Advances, Applications, and Translation in Nutrition and Epidemiology
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Mediterranean diet, olive oil, and HDL functionality in the PREDIMED trial

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Despite regional variations,
the use of these 3 products
is common in all Mediterranean countries.

Wheat

Olive tree

Vineyard

The Mediterranean diet trilogy

The underlying mechanisms by which a healthy diet can exert its beneficial effects on CVD are not fully understood.

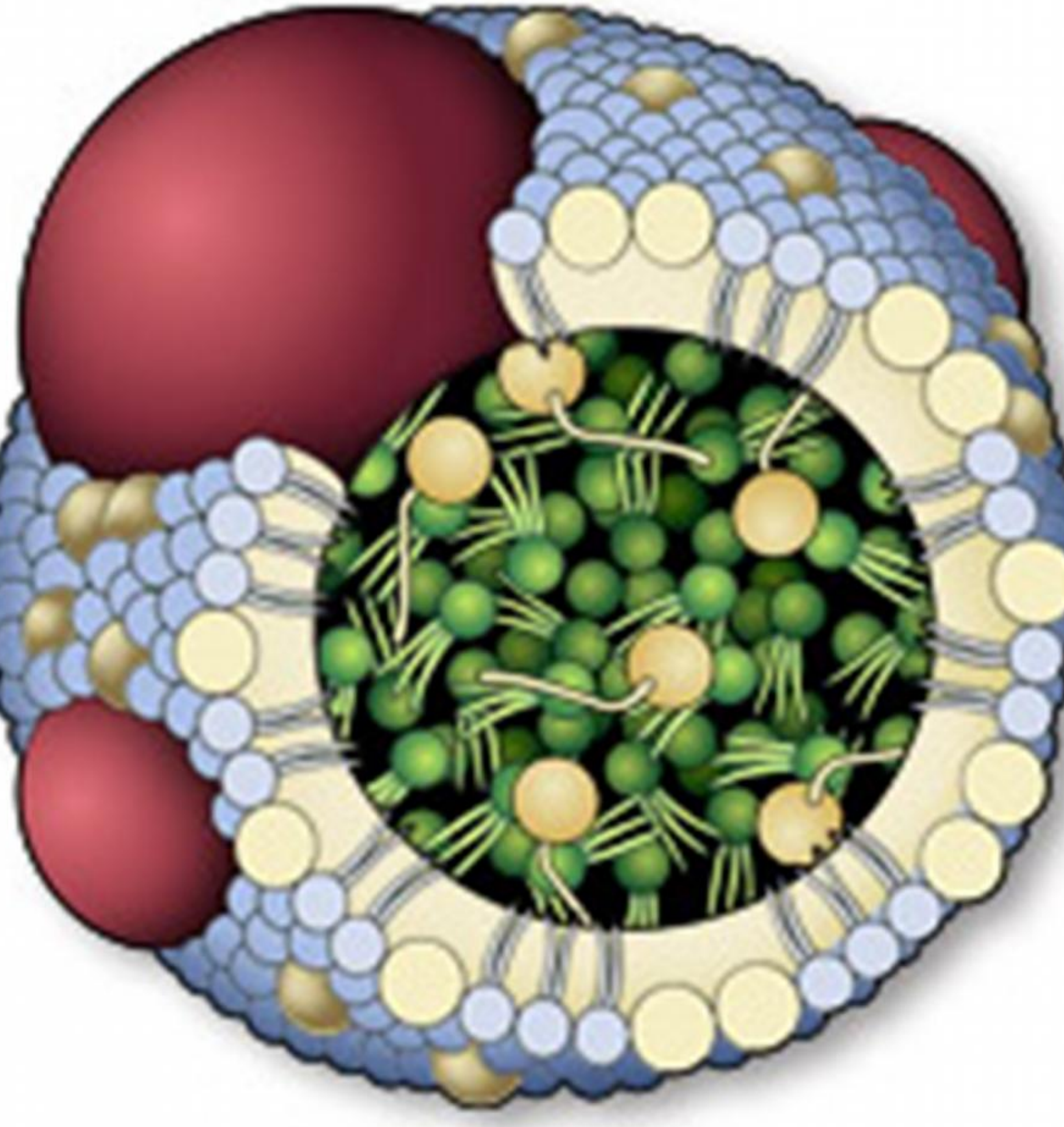
The benefits of the Mediterranean diet have been traditionally attributed to its richness in antioxidants, due to a high vegetal food intake.

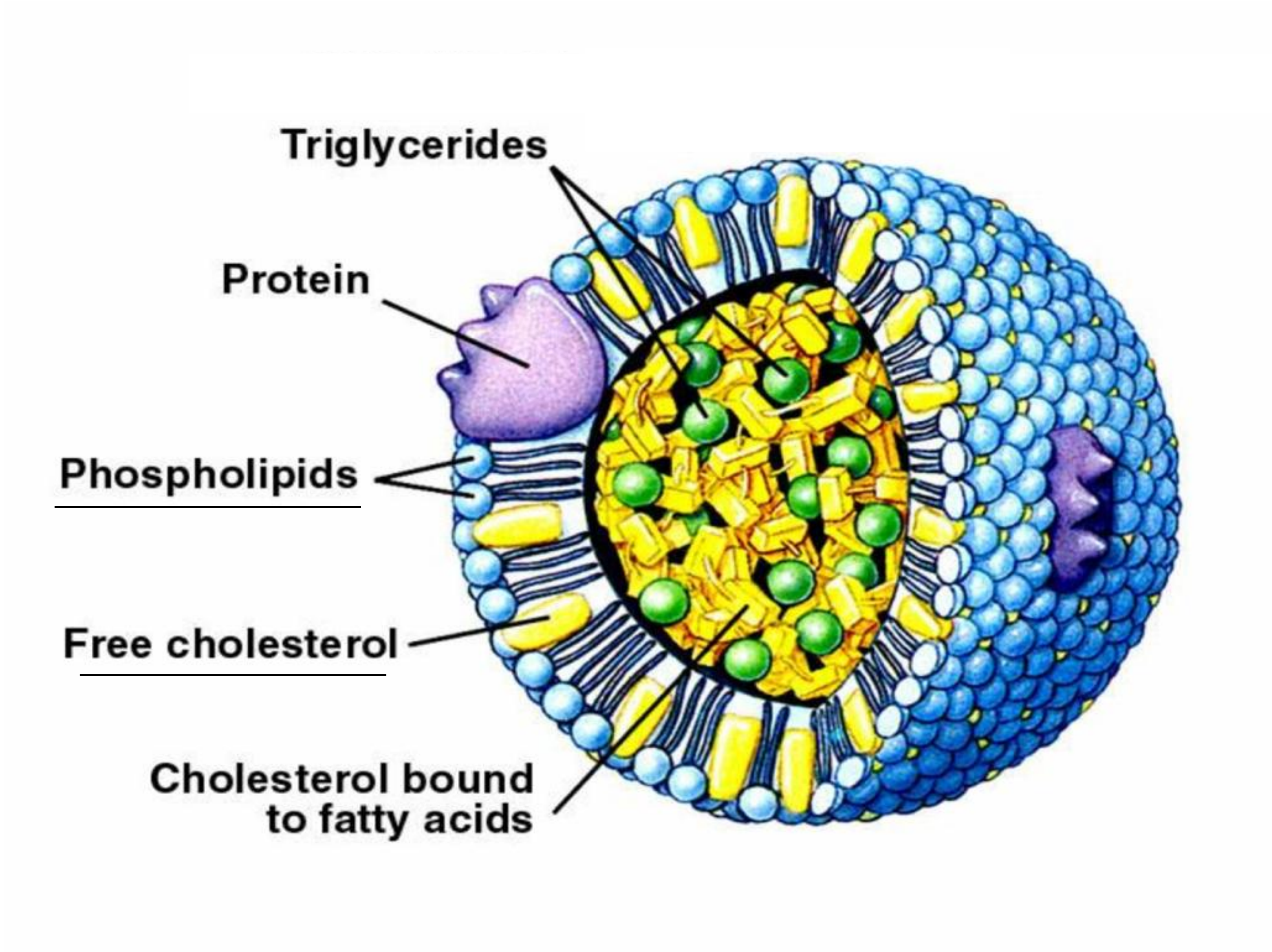
De Sosa et al.
Curr Vasc Pharmacol 2010.

The Mediterranean diet pattern has been shown to improve **lipid profile**.

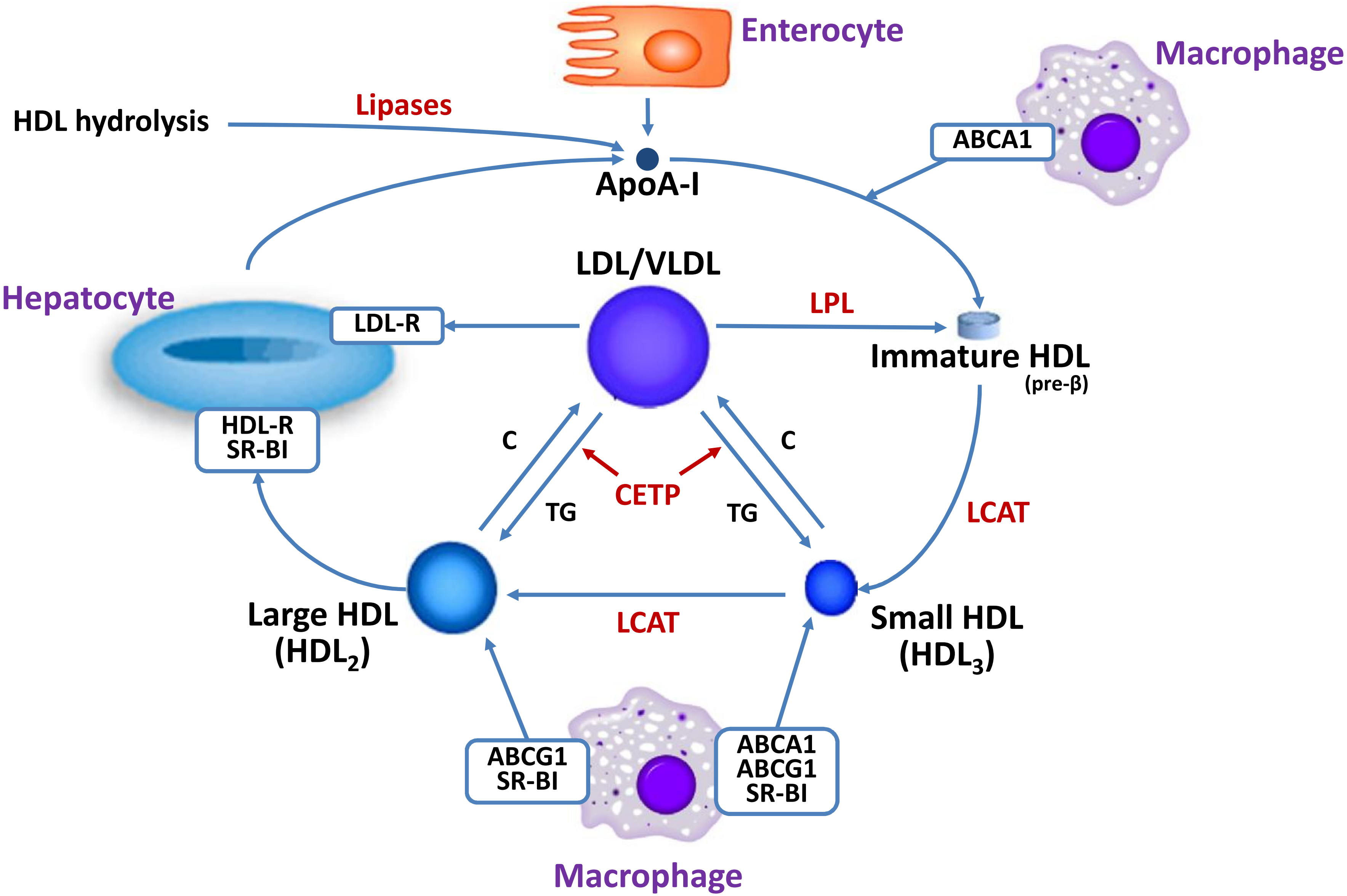
Nevertheless, it is becoming increasingly more accepted that the information provided by **HDL functionality and LDL pro-atherogenic traits** can be more informative with respect to the unexplained cardiovascular risk of an individual.





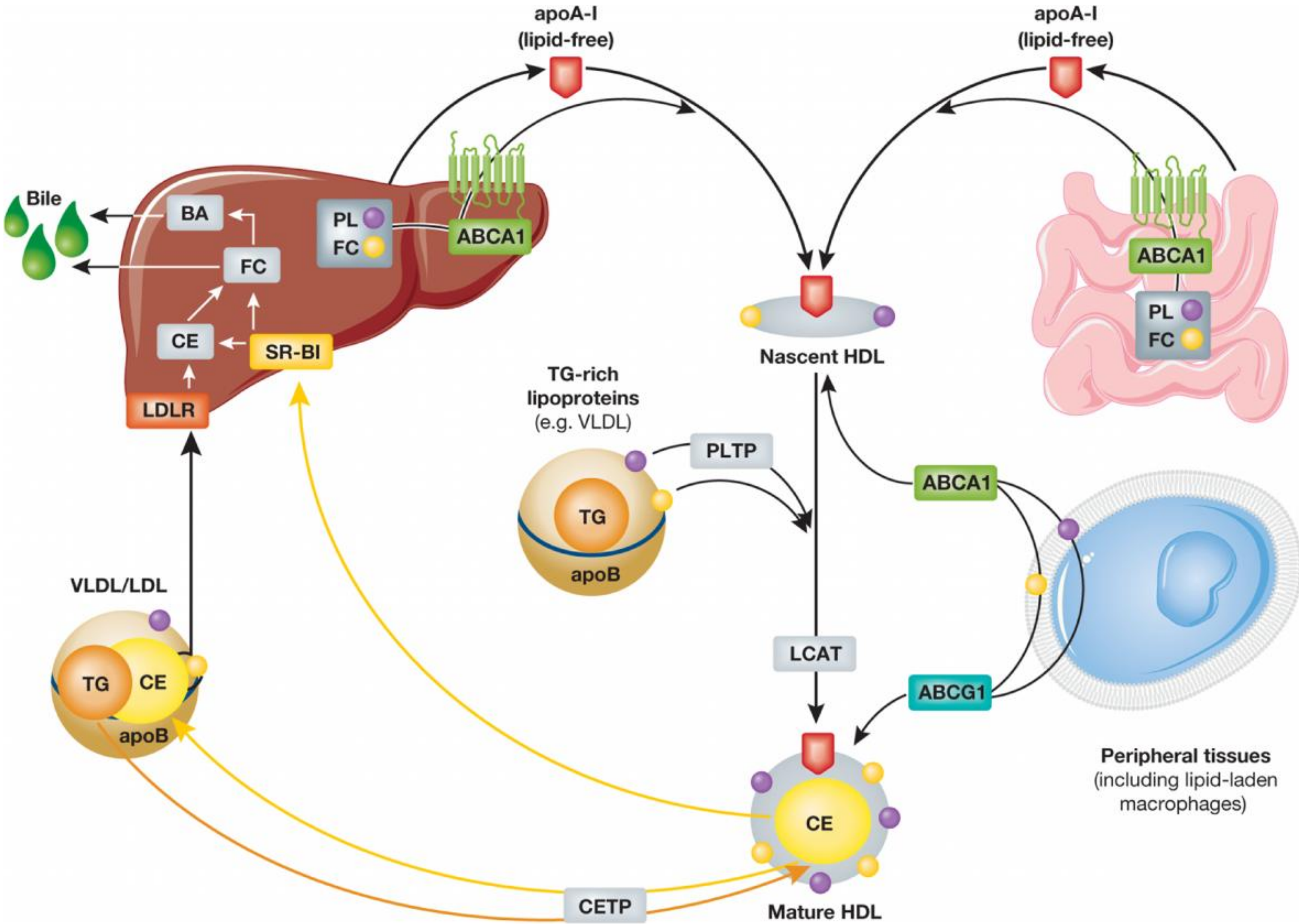
Lipoproteins are a type of micelles in which the polar plasma lipids are located on the hydrophilic surface and the non-polar lipids present in plasma are placed into the hydrophobic core.

