MJ, et al. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med. 1991;325(6):373-81*
- *Sweetnam PM, et al. Associations of the HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. The Caerphilly and Speedwell Collaborative Heart Disease Studies. Circulation. 1994;90(2):769-74..*
- *Lamarche B, et al. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. Arterioscler Thromb Vasc Biol. 1997;17(6):1098-105.*
- *Yu S, et al. High density lipoprotein subfractions and the risk of coronary heart disease: 9-years follow-up in the Caerphilly Study. Atherosclerosis. 2003;166(2):331-8.*
- *Hirano T, et al. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. J Lipid Res. 2008;49(5):1130-6.*

Conclusion

- This study investigated the potential associations between HDL-C and its subclasses with incident CVD and CHD among the Iranian population and showed that:

✓ HDL-C and HDL3-C had significant inverse relationships with incident CVD and CHD in the presence of a large set of confounders, including non-HDL-C and TG.

✓ These associations were more prominent for CHD.

✓ No significant association was found between HDL2-C and CVD/CHD.

✓ We found a signal that the increasing value of HDL2-C/HDL3-C was accompanied by a higher risk of CHD, as the 4th quartile (ratio ≥ 0.60) had more than 90% higher risk for the event.

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Thank you for your attention