HDL biological cycle: **Formation of HDL**



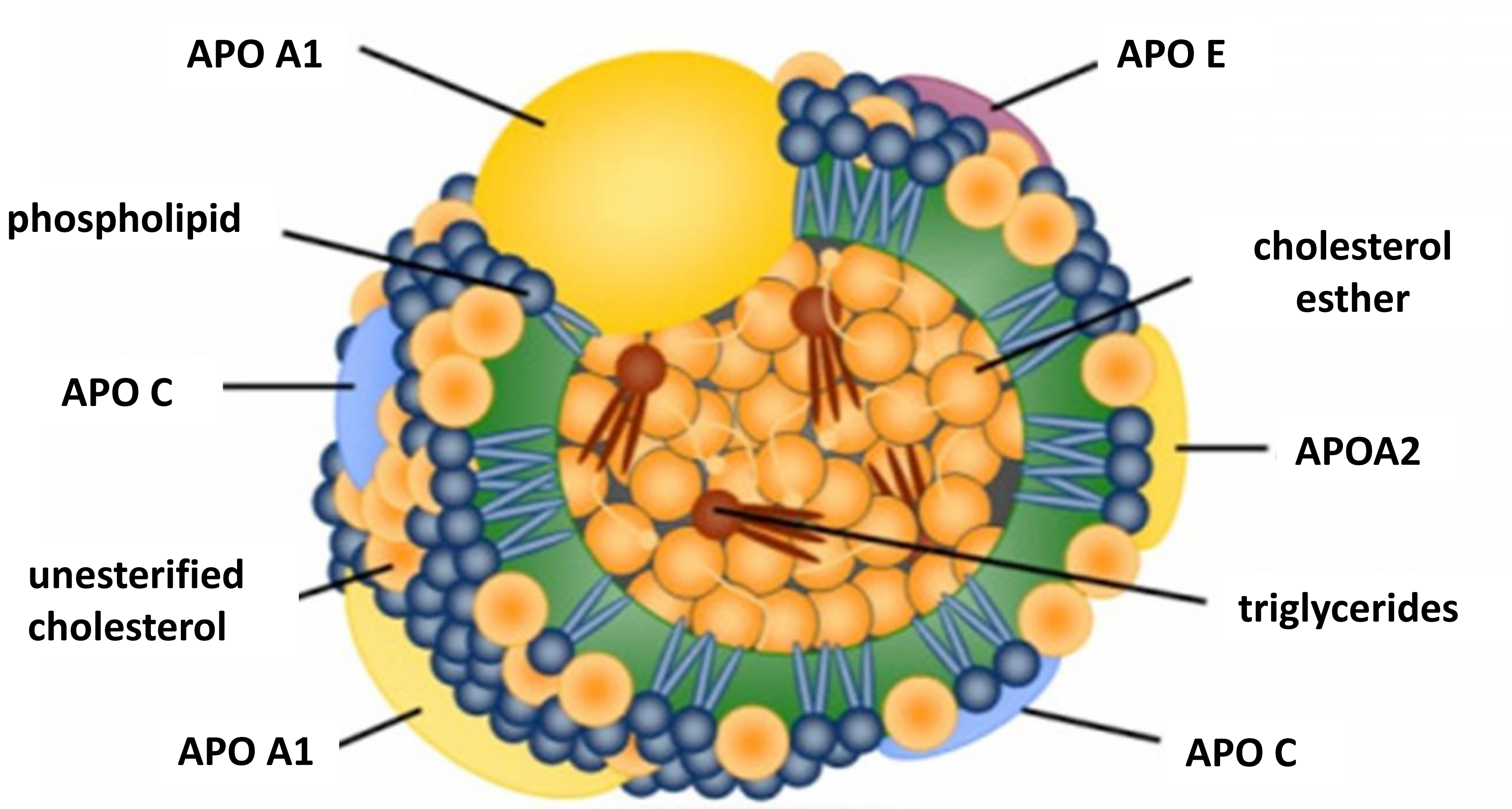
Camont L et al. *Trends Mol Med.* 2011

HDL: biological cycle



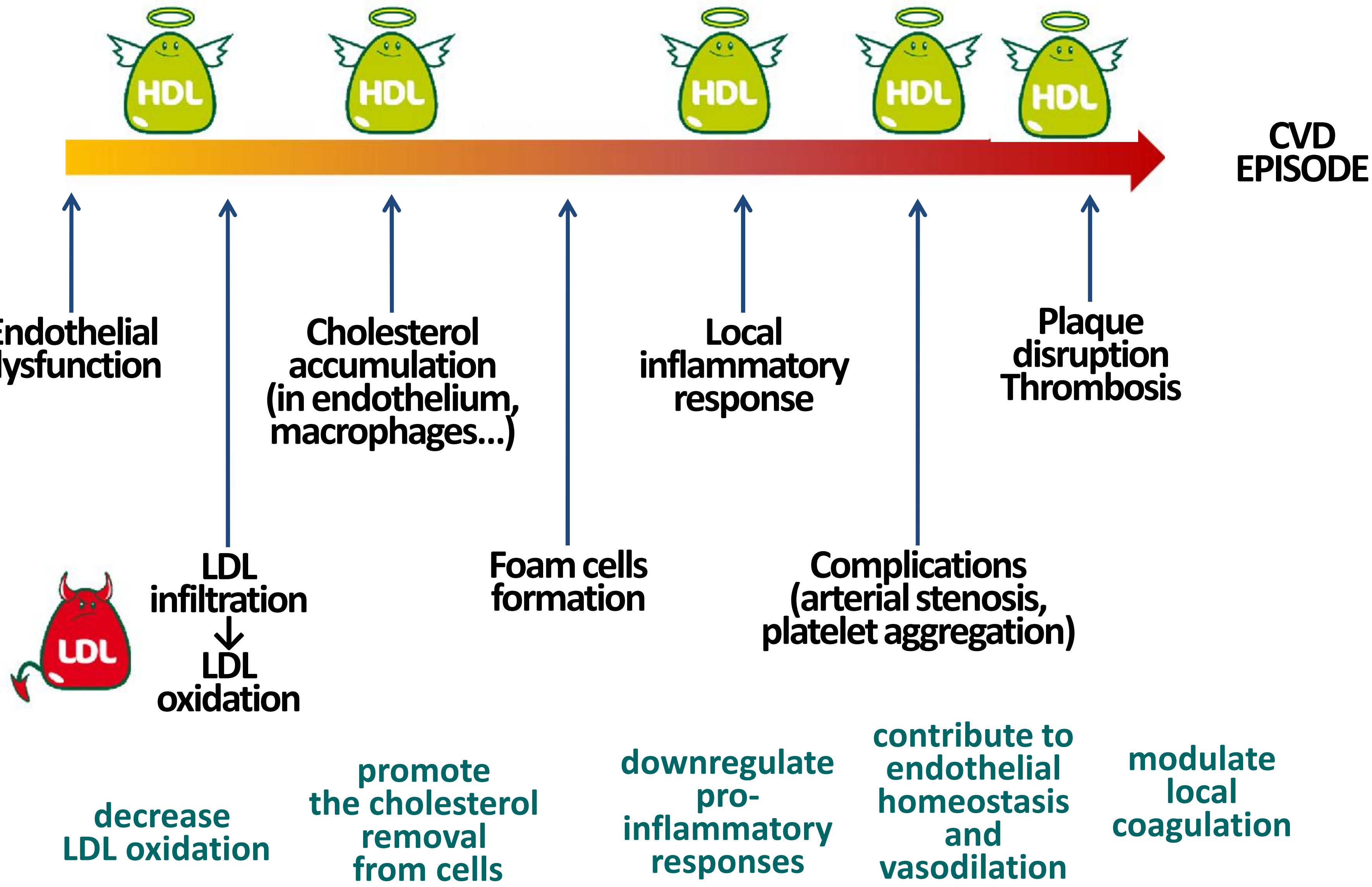
Besler C et al, *EMBO Mol Med*, 2012

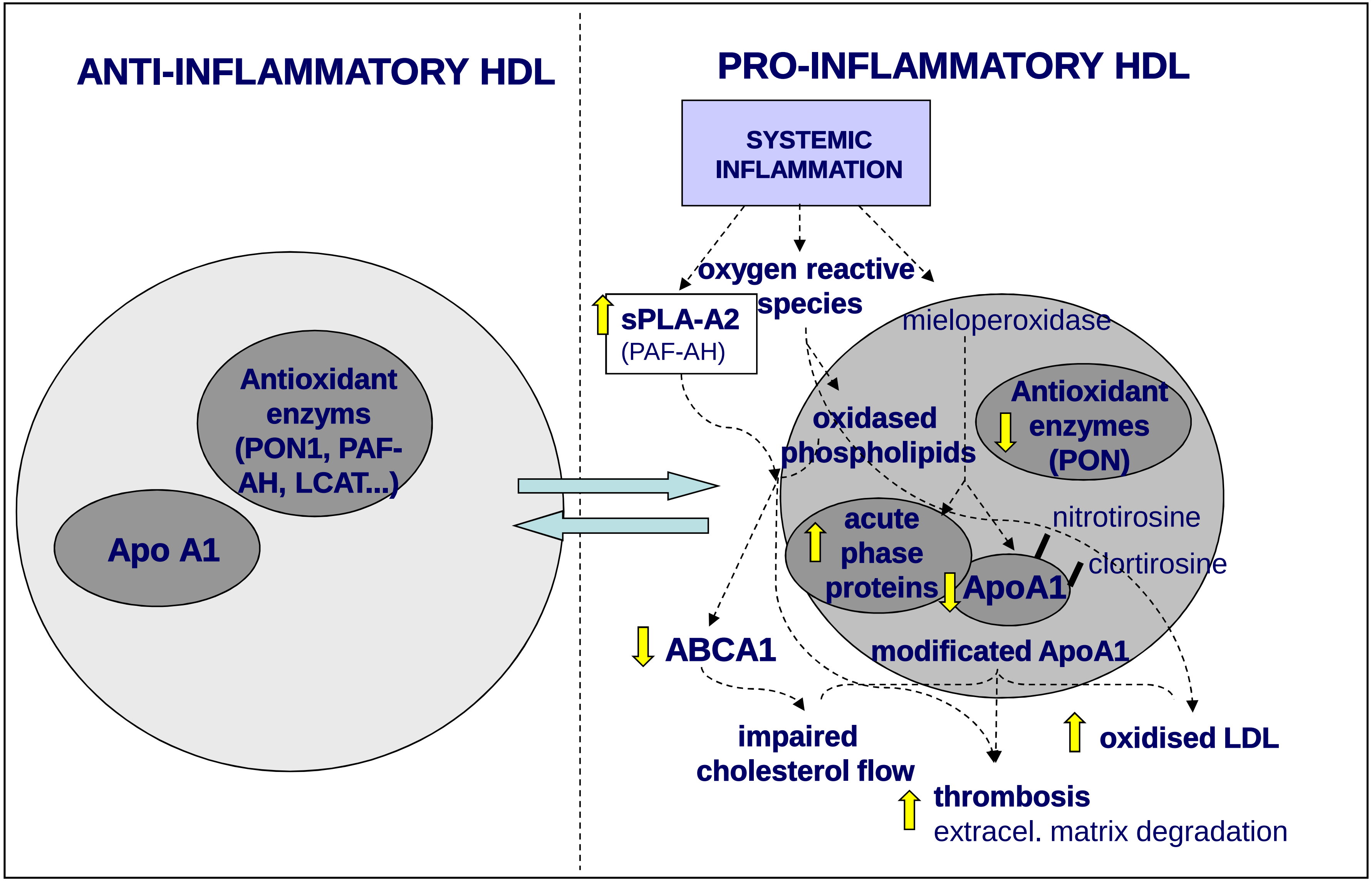
HDL structure



HDL presents the micellar form and, unlike LDL,
has several types of apolipoproteins on its surface.

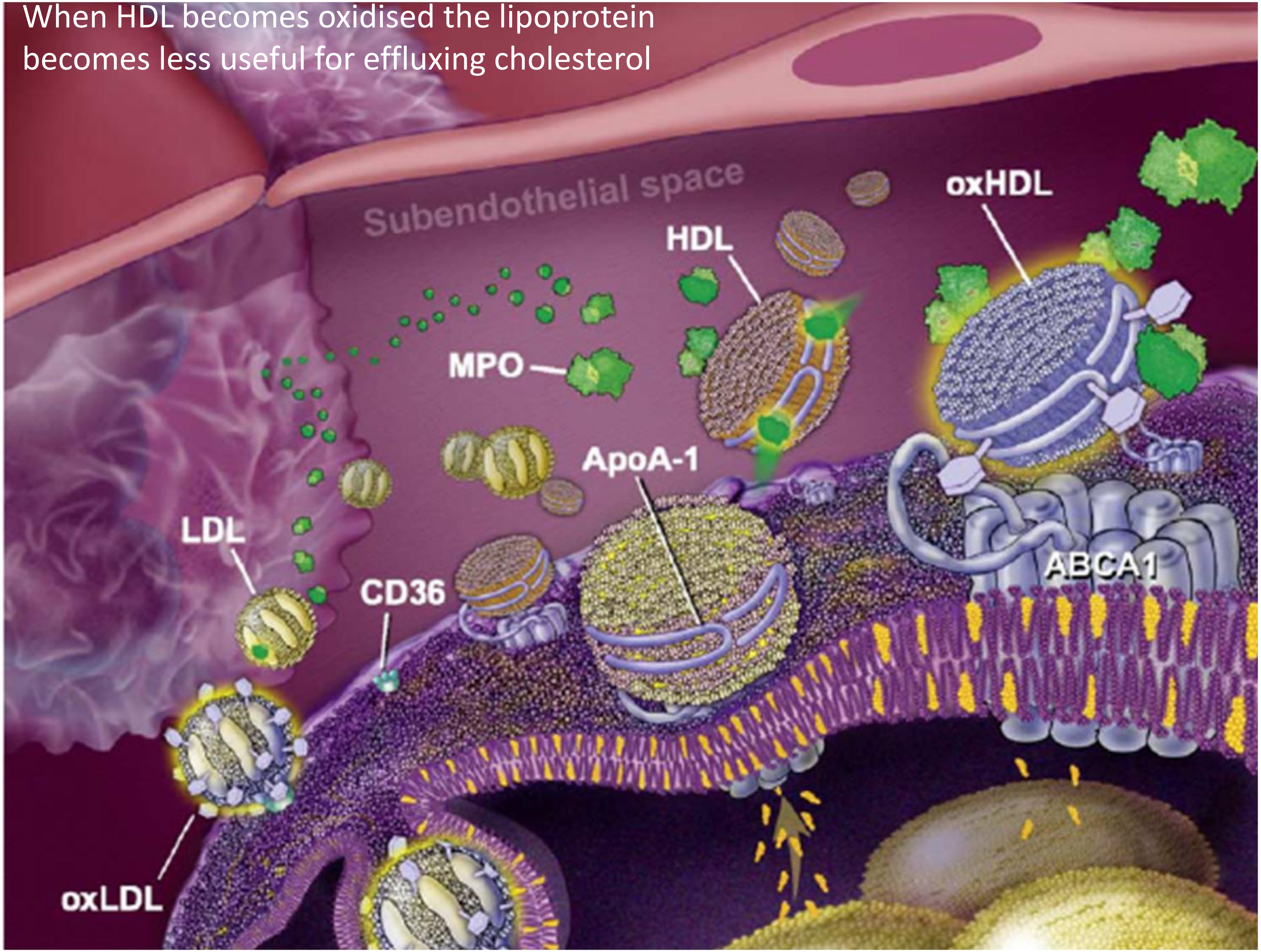
HDL, LDL and **Atherosclerosis onset and development**





Chemical modifications (by oxidation and inflammation) in HDL can affect their physiological properties and reduce their functionality.

When HDL becomes oxidised the lipoprotein becomes less useful for effluxing cholesterol



HDL: epidemiological evidences

Low HDL cholesterol levels are an independent cardiovascular risk factor

- 43% coronary events: HDL-C <40 mg/dL
  Castelli WP et al, *JAMA,* 1986
- Predictive role even in countries with low coronary event incidence (ACS)
  Pintó X et al, *Clin Cardiol,* 2007
- Predictive role for cerebrovascular diseases
  Amarenco P et al, *Atherosclerosis,* 2008

Nevertheless, increasing HDL cholesterol levels do not always decrease cardiovascular risk

- Use of drugs that increase HDL-C levels + statins → No extra protective effects (relative to statins)
  Keene D et al, *BMJ, 2014*
- Individuals with genetic predisposition to have high HDL-C levels → No lower CHD risk
  Voight BF et al, *Lancet,* 2012

New perspective:

HDL FUNCTIONAL PROPERTIES CAN ADD VALUABLE INFORMATION

HDL: functions

Predictive value

Cholesterol efflux capacity

Rohatgi A et al,
N Engl J Med, 2015
(CHD)

Khera AV et al,
N Engl J Med, 2011
(subclinical)

HDL role in other steps of reverse cholesterol transport

Cholesterol ester transfer protein (CETP)

Lecithin cholesterol acyltransferase (LCAT)

HDL antioxidant capacity

Antioxidant enzymes (paraoxonase-1, arylesterase)

Direct antioxidant capacity

Tang WH et al,
Circ Heart Fail 2011
(HF)

HDL endothelial properties

Other HDL functions

Mediterranean Diet and HDL functionality: state of the art

Mediterranean Diet and HDL functionality

- Mediterranean Diet → Better HDL-related lipid profile

  Estruch R et al, Ann Intern Med, 2006

  Solà R et al, *Atherosclerosis,* 2011

- Antioxidant-rich dietary interventions → ↑ HDL functions

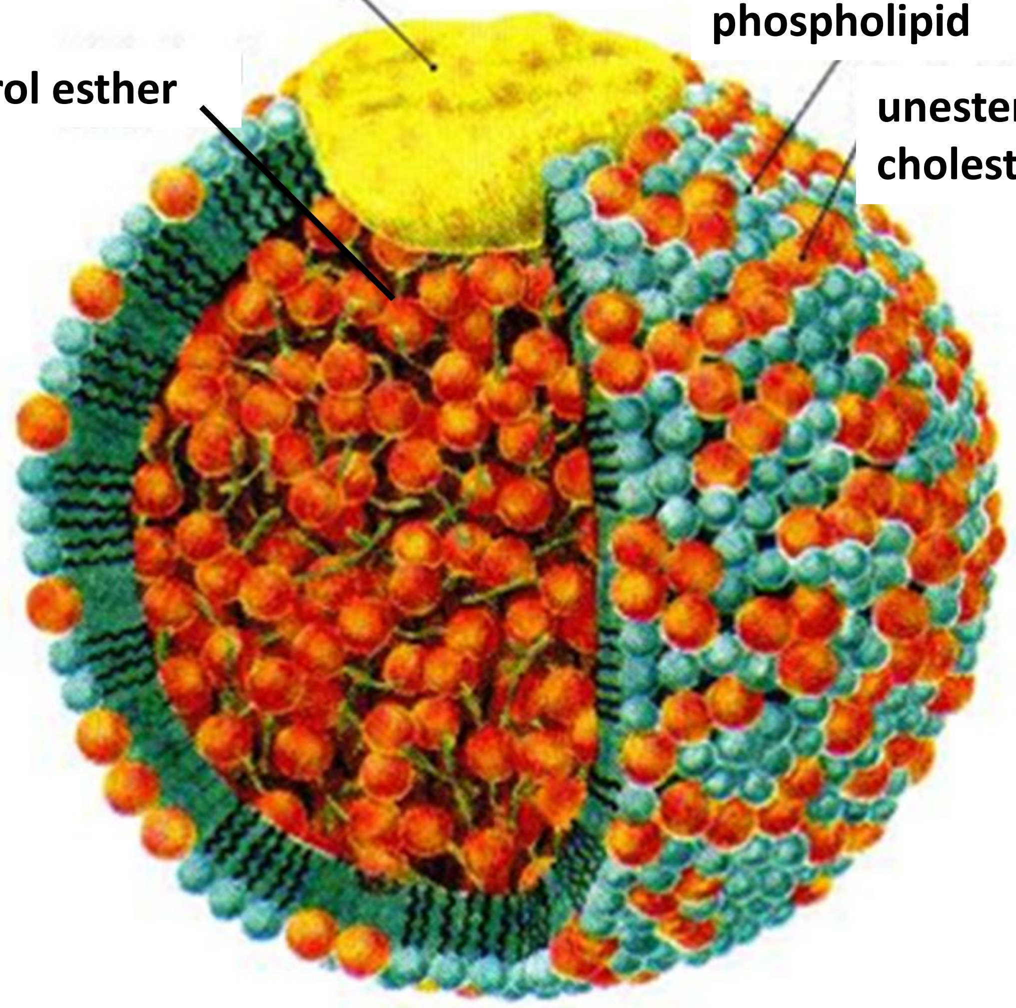
  Qin Y et al, *Am J Clin Nutr,* 2009

  Zhu Y et al, *J Clin Endocrinol Metab,* 2014

  McEneny J et al, *J Nutr Biochem*, 2013

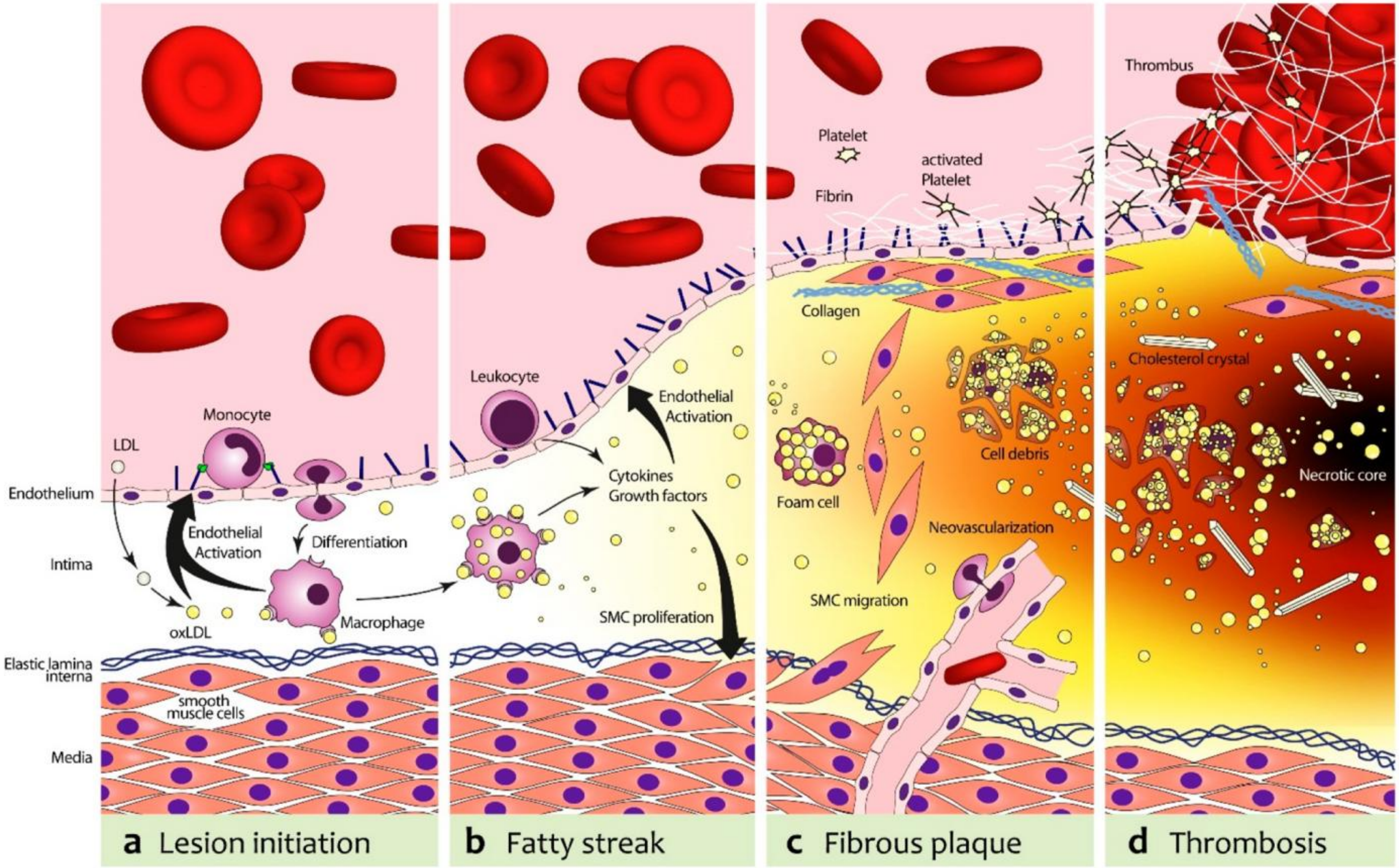
  Daniels JA et al, *Cardiovasc Diabetol*, 2014

LDL structure



With regard to LDL, it also presents the micellar form and, unlike HDL, has only one lipoprotein on its surface (apoB).

The main pathophysiologic process responsible for CVDs is Atherosclerosis



Endothelial dysfunction

Steinl DC, *Int J Mol Sci*, 2015

LDL: epidemiological evidences

High LDL-C levels: cardiovascular risk factor

- Increases in 1% LDL-C levels: ↑ 2% CHD incidence
  Wilson P, *Am J Cardiol,* 1990
- Decreases in LDL-C levels due to statin use → ↓ Incidence of major cardiovascular diseases
  Thavendiranathan P et al, *N Engl J Med,* 2006

Low LDL-C levels: RESIDUAL CARDIOVASCULAR RISK

- Most drugs that decrease LDL-C levels + statins → No extra protective effects (relative to statins)
  Ip C et al, *Int J Cardiol, 2015*
- High CV risk individuals with LDL-C <50 mg/dL → Unexplained residual CV risk
  Malave H et al, *Am J Cardiol,* 2012

LDL pro-atherogenic characteristics: atherogenicity

LDL: atherogenic characteristics

Predictive value

Modifications of LDL structure

Meisinger C et al, *Circulation*, 2005

LDL oxidation

LDL resistance against oxidation

Gómez M et a. Atherosclerosis

Glycation, acetylation, immune complexes, aggregation

LDL size

Hoogeveen RC et al, *ATBV*, 2014 (sdLDL)

LDL particle number

Cromwell WC et al, *J Clin Lipidol*, 2007

LDL composition

LDL electronegativity

LDL *ex vivo* cytotoxicity

Mediterranean Diet and LDL atherogenicity: state of the art

Mediterranean Diet and LDL atherogenicity

- Mediterranean Diet → ↓ LDL-C/HDL-C

  Estruch R et al, Ann Intern Med, 2006

- Richard C, Br J Nutr 2012

- Mediterranean Diet → ↓ LDL atherogenic traits, ↓ vsLDL, large LDL counts

  Fitó M et al, *Arch Intern Med*, 2007

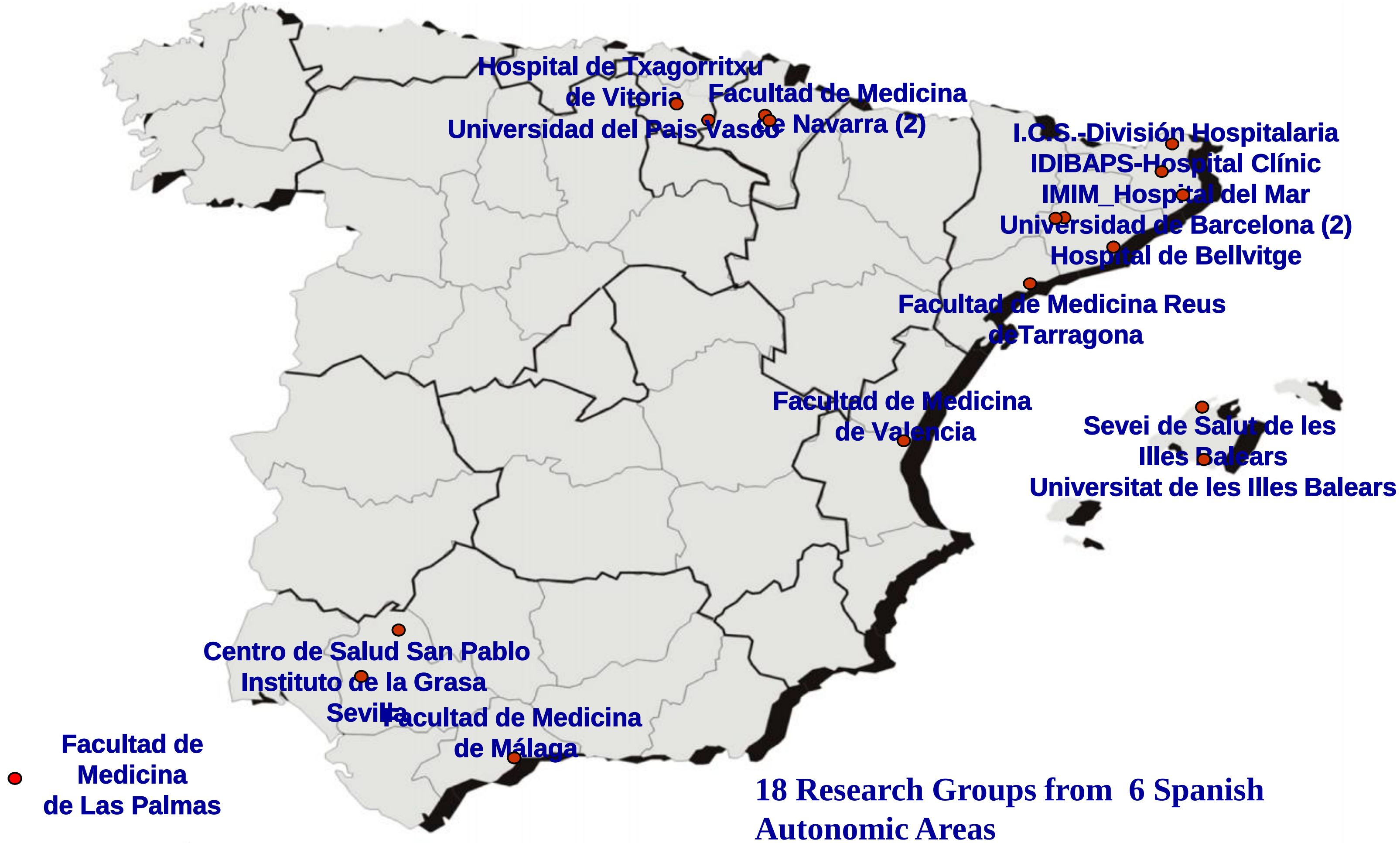
  Damasceno NRT et al, *Atherosclerosis*, 2013

In this context, our aim was to study the effects of a whole Mediterranean diet pattern intervention on HDL functionality and LDL pro-atherogenic traits,
in high cardiovascular risk individuals, within the frame of the PREDIMED Study.

PREDIMED Study (Coordinator: Ramon Estruch)

Primary Prevention of Cardiovascular Disease

The PREDIMED Study, is a large, parallel-group, multicenter, randomised, controlled, **trial that aimed to assess the effects of the Traditional Mediterranean Diet on the Primary Prevention of Cardiovascular Disease**



18 Research Groups from 6 Spanish Autonomic Areas

7,447 participants (at high CV risk) were recruited and **assigned to 3 interventions: TMD with VOO, TMD with mixed nuts, and a low-fat diet control group.**

- Men: 55-80 yr
- Women: 60-80 yr
- High CV risk without CVD
  - type 2 diabetics
  - 3+ risk factors

1. Smoking
2. Hypertension
3. ë cLDL
4. í cHDL
5. Overweight/obese
6. Family history

All free of CVD at baseline

random

Mediet +
Virgin Olive Oil

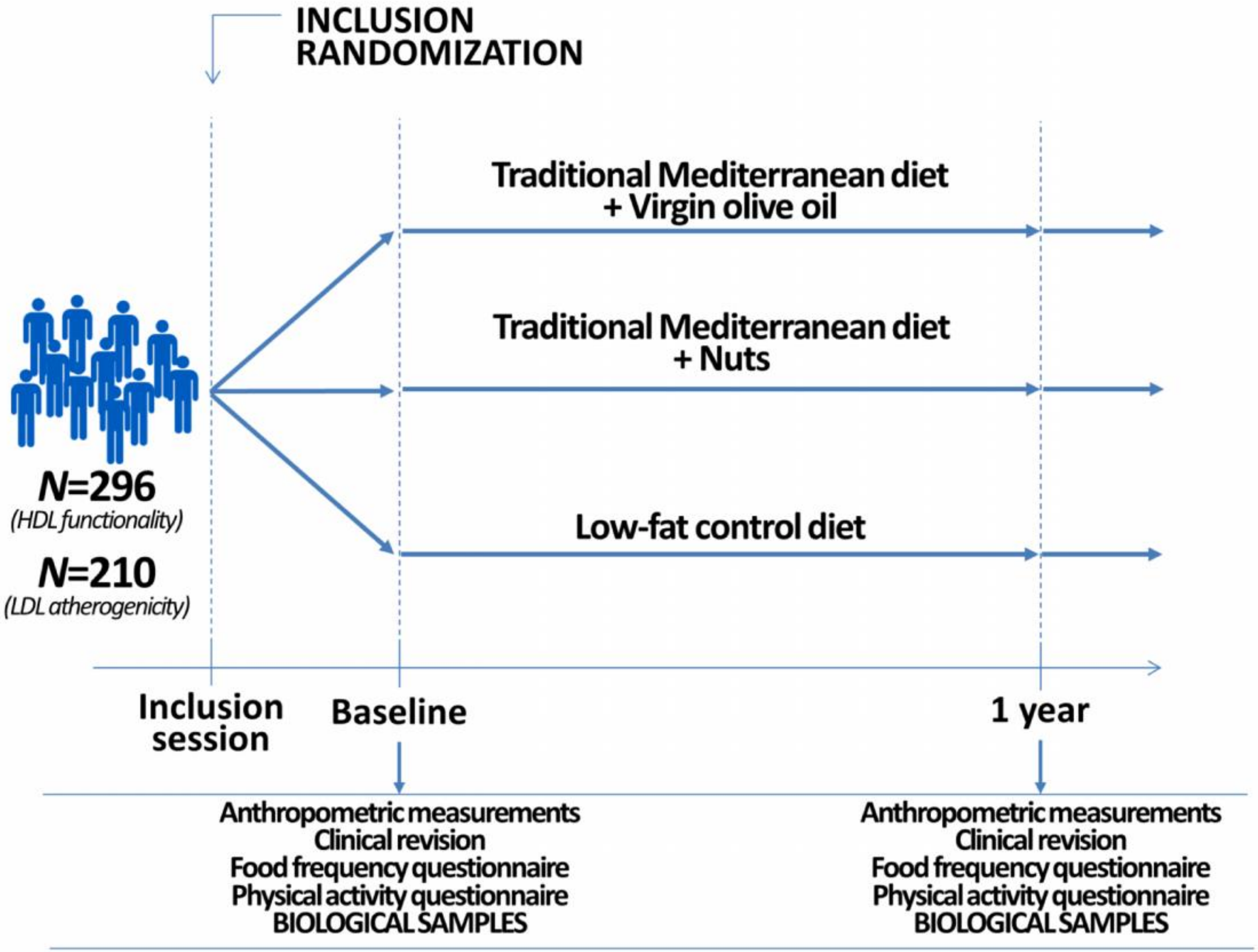
1L/week

Mediet +
Nuts

30g/day

Control
Low-fat

Subprojects about lipoprotein state and their properties



Lipoproteins-PREDIMED Study: design and methodologies

Biological samples were obtained at the start of the study and 1-year after

ApoB-deplected plasma

- ApoB-deplected **plasma composition**
- **Cholesterol efflux**
- **HDL antioxidant properties**
  HII: dichlorodihydrofluorescein diacetate (H2DCF-DA)
  MDA equivalents
- **HDL vasodilatory Property** (DAF) diaminofluorescein diacetate (DAF-2DA)

Plasma

- **Lipid profile**
- **Apolipoproteins**

Serum

- **HDL metabolic enzymes: CETP, LCAT**

HDL and LDL lipoproteins

- **Conjugated diens**
- **LDL citotoxicity** Thiazolyl Blue Tetrazolium bromide (MTT)

Lipoproteins-PREDIMED Study: design and methodologies

Biological samples were obtained at the start of the study and 1-year after

ApoB-deplected plasma

Cholesterol efflux

THP1 monocytes
(phorbol-myristate-acetate)
H(N)]-cholesterol

HDL vasodilatory Property (DAF)
diaminofluorescein diacetate (DAF-2DA)

HUVEC

Plasma

Serum

HDL and LDL lipoproteins

LDL citotoxicity
Thiazolyl Blue
Tetrazolium bromide (MTT)

THP1 monocytes
(phorbol-myristate-acetate)

HDL RESULTS

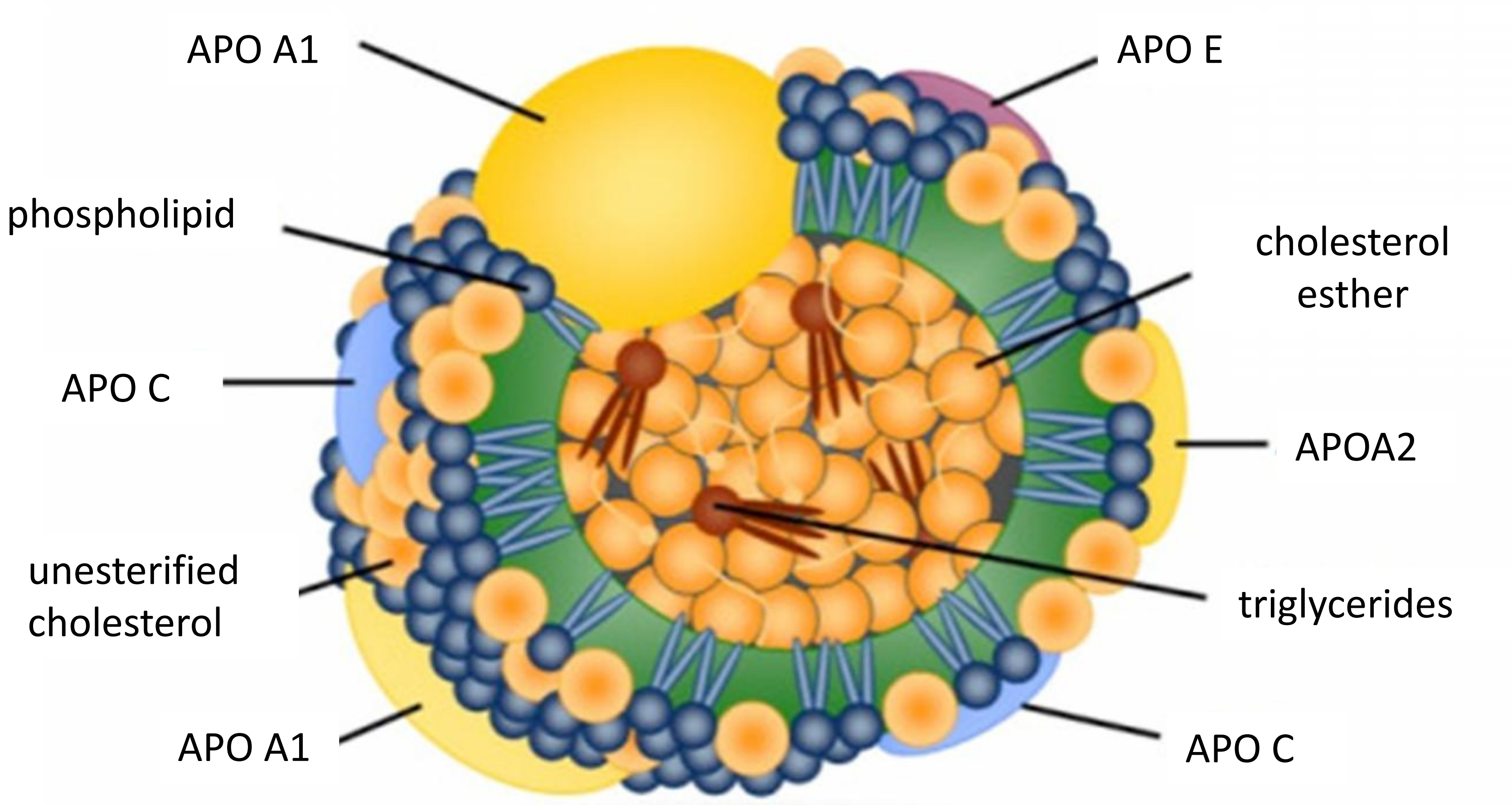

APO A1
APO E
phospholipid
cholesterol
esther
APO C
APOA2
unesterified
cholesterol
triglycerides
APO A1
APO C

Table. Baseline Characteristics of the Volunteers in the 3 Intervention Groups of the Study

Variables	TMD-VOO (n=100)	TMD-Nuts (n=100)	Low-Fat Diet (n=96)	*P* Value
Age, y	66.3 (5.78)*	66.4 (6.93)*	65.0 (6.49)*	0.247
Male, %	56.0	47.0	50.0	0.432
Body mass index, kg/m^2	30.1 (3.85)	29.0 (3.76)	29.9 (3.87)	0.087
Waist circumference, cm	100 (10.7)	101 (10.3)	102 (11.2)	0.469
Leisure-time physical activity, METs·min/d	176 (69.3–284)†	175 (68.3–408)†	174 (41.3–362)†	0.870
Smoking status, %	15.0	10.0	12.5	0.565
Type 2 diabetes mellitus, %	48.0	52.0	46.9	0.751
Hypertension, %	78.0	78.0	80.2	0.910
Dyslipidemia, %	79.0	70.0	83.3	0.074
Glucose, mg/dL	110 (93.8–137)	108 (92.5–140)	108 (94.0–131)	0.982
Triglycerides, mg/dL	207 (36.8)	196 (36.2)	204 (36.8)	0.112
Total cholesterol, mg/dL	110 (93.2–158)	100 (72.5–144)	113 (83.0–140)	0.160
HDL cholesterol, mg/dL	50.2 (12.3)	49.8 (10.3)	49.1 (11.6)	0.777
LDL cholesterol, mg/dL	130 (28.5)	123 (30.4)	130 (31.7)	0.181
ApoA-I, mg/dL	138 (23.5)	134 (20.2)	131 (19.4)	0.232
Apolipoprotein B, mg/dL	106 (21.6)	98.2 (18.9)	103 (21.8)	0.062

ApoA-I indicates apolipoprotein A-I; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent of task; TMD-Nuts, traditional Mediterranean diet enriched with nuts; and TMD-VOO, traditional Mediterranean diet enriched with virgin olive oil.

*Mean (SD).

†Median (first–third quartiles).

Differences between the Traditional Mediterranean Diet interventions and the low-fat control diet in the dietary profile of the volunteers.

VARIABLES	TMD-VOO vs. Low-fat control diet Coefficient β [CI 95%]*	P-value	TMD-Nuts vs. Low-fat control diet Coefficient β [CI 95%]*	P-value
Adherence to TMD (score)	1.23 [0.80;1.66]	<0.001	1.30 [0.85;1.75]	<0.001
Total energy intake (kcal/day)	-8.24 [-135;119]	0.899	-0.72 [-133;132]	0.992
Carbohydrates (g/day)	-19.9 [-38.2;-1.58]	0.034	-27.7 [-46.9;-8.55]	0.005
Proteins (g/day)	2.80 [-2.60;8.20]	0.311	2.18 [-3.45;7.82]	0.449
Total fats (g/day)	7.47 [1.58;13.4]	0.014	12.0 [5.81;18.2]	<0.001
MUFAs (g/day)	6.11 [2.97;9.25]	<0.001	6.38 [3.08;9.67]	<0.001
SFAs (g/day)	0.58 [-1.16;2.32]	0.516	1.15 [-0.68;2.98]	0.218
PUFAs (g/day)	1.03 [-0.34;2.40]	0.143	4.53 [3.08;5.97]	<0.001
Fiber (g/day)	-0.12 [-2.38;2.14]	0.918	0.11 [-2.25;2.48]	0.925
Total olive oil (g/day)	10.3 [6.72;13.9]	<0.001	3.38 [-0.40;7.15]	0.081
Virgin olive oil (g/day)	26.2 [21.3;31.1]	<0.001	3.66 [-1.49;8.81]	0.165
Refined olive oil (g/day)	-15.2 [-19.3;-11.1]	<0.001	0.83 [-3.44;5.09]	0.705

Physical activity did not change throughout the study.

Volunteers' compliance was correct according to TMD adherence scores, the 1-year food frequency questionnaire, and biomarkers of compliance.

Hernáez A et al, Circulation 2017

Differences between the Traditional Mediterranean Diet interventions and the low-fat control diet in the biochemical profile of the volunteers.

VARIABLES	TMD-VOO vs. Low-fat control diet Coefficient β [CI 95%]*	TMD-VOO vs. Low-fat control diet P-value	TMD-Nuts vs. Low-fat control diet Coefficient β [CI 95%]*	TMD-Nuts vs. Low-fat control diet P-value
Glucose (mg/dL)†	-0.022 [-0.079;0.036]	0.460	0.013 [-0.049;0.074]	0.683
Triglycerides (mg/dL)†	$-2\cdot10^{-4}$ [-0.091;0.091]	0.997	-0.068 [-0.16;0.029]	0.170
Total cholesterol (mg/dL)	10.5 [2.86;18.1]	0.007	0.056 [-8.02;8.13]	0.989
HDL cholesterol (mg/dL)	0.73 [-1.17;2.62]	0.453	-1.25 [-3.28;0.77]	0.227
LDL cholesterol (mg/dL)	9.56 [3.16;16.0]	0.004	1.21 [-5.63;8.04]	0.729
Apolipoprotein A-I (mg/dL)	0.50 [-3.44;4.44]	0.804	-0.41 [-4.52;3.69]	0.844
Apolipoprotein B (mg/dL)	1.69 [-2.90;6.27]	0.472	-1.96 [-6.69;2.77]	0.419
HDL cholesterol/Apolipoprotein A-I (unitless ratio)	0.001 [-0.006;0.009]	0.750	-0.005 [-0.013;0.003]	0.200
Apolipoprotein B/Apolipoprotein A-I (unitless ratio)	0.007 [-0.032;0.046]	0.721	-0.015 [-0.056;0.025]	0.464

*: β coefficient of the multivariate linear regression model, adjusted for: sex; age; volunteer's center of origin; baseline value of the variable; and changes in the presence of dyslipidemia, hypertension, diabetes, and smoking habit. †: log-transformed variables. *TMD-VOO*: Traditional Mediterranean Diet enriched with virgin olive oil. *TMD-Nuts*: Traditional Mediterranean Diet enriched with mixed nuts

A decline in total cholesterol concentrations after the low-fat control diet, versus baseline and TMD-VOO intervention was observed.

This was due mainly to a decrease in LDL cholesterol concentrations.

No significant changes in HDL cholesterol or ApoA-I concentrations were observed.

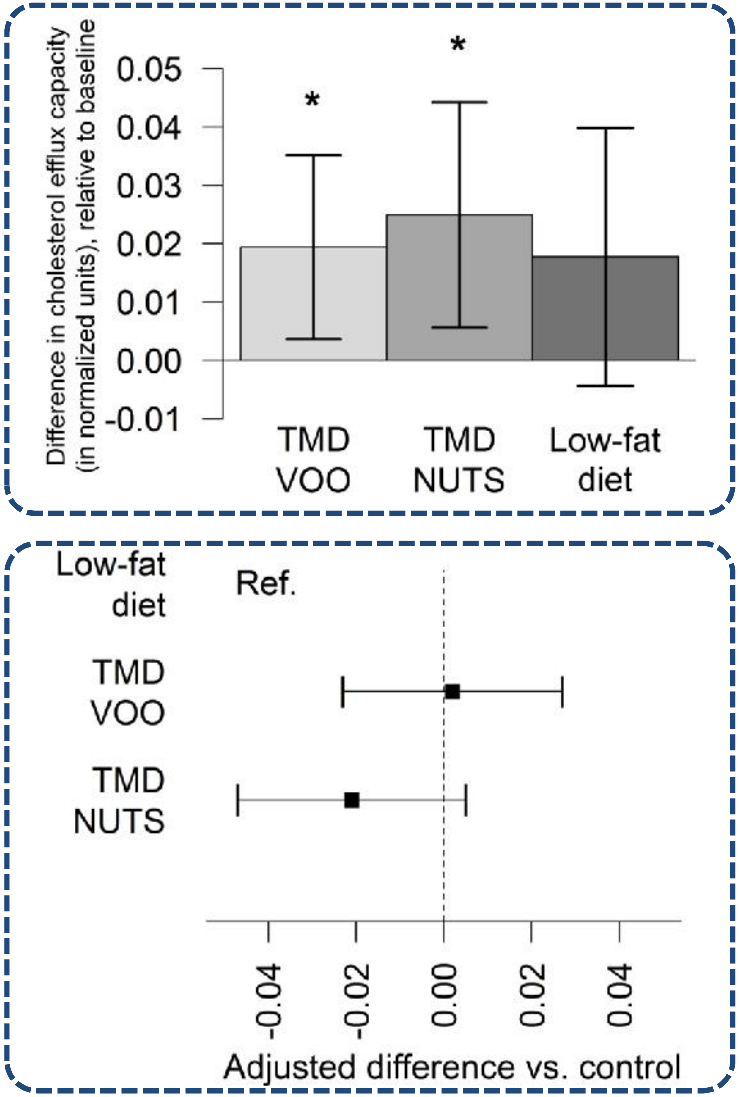
Hernáez A et al, Circulation 2017

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

↑ Cholesterol efflux capacity

Post- vs. pre- intervention:

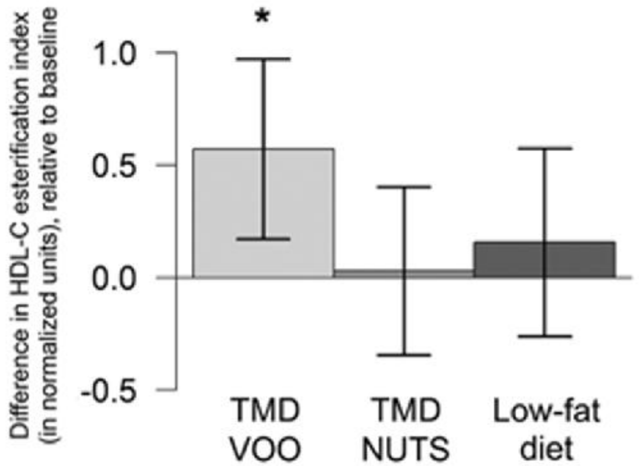
Changes after the TMD interventions vs. the low-fat diet:



*: *P*<0.05; **: P<0.01; ***: P<0.001

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



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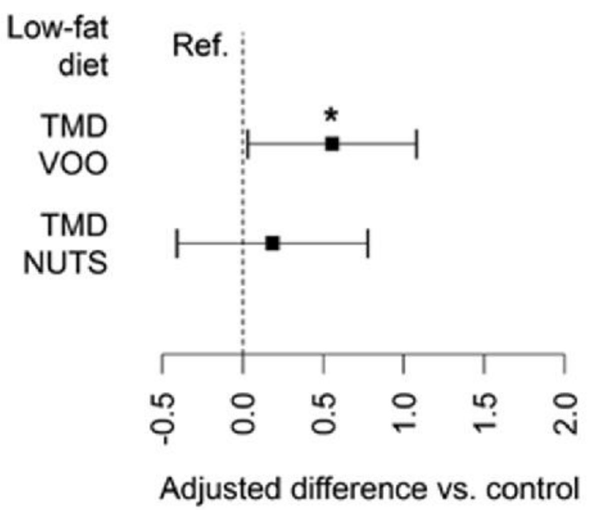

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Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

↑ Cholesterol efflux capacity

↑ HDL cholesterol esterification index

Hernáez A et al, Circulation 2017

Post- vs.
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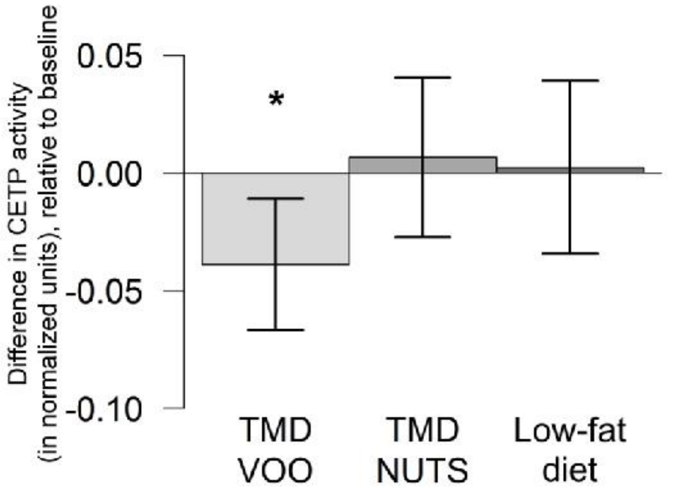

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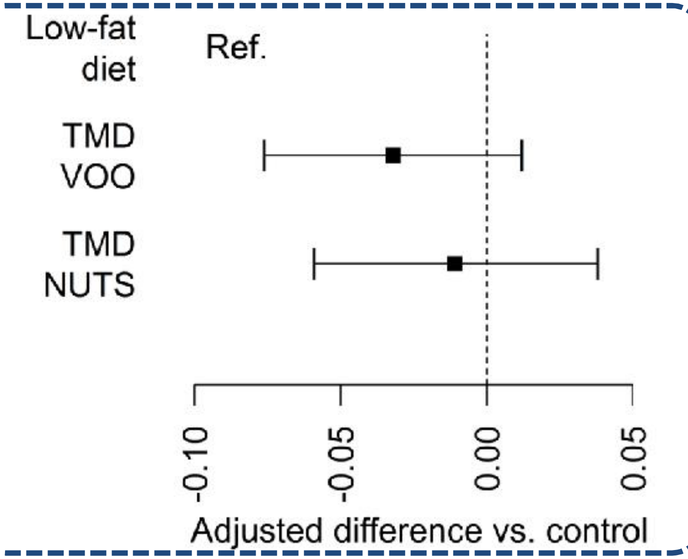

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Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

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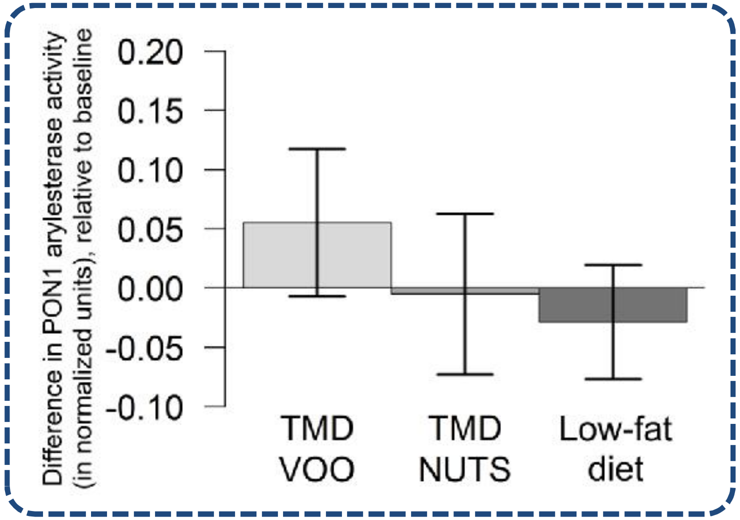
↑ HDL cholesterol esterification index

↓ CETP activity

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



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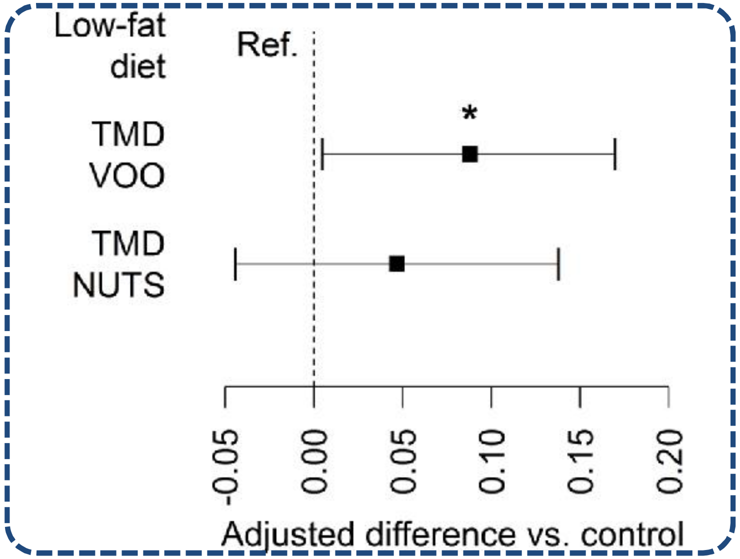
TMD interventions
vs. the low-fat diet:



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Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

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↑ Paraoxonase-1 activity

Hernáez A et al, Circulation 2017

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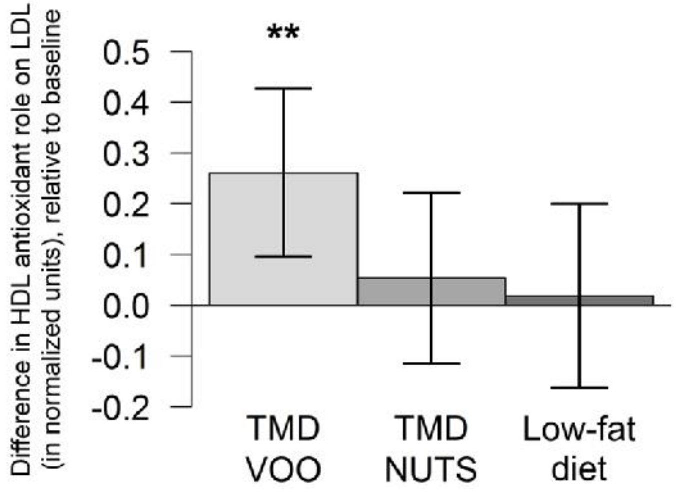
pre- intervention:



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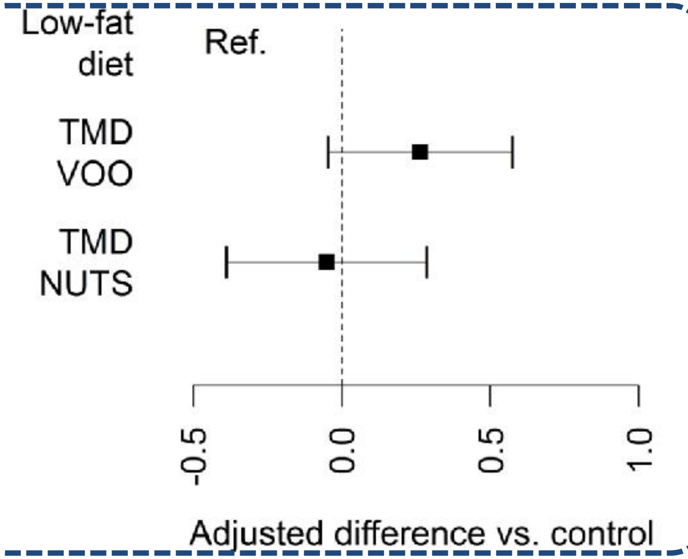

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Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

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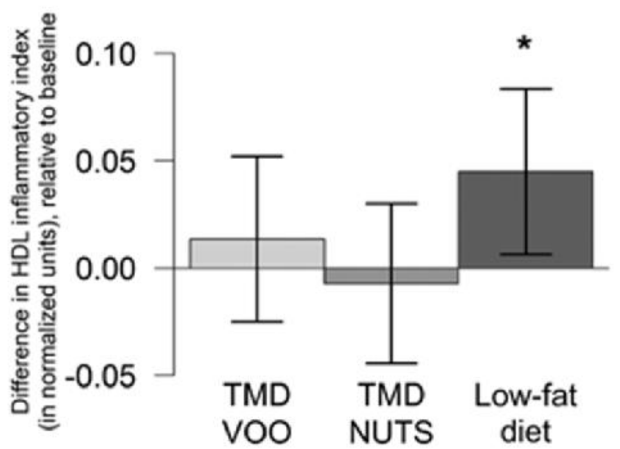
↓ CETP activity

↑ Paraoxonase-1 activity

↑ HDL antioxidant capacity on LDLs

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



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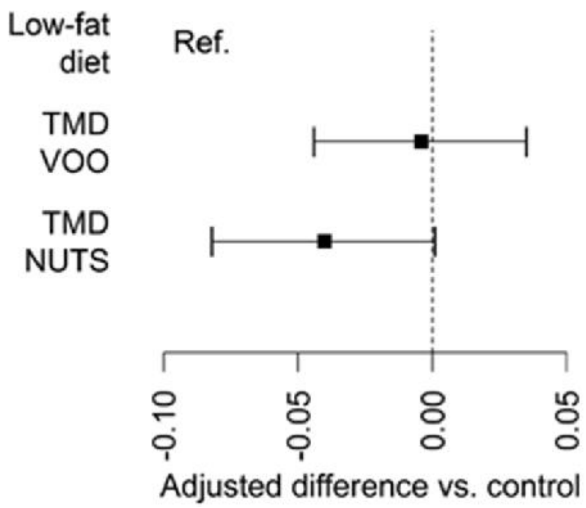

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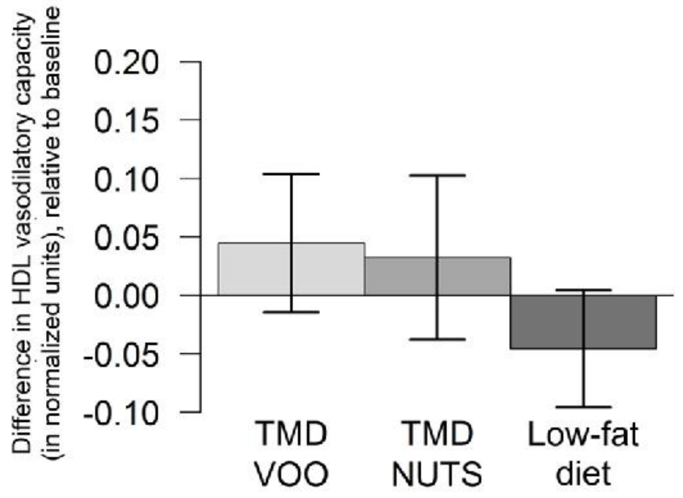

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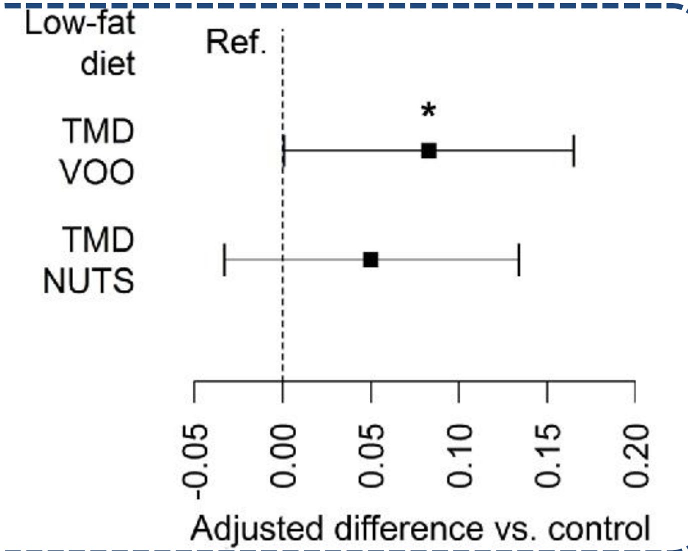


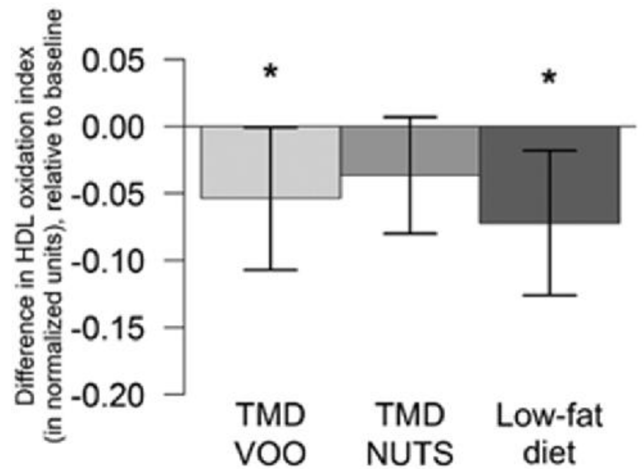
***: *P*<0.05; **: P<0.01; ***: P<0.001**

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

- ↑ Cholesterol efflux capacity
- **↑ HDL cholesterol esterification index**
- ↓ CETP activity
- **↑ Paraoxonase-1 activity**
- ↑ HDL antioxidant capacity on LDLs
- **↑ HDL vasodilatory capacity**

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



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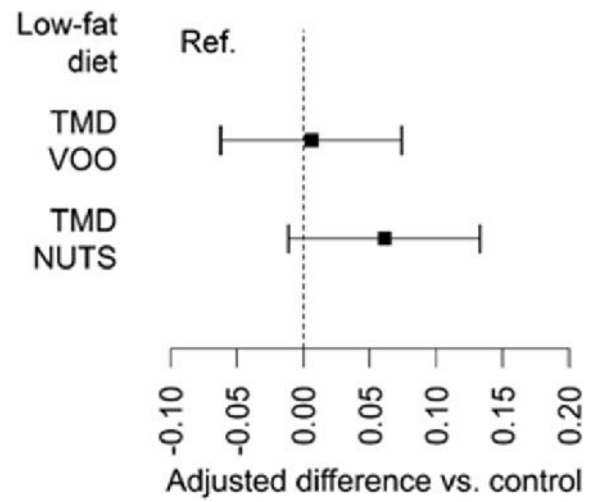
vs. the low-fat diet:



*: *P*<0.05; **: P<0.01; ***: P<0.001

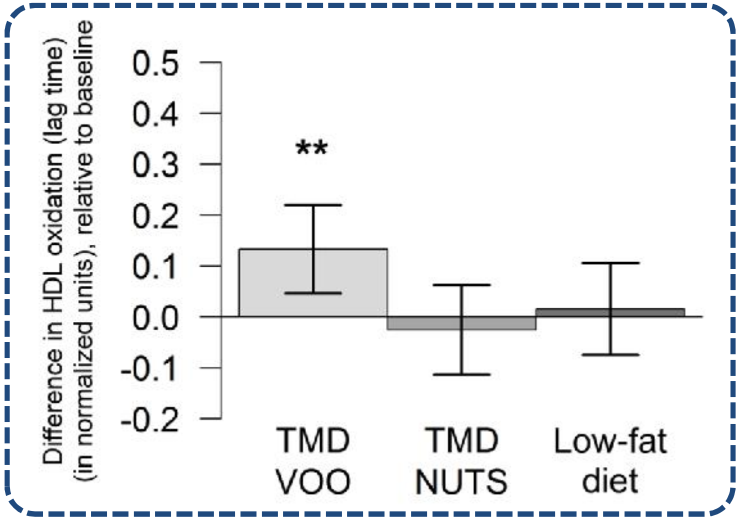
Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

- ↑ Cholesterol efflux capacity
- **↑ HDL cholesterol esterification index**
- ↓ CETP activity
- **↑ Paraoxonase-1 activity**
- ↑ HDL antioxidant capacity on LDLs
- **↑ HDL vasodilatory capacity**
- ↓ HDL oxidation index

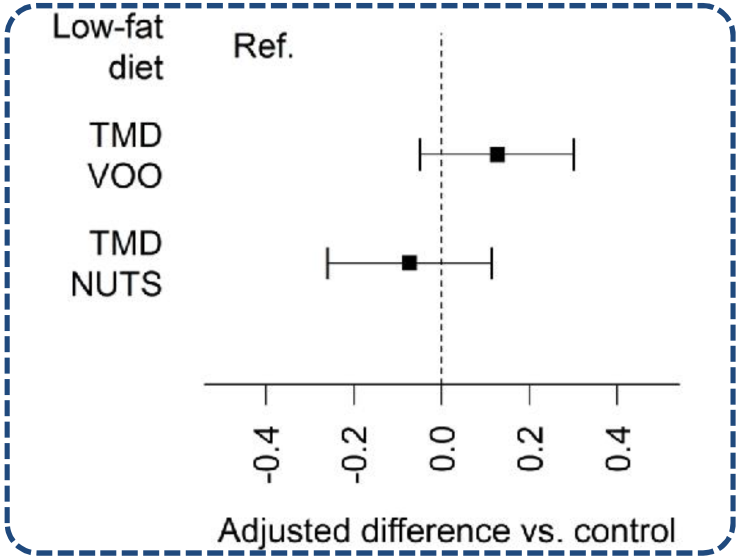
Hernáez A et al, Circulation 2017

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:

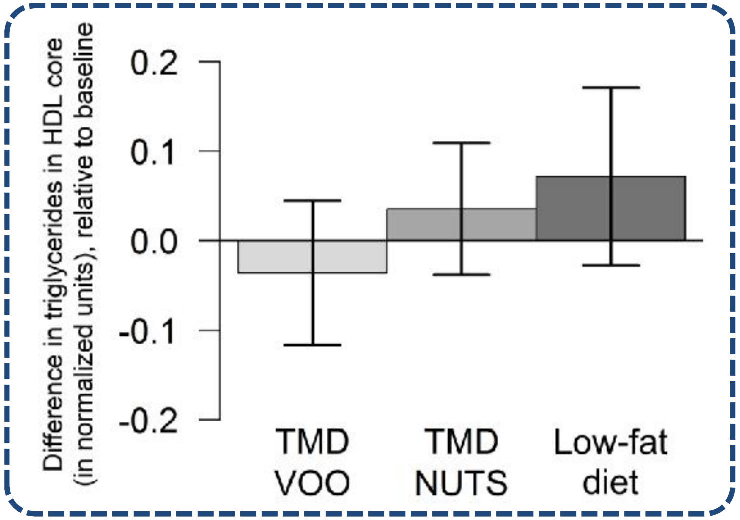


***: *P*<0.05; **: P<0.01; ***: P<0.001**

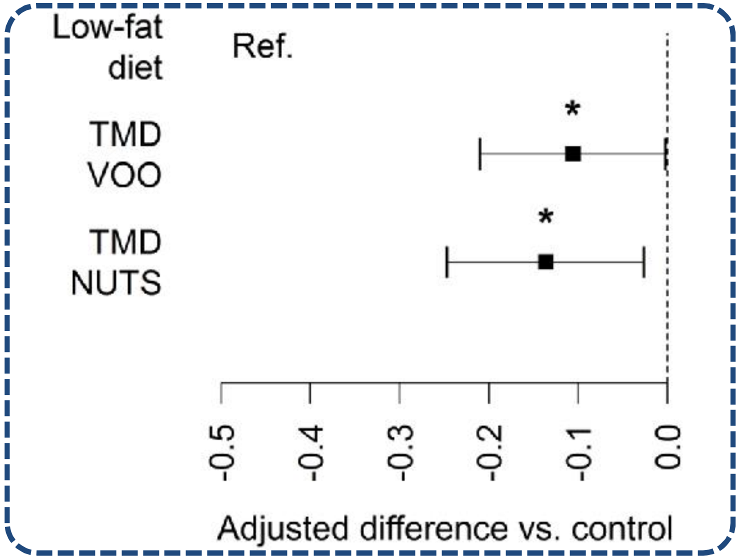
- ↑ Cholesterol efflux capacity
- **↑ HDL cholesterol esterification index**
- ↓ CETP activity
- **↑ Paraoxonase-1 activity**
- ↑ HDL antioxidant capacity on LDLs
- **↑ HDL vasodilatory capacity**
- ↓ HDL oxidation index
- ↑ HDL resistance against oxidation

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



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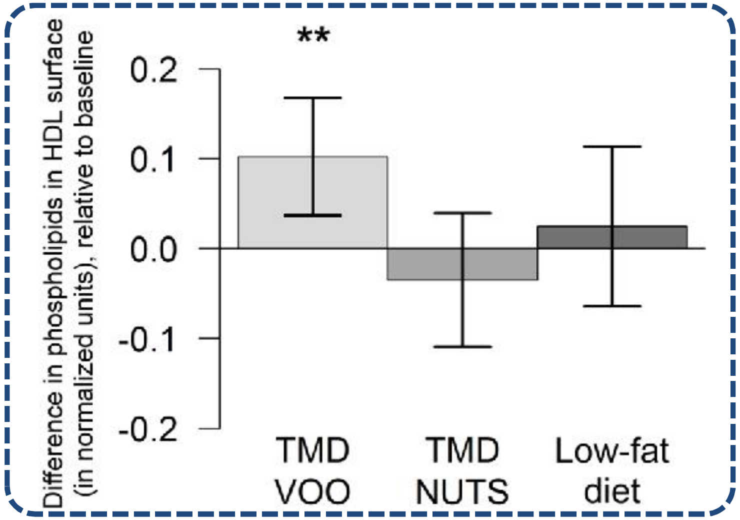
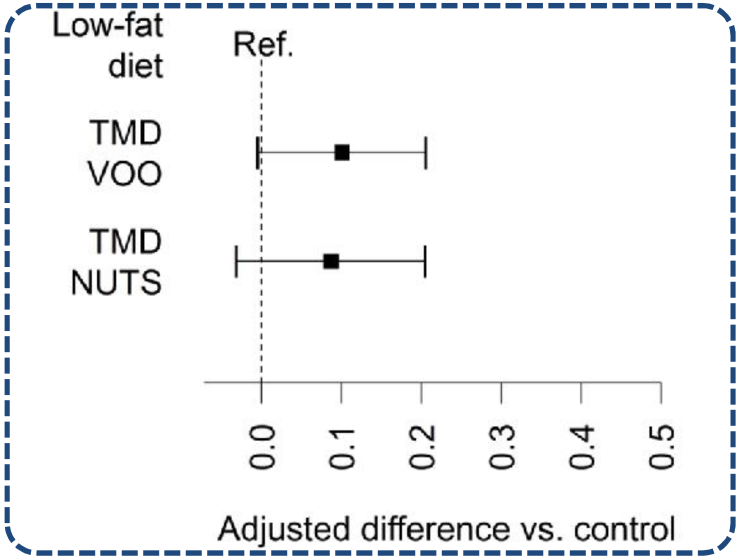
- ↓ HDL oxidation index
- ↑ HDL resistance against oxidation
- **↓ Triglycerides in HDL core**

Hernáez A et al, Circulation 2017

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



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Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

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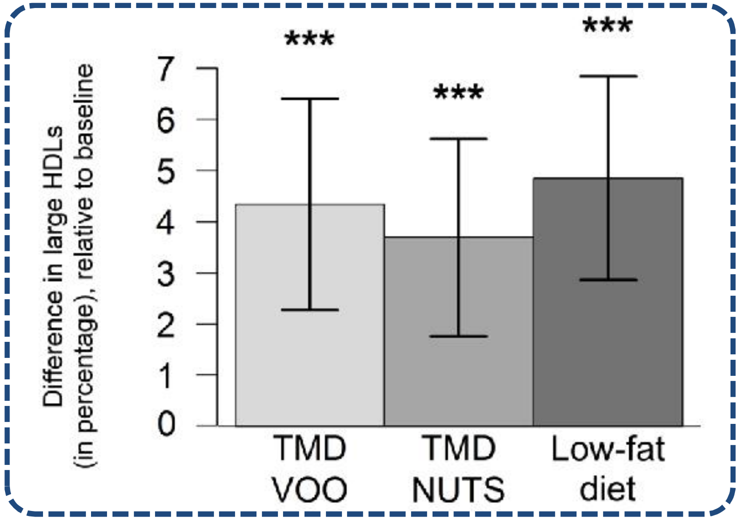
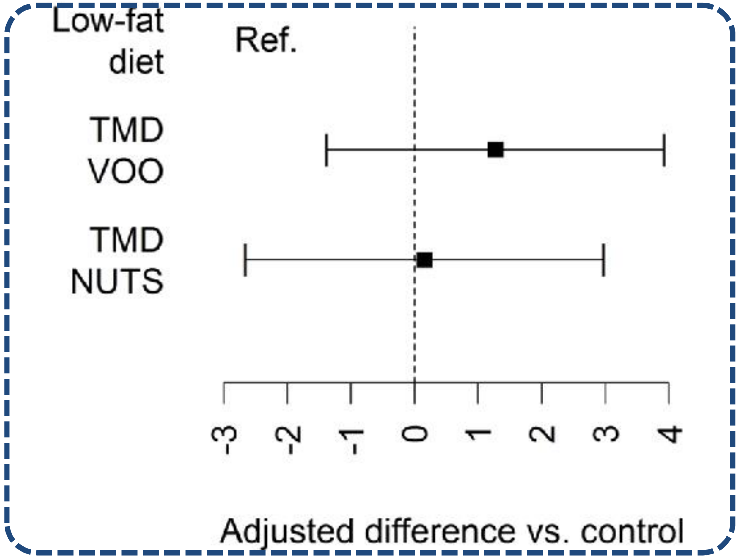
- ↑ HDL antioxidant capacity on LDLs
- **↑ HDL vasodilatory capacity**
- ↓ HDL oxidation index
- ↑ HDL resistance against oxidation
- **↓ Triglycerides in HDL core**
- ↑ Phospholipids in HDL surface

Hernáez A et al, Circulation 2017

Post- vs.
pre- intervention:



Changes after the
TMD interventions
vs. the low-fat diet:

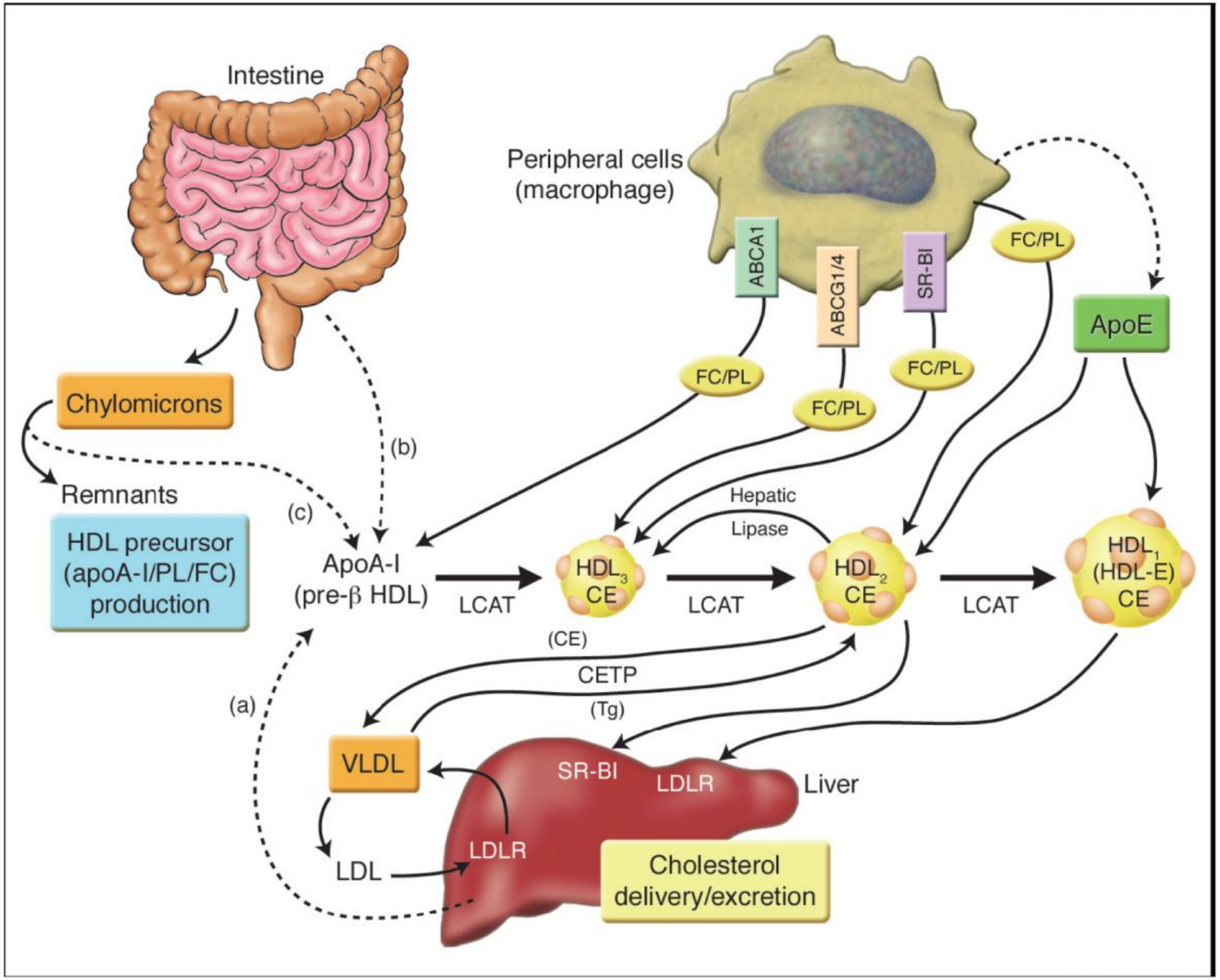


*: *P*<0.05; **: P<0.01; ***: P<0.001

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

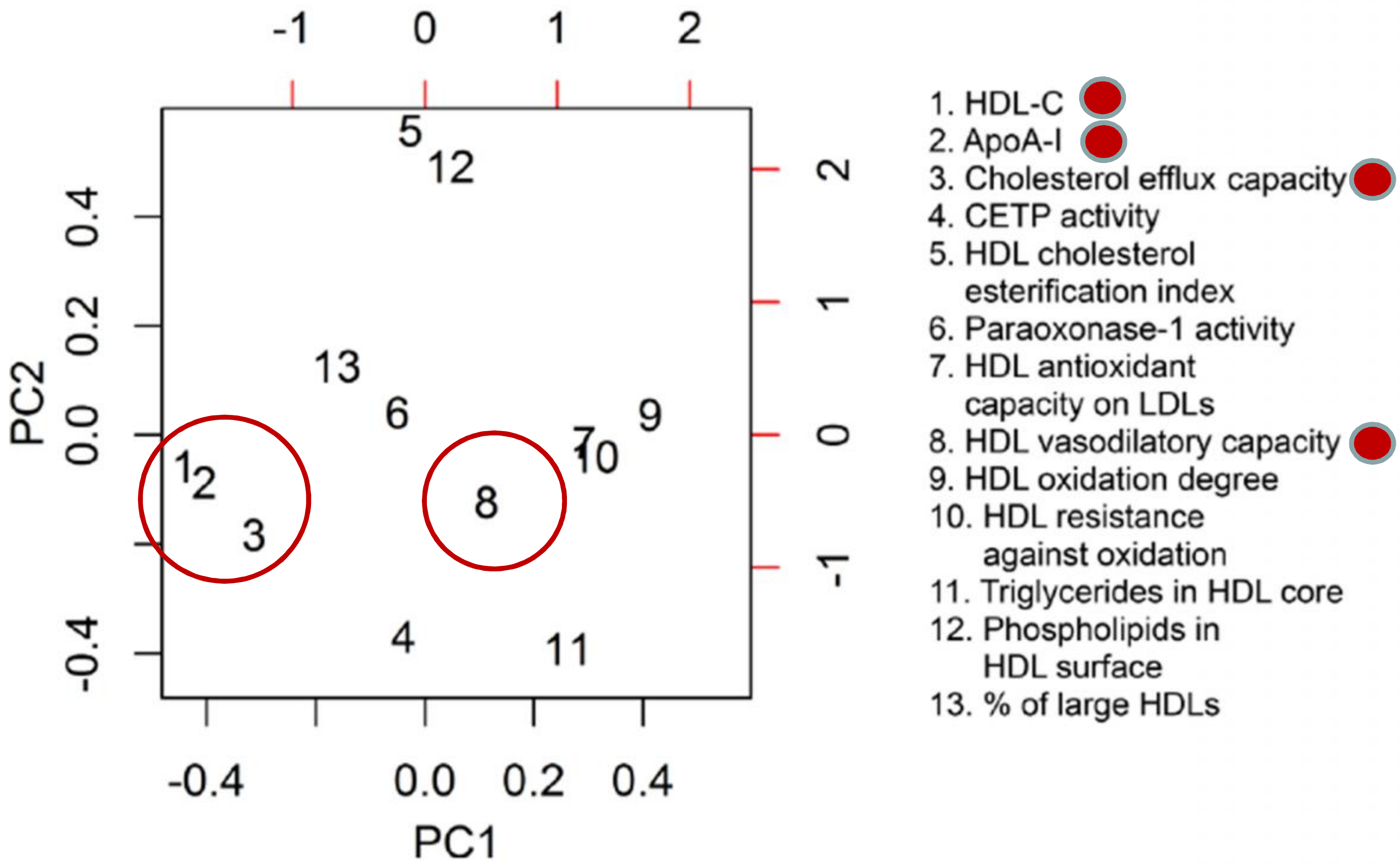
- ↑ Cholesterol efflux capacity
- **↑ HDL cholesterol esterification index**
- ↓ CETP activity
- **↑ Paraoxonase-1 activity**
- ↑ HDL antioxidant capacity on LDLs
- **↑ HDL vasodilatory capacity**
- ↓ HDL oxidation index
- ↑ HDL resistance against oxidation
- **↓ Triglycerides in HDL core**
- ↑ Phospholipids in HDL surface
- ↑ Large HDLs

Hernáez A et al, Circulation 2017



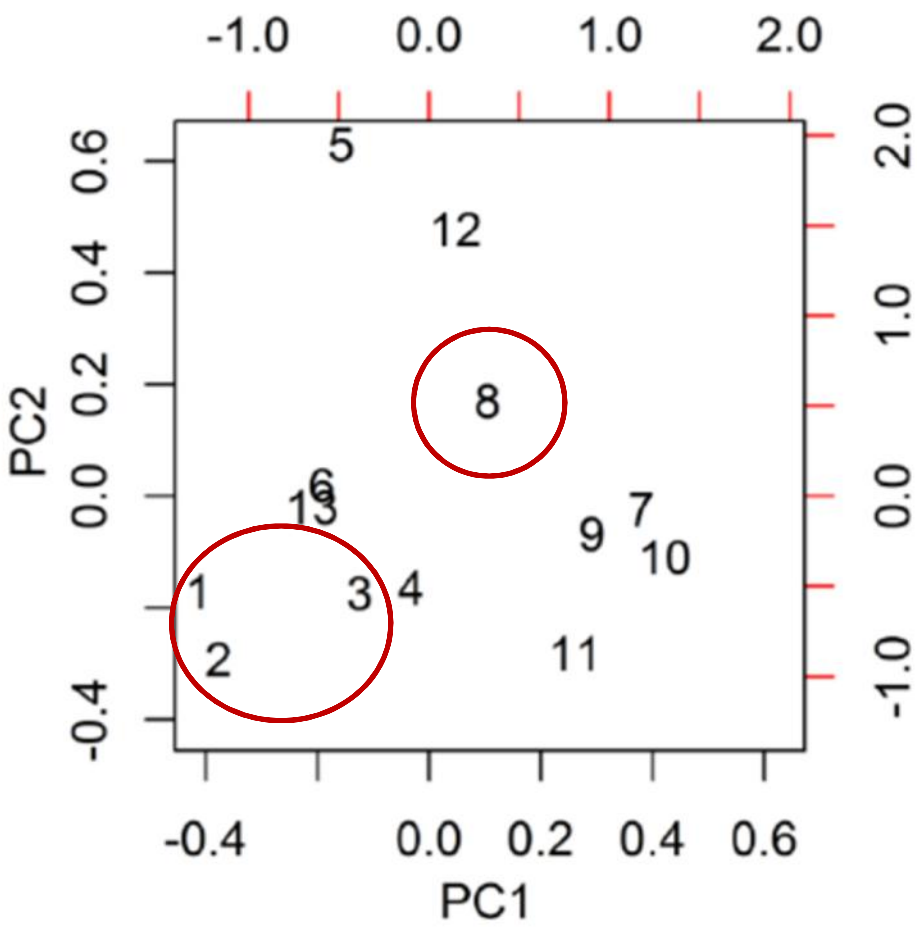
Low levels of large HDL and/or high levels of small HDL are present in coronary heart disease, ischemic stroke, type-II diabetes mellitus and peripheral arterial disease patients.

Bi-dimensional plot of the distribution of HDL-related variables at baseline according to the two main components of the principal component analysis.



The two main components of this analysis were able to explain 75.3% of total variance of the data.

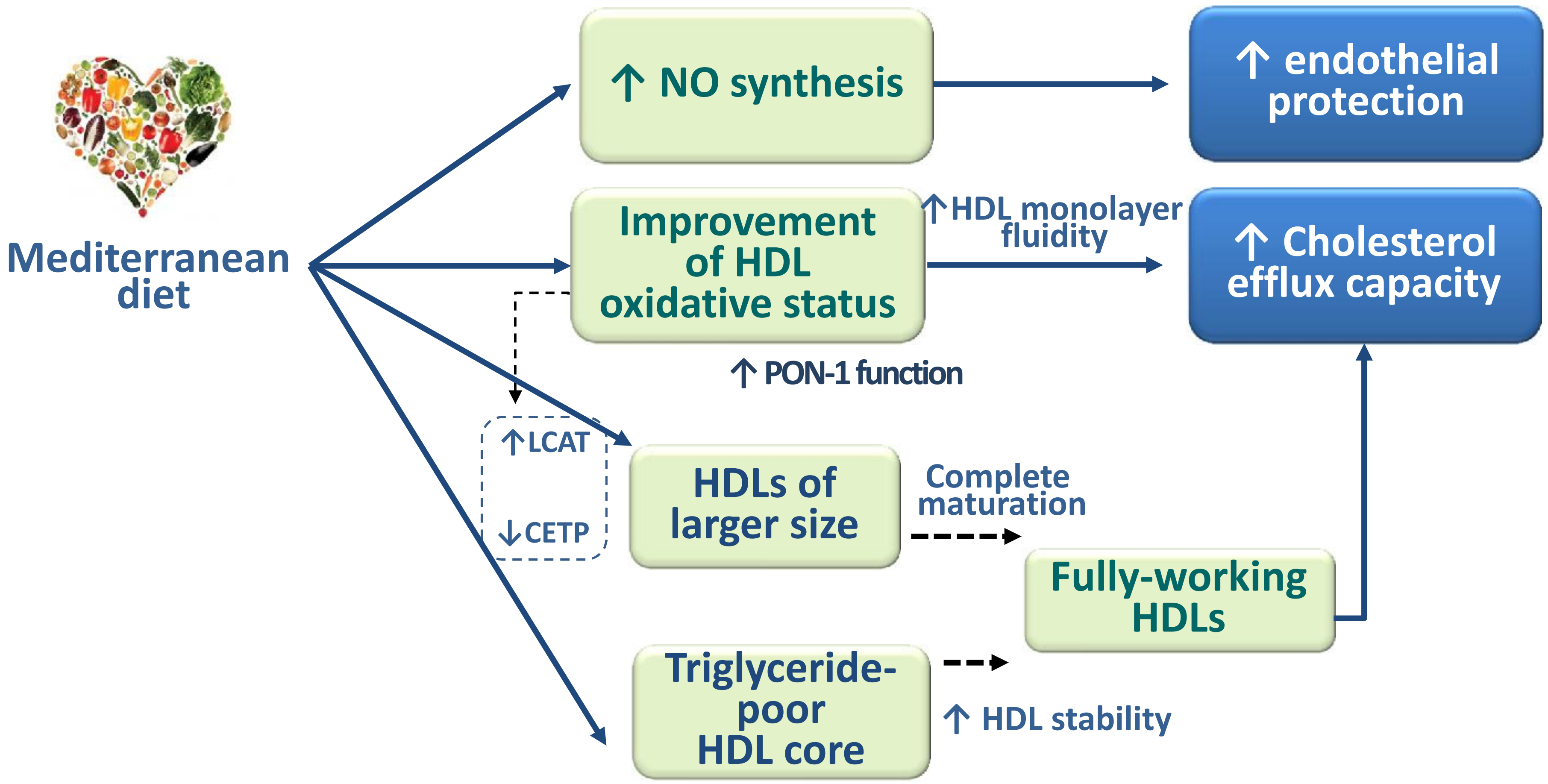
Bi-dimensional plot of the distribution of the changes in HDL-related variables after the TMD-VOO intervention according to the two main components of the principal component analysis.



1. HDL-C
2. ApoA-I
3. Cholesterol efflux capacity
4. CETP activity
5. HDL cholesterol esterification index
6. Paraoxonase-1 activity
7. HDL antioxidant capacity on LDLs
8. HDL vasodilatory capacity
9. HDL oxidation degree
10. HDL resistance against oxidation
11. Triglycerides in HDL core
12. Phospholipids in HDL surface
13. % of large HDLs

The two main components of this analysis were able to explain 64.1% of total variance of the data.

HDL FUNCTIONALITY: The MD intervention improves NO synthesis, oxidative status, increased HDL size, and promoted a greater HDL stability reflected as a triglyceride-poor core. Overall produces an improvement of endothelial protection and cholesetrol efflux



Strengths.

First, the involves **a large sample size** (n=296).

Second, it presents a **randomized** design with the presence of an active comparator (low-fat diet).

Third, its duration is **long** (1 year of follow-up).

Last, it comprehensively assesses **diverse HDL functions and HDL quality-related characteristics.**

Limitations.

The participants of the trial were **elderly people** at high cardiovascular risk, which hinders the extrapolation of our results to the general population.

As expected, we found **only slight** differences because the trial is based on modest real-life modifications **of the diet and the control diet is already a well-known healthy dietary pattern**.

The use of **cellular models**, although a noninvasive alternative to test relevant physiological functions, **may not have demonstrated the effect of contraregulatory mechanisms, which can modify the final in vivo outcome** in humans.

Conclusions

Long-term adherence to a traditional Mediterranean Diet, especially when supplemented with virgin olive oil, in high cardiovascular risk individuals, is able to improve HDL atheroprotective functions:

a. cholesterol efflux capacity,
b. HDL-C metabolism,
c. HDL antioxidant/anti-inflammatory properties,
d. and vasoprotective effects

Both TMDs increased cholesterol efflux capacity relative to baseline.

The TMD-VOO intervention decreased cholesteryl ester transfer protein activity relative to baseline

Conclusions

And the TMD-VOO intervention increased relative to the control group:
HDL ability to esterify cholesterol,
paraoxonase-1 arylesterase activity,
and HDL vasodilatory capacity

Adherence to a TMD can induce these beneficial changes by improving HDL oxidative status, composition, and size.

The 3 diets increased the percentage of large HDL particles relative to baseline.

LDL RESULTS

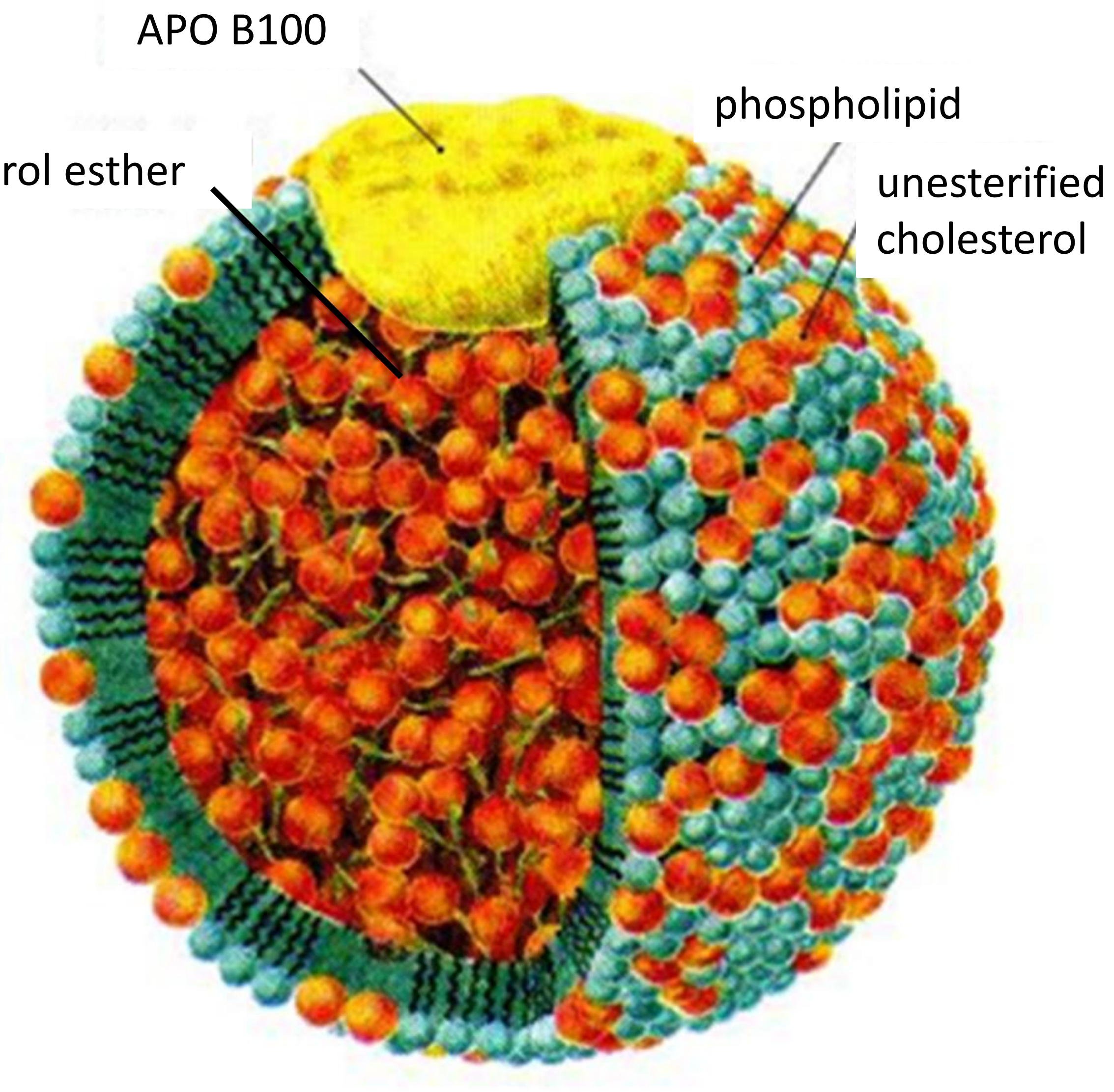

APO B100
phospholipid
cholesterol esther
unesterified
cholesterol

Table 1. Baseline characteristics of the volunteers in the three intervention groups

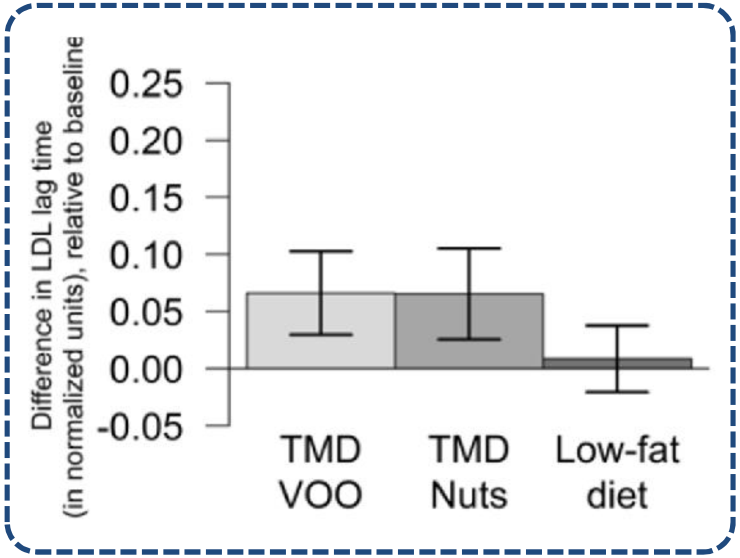
VARIABLES	TMD-VOO $N = 71$	TMD-Nuts $N = 68$	Low-fat diet $N = 71$	*p*-value
Age (years)	66.5 ± 6.34	65.1 ± 6.85	64.7 ± 6.58	0.270
Sex (% male)	45.1%	61.8%	47.9%	0.111
Body mass index (kg/m^2)	30.2 ± 3.96	29.2 ± 3.92	29.7 ± 3.98	0.386
Waist circumference (cm)	99.8 ± 10.7	102 ± 10.2	101 ± 11.5	0.489
Leisure-time physical activity (MET·min/day)	156 (67.5–247)	169 (59.1–323)	150 (15.5–332)	0.782
Smoking status (% of smokers)	16.9%	11.8%	12.7%	0.642
Type 2 diabetes (% of diabetic patients)	76.1%	76.5%	84.5%	0.380
Hypertension (% of hypertensive patients)	47.9%	55.9%	38.0%	0.107
Dyslipidemia (% of dyslipidemic patients)	83.1%	77.9%	85.9%	0.458
Fasting glucose (mg/dL)	105 (92.5–127)	118 (96.0–140)	105 (94.0–128)	0.470
Triglycerides (mg/dL)	108 (90.7–157)	105 (73.0–147)	115 (97.0–140)	0.610
Total cholesterol (mg/dL)	206 ± 39.1	198 ± 35.9	210 ± 38.4	0.231
HDL cholesterol (mg/dL)	49.8 ± 11.8	49.2 ± 10.8	49.2 ± 10.6	0.932
LDL cholesterol (mg/dL)	129 ± 30.0	125 ± 30.1	135 ± 33.0	0.190
Apolipoprotein B (mg/dL)	104 ± 22.0	97.6 ± 17.1	105 ± 22.7	0.121
Apolipoprotein B/A-I ratio (unitless)	0.78 ± 0.16	0.75 ± 0.16	0.82 ± 0.22	0.123

Variables are expressed as percentages (categorical variables), means ± SD (normally distributed variables) or median (1st–3rd quartile) (non-normally distributed variables). *MET*, metabolic equivalent of task; *TMD-Nuts*, Traditional Mediterranean Diet enriched with mixed nuts; *TMD-VOO*, Traditional Mediterranean Diet enriched with virgin olive oil.

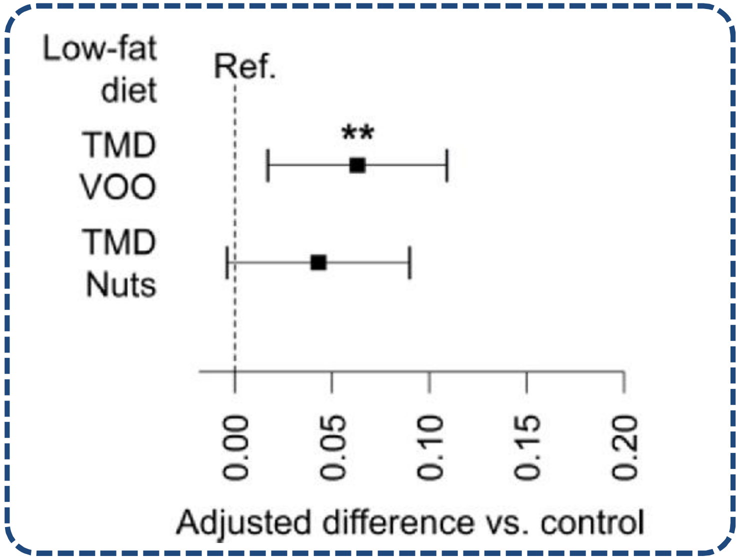
Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

↑ LDL resistance against oxidation

Post- vs. pre- intervention:



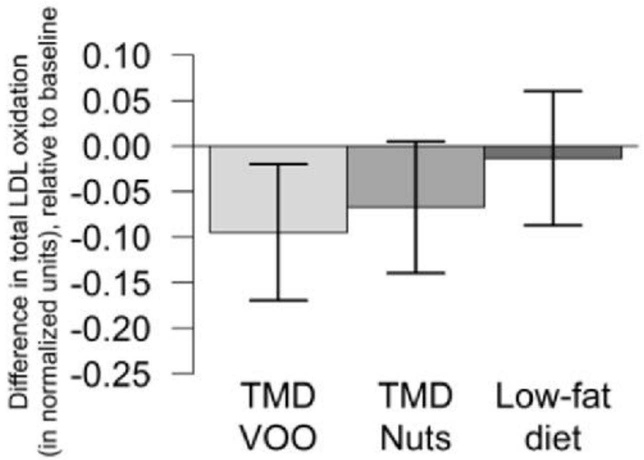
Changes after the TMD interventions vs. the low-fat diet:



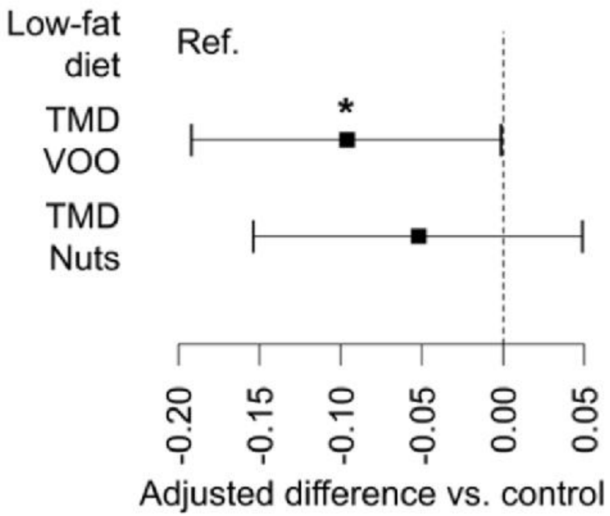
***: *P*<0.05; **: P<0.01; ***: P<0.001**

Hernáez A et al, MNFR 2017

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



***: *P*<0.05; **: P<0.01; ***: P<0.001**

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

↑ LDL resistance against oxidation

↓ total LDL oxidation

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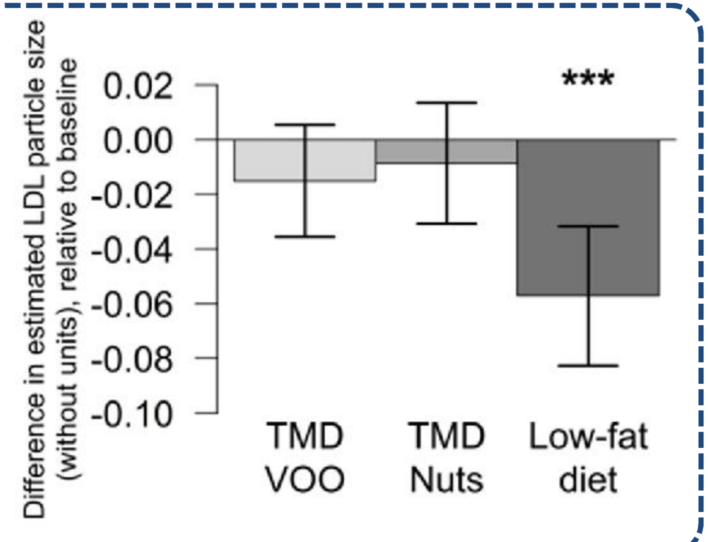
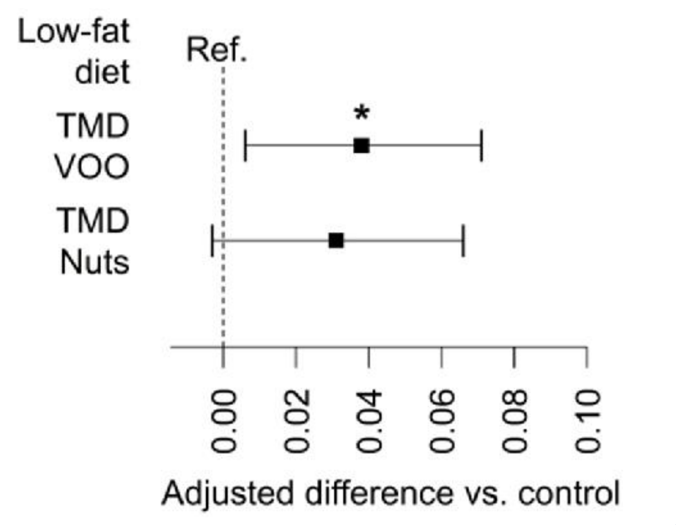
Post- vs.
pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



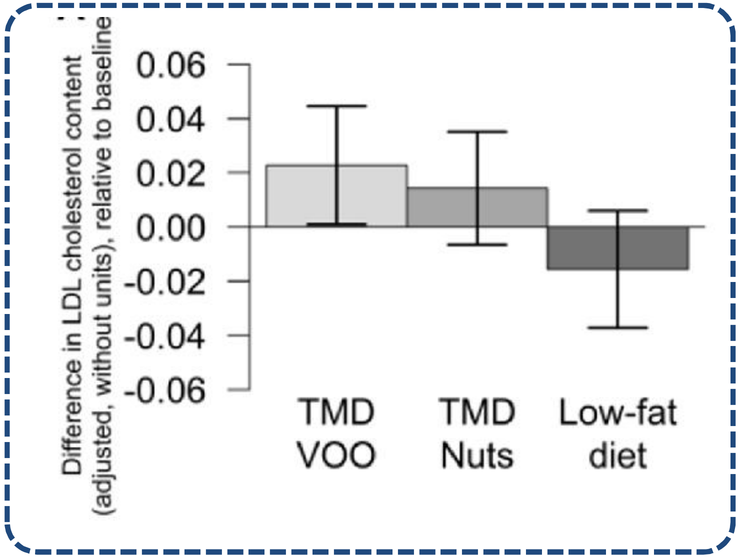
***: *P*<0.05; **: P<0.01; ***: P<0.001**

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

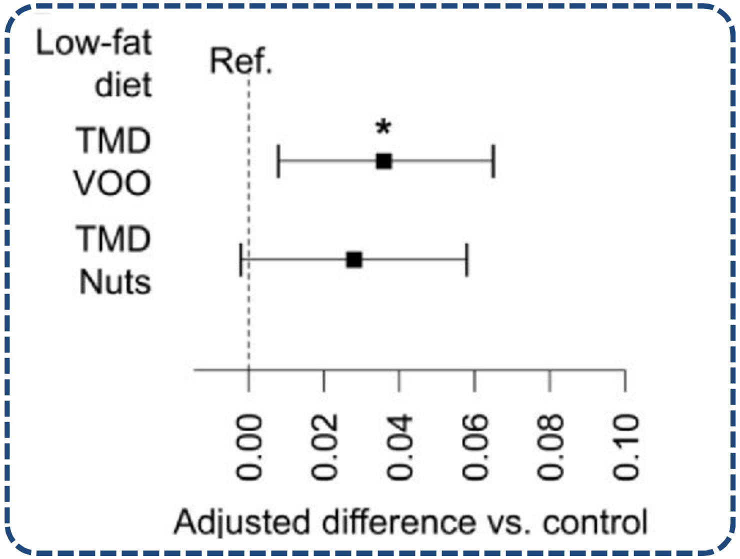
- ↑ LDL resistance against oxidation
- ↓ total LDL oxidation
- ↑ Indicator of LDL size

Hernáez A et al, MNFR 2017

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



*: *P*<0.05; **: P<0.01; ***: P<0.001

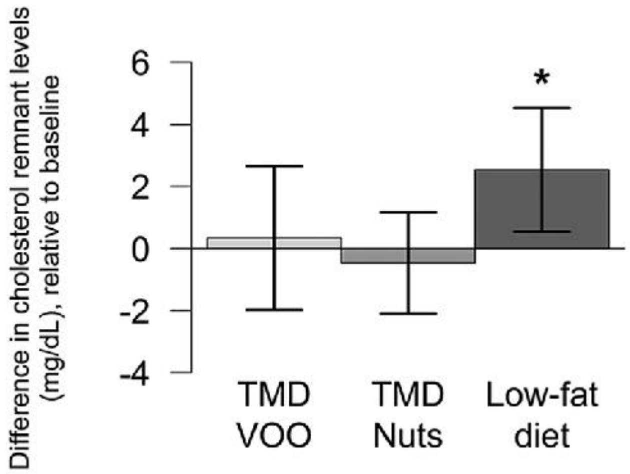
Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

- ↑ LDL resistance against oxidation
- ↓ total LDL oxidation
- ↑ Indicator of LDL size
- ↑ LDL cholesterol content

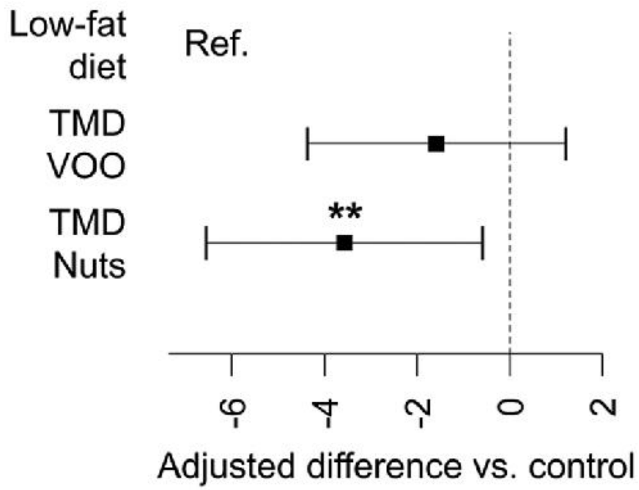
Hernáez A et al, MNFR 2017

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:

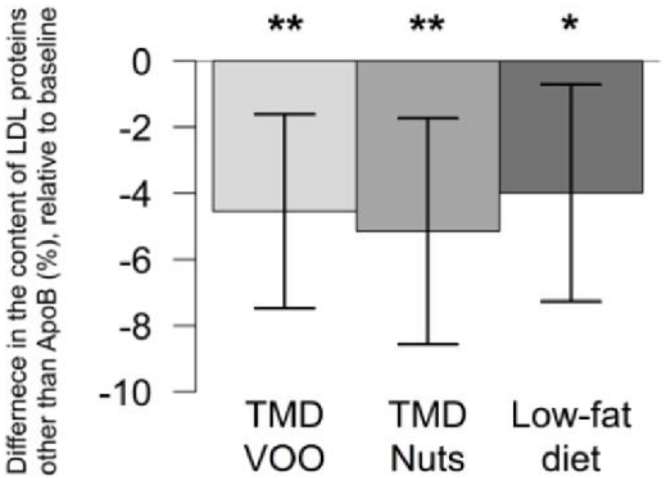


*: *P*<0.05; **: P<0.01; ***: P<0.001

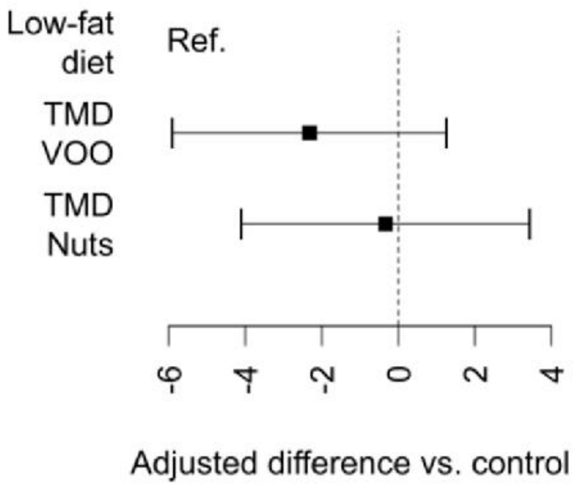
- ↑ LDL resistance against oxidation
- ↓ total LDL oxidation
- ↑ Indicator of LDL size
- ↑ LDL cholesterol content
- ↓ remnant cholesterol

Hernáez A et al, MNFR 2017

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:



*: *P*<0.05; **: P<0.01; ***: P<0.001

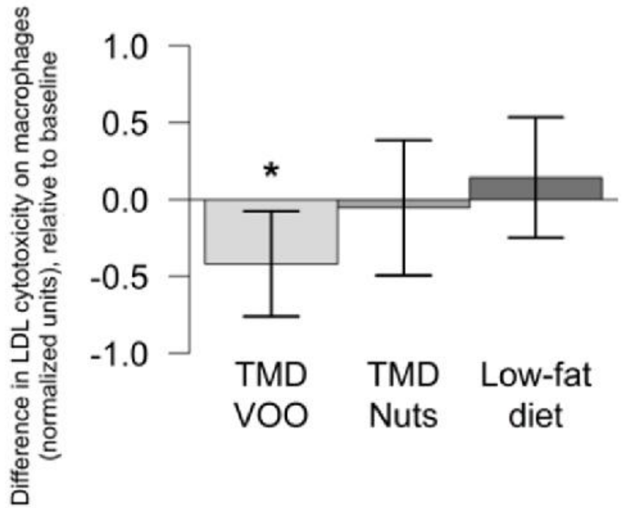
Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

- ↑ **LDL resistance against oxidation**
- ↓ **total LDL oxidation**
- ↑ **Indicator of LDL size**
- ↑ **LDL cholesterol content**
- ↓ **remnant cholesterol**
- ↓ protein other than ApoB content

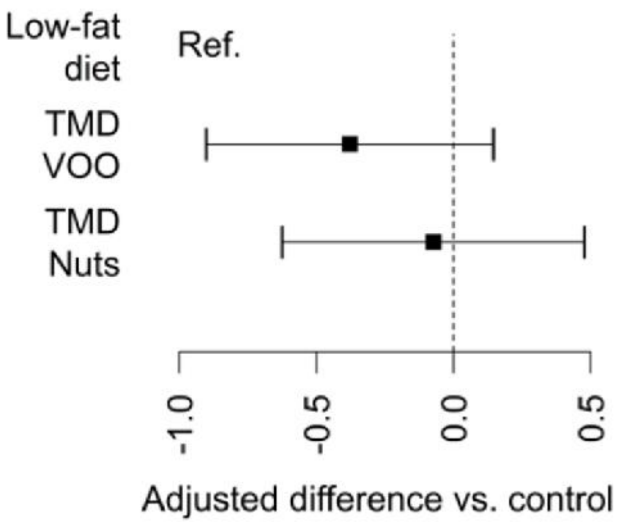
Hernáez A et al, MNFR 2017

Adherence to a traditional Mediterranean Diet (supplemented with virgin olive oil or nuts), for 1 year, induced:

Post- vs. pre- intervention:



Changes after the TMD interventions vs. the low-fat diet:

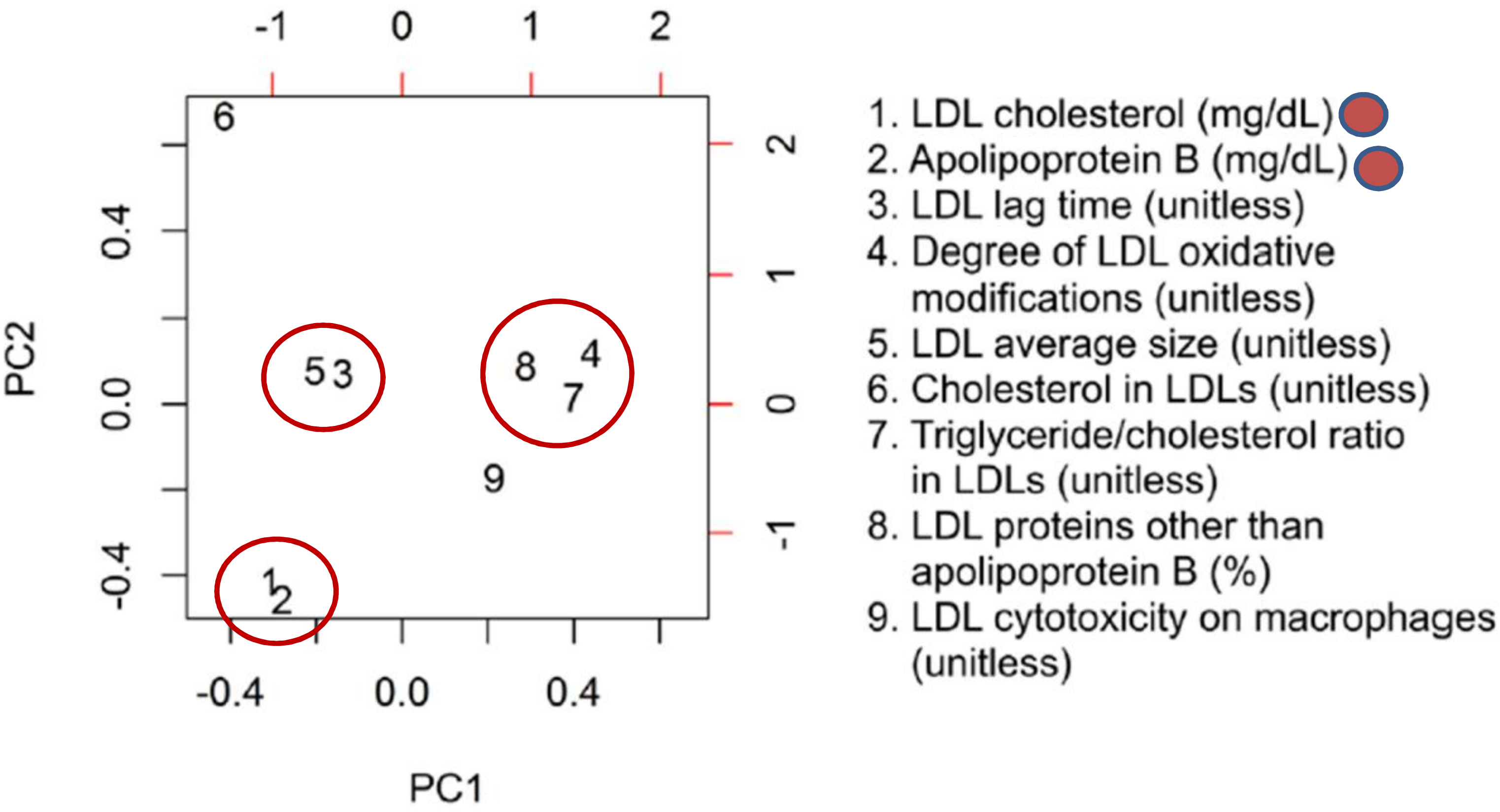


*: *P*<0.05; **: P<0.01; ***: P<0.001

- ↑ LDL resistance against oxidation
- ↓ total LDL oxidation
- ↑ Indicator of LDL size
- ↑ LDL cholesterol content
- ↓ remnant cholesterol
- ↓ protein other than ApoB content
- ↓ LDL cytotoxicity on macrophages

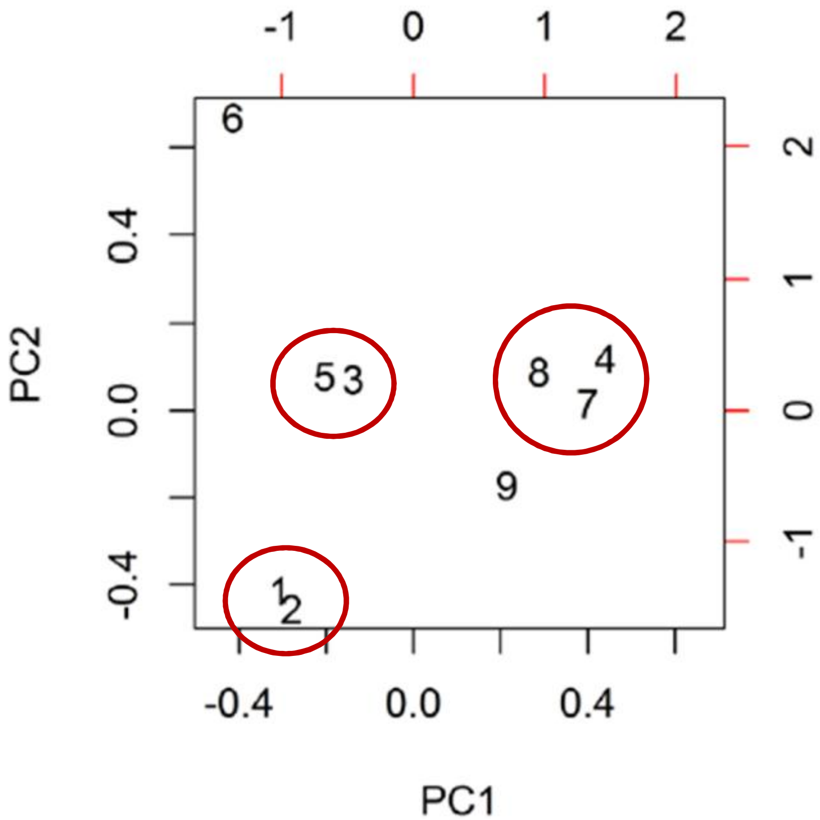
Hernáez A et al, MNFR 2017

Supplemental Figure 2. Bi-dimensional plot of the distribution of the changes in LDL atherogenicity traits variables after the TMD-VOO intervention, according to the two main components of the principal component analysis.



The two main components of this analysis are able to explain 83.9% of total variance of the data.

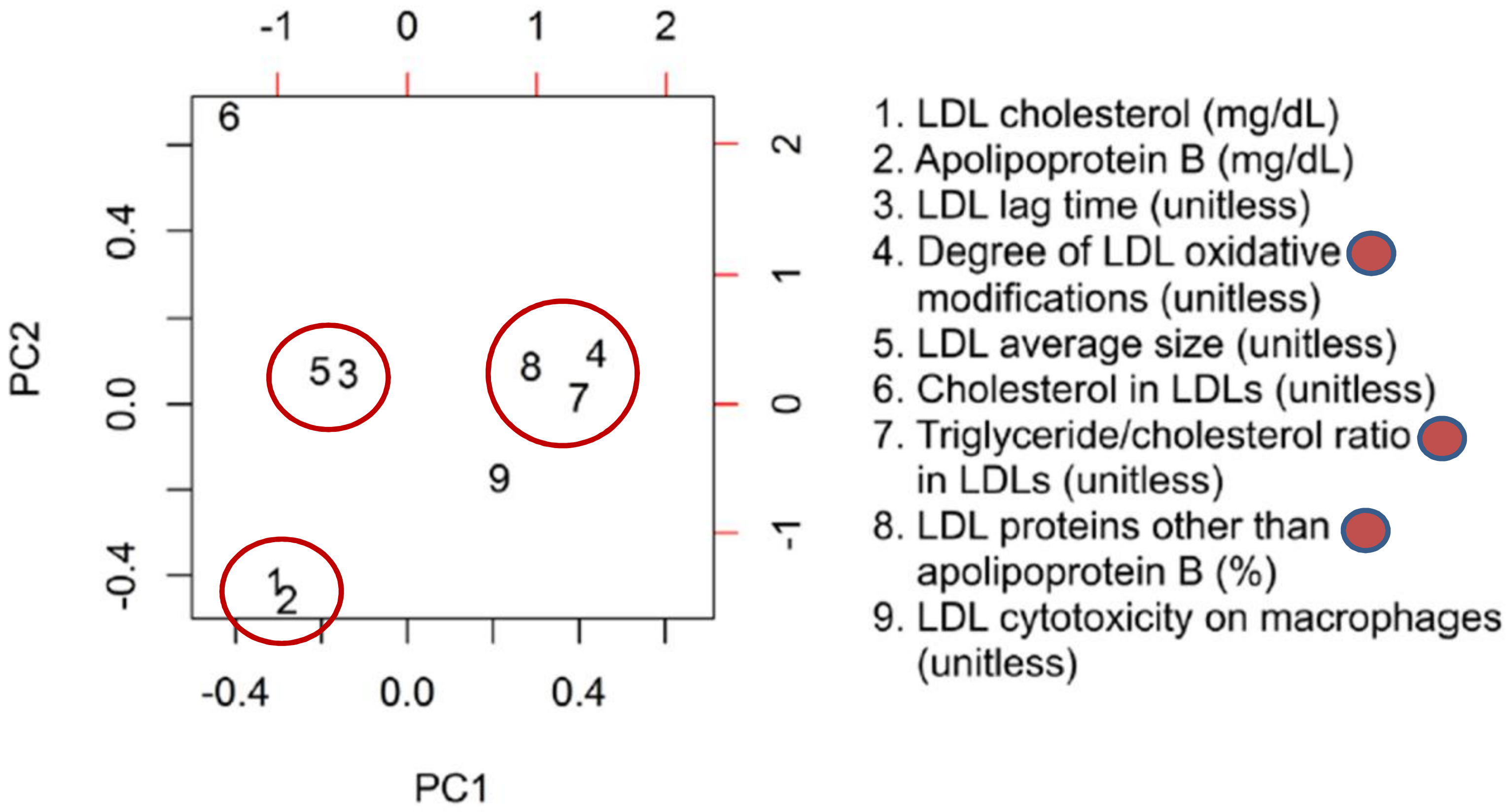
Supplemental Figure 2. Bi-dimensional plot of the distribution of the changes in LDL atherogenicity traits variables after the TMD-VOO intervention, according to the two main components of the principal component analysis.



1. LDL cholesterol (mg/dL)
2. Apolipoprotein B (mg/dL)
3. LDL lag time (unitless)
4. Degree of LDL oxidative modifications (unitless)
5. LDL average size (unitless)
6. Cholesterol in LDLs (unitless)
7. Triglyceride/cholesterol ratio in LDLs (unitless)
8. LDL proteins other than apolipoprotein B (%)
9. LDL cytotoxicity on macrophages (unitless)

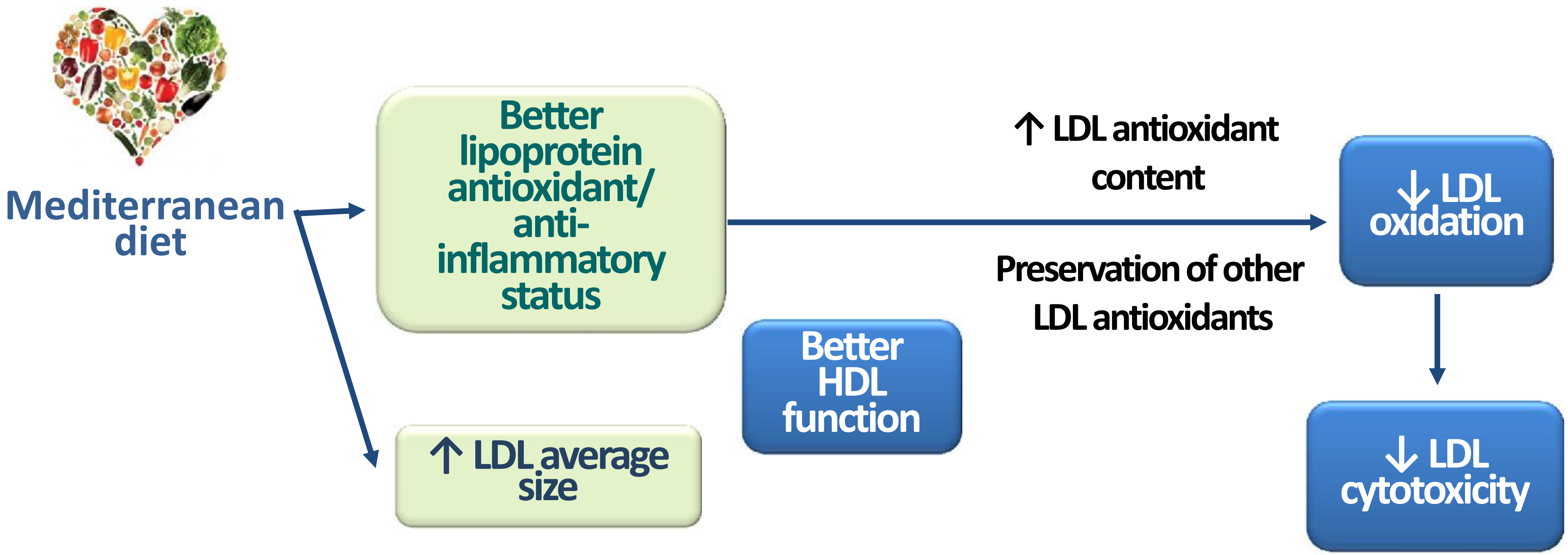
The two main components of this analysis are able to explain 83.9% of total variance of the data.

Supplemental Figure 2. Bi-dimensional plot of the distribution of the changes in LDL atherogenicity traits variables after the TMD-VOO intervention, according to the two main components of the principal component analysis.



The two main components of this analysis are able to explain 83.9% of total variance of the data.

LDL ATHEROGENICITY: The MD intervention improve the oxidative/inflammatory status, increased LDL size, and promoted a decrease of LDL oxidation. Overall produces a decrease of LDL cytotocicity and atherogenicity.



Conclusions

Long-term adherence to a traditional Mediterranean Diet is able to reduce LDL atherogenicity in high cardiovascular risk individuals:

* it improve LDL resistance against oxidation and LDL oxidation
* decreases LDL size,
* improves LDL composition,
* and lowers LDL cytotoxicity in macrophages.

Conclusions

The Mediterranean Diet pattern, including virgin olive oil as it main source of fat, is a useful tool for protecting against cardiovascular risk linked to lipid homeostasis.

Mechanisms beyond an improvement of basic lipid profile can be the enhance of cholesterol efflux promoted by HDLs, together with endothelial protection, and antioxidant actions exerted by this particle.

Changes towards a less atherogenic LDL profile can also play a role in the amelioration of cardiovascular risk.

Particle composition and oxidative/inflammatory status can be closely involved in HDL functionality and LDL atherogenicity.

Agraïments

Predimed
Prevención con Dieta Mediterránea

ciberobn
Centro de Investigación Biomédica En Red
Fisiopatología de la Obesidad y Nutrición

cnic
Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III

Instituto de Salud Carlos III

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN

FONDO DE INVESTIGACIÓN SANITARIA, MINISTERIO DE CIENCIA E INNOVACIÓN, FUNDACIÓN MAPHRE, CONSEJERIA DE SALUD DE LA JUNTA DE ANDALUCÍA, DEPARTAMENT DE SALUT DE LA GENERALITAT DE CATALUNYA, GENERALITAT DE VALENCIA Y GOBIERNO REGIONAL DE NAVARRA.

PATRIMONIO COMUNAL OLIVARERO FUNDACIÓN

BORGES
DA BUENOS FRUTOS

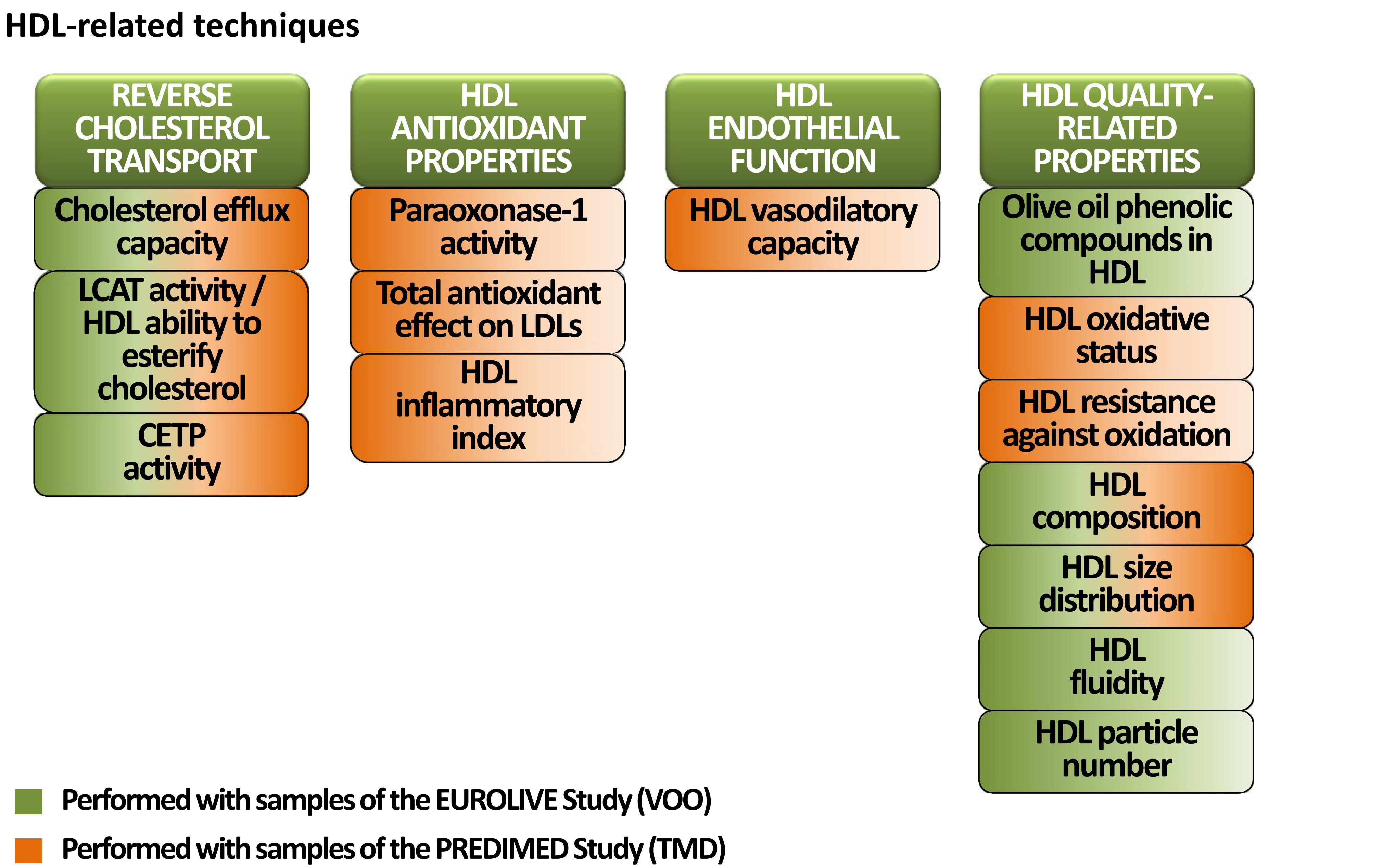
LA MORELLA NUTS

CALIFORNIA Walnuts

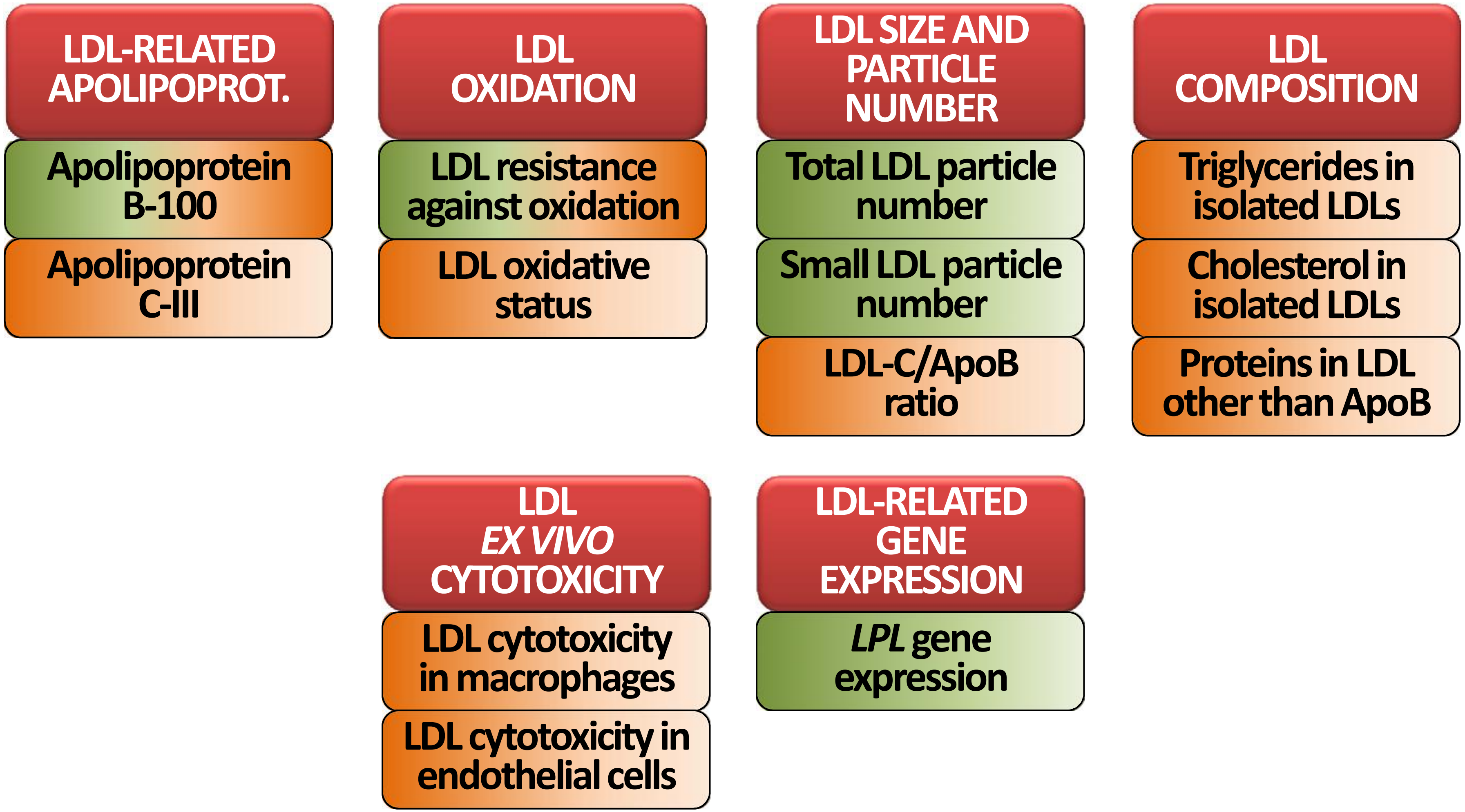
LA DONACIÓN POR PARTE DE LAS EMPRESAS ALIMENTARIAS DEL ACEITE DE OLIVA VIRGEN EXTRA Y LOS FRUTOS SECOS ES UNA CONTRIBUCIÓN SUSTANCIAL AL ESTUDIO. NINGUNA DE ESTAS COMPAÑÍAS HA DESEMPEÑADO NINGÚN PAPEL EN EL DISEÑO, RECOGIDA, ANÁLISIS NI INTERPRETACIÓN DE LOS DATOS.

IMIM
hospitaldelmar

Barcelona
Biomedical
Research
Park


HDL-related techniques
REVERSE CHOLESTEROL TRANSPORT
Cholesterol efflux capacity
LCAT activity / HDL ability to esterify cholesterol
CETP activity
HDL ANTIOXIDANT PROPERTIES
Paraoxonase-1 activity
Total antioxidant effect on LDLs
HDL inflammatory index
HDL ENDOTHELIAL FUNCTION
HDL vasodilatory capacity
HDL QUALITY-RELATED PROPERTIES
Olive oil phenolic compounds in HDL
HDL oxidative status
HDL resistance against oxidation
HDL composition
HDL size distribution
HDL fluidity
HDL particle number
Performed with samples of the EUROLIVE Study (VOO)
Performed with samples of the PREDIMED Study (TMD)

LDL-related techniques



Performed with samples of the EUROLIVE Study (VOO)

Performed with samples of the PREDIMED Study (TMD)

Small LDL particles are also more atherogenic [22]:

* they remain longer in circulation (they interact more poorly with LDL receptors),

* they are more easily oxidized,

* and tend to traverse the endothelial barrier more than large ones [23].

Low fat diet intervention decreases total and LDL cholesterol, decreases the HDL oxidation state, and increase the HDL size (also increase in a non significative way the cholesterol efflux) but:

- Increases tryglicerides core-content in HDL
- Increases the HDL inflammatory index

- Increases remnants of cholesterol
- decreases LDL size

Nitrolipids

plant- and marine-derived omega-3 PUFA

nitrates - nitrites (acid pH)

(Green leafy vegetables, characteristic of the TMD, are rich sources of nitrates)

↓

nitrolipids or nitro-fatty acids

(nitration)

↓

NITRIC OXIDE

Nitric oxide generation from the **nitrate-nitrite-nitric oxide pathway** appears to be an alternative source for nitric oxide synthase-dependent nitric oxide production, ensuring nitric oxide bioavailability when the endogenous 1-arginine/NO synthase pathway is dysfunctional in local hypoxic conditions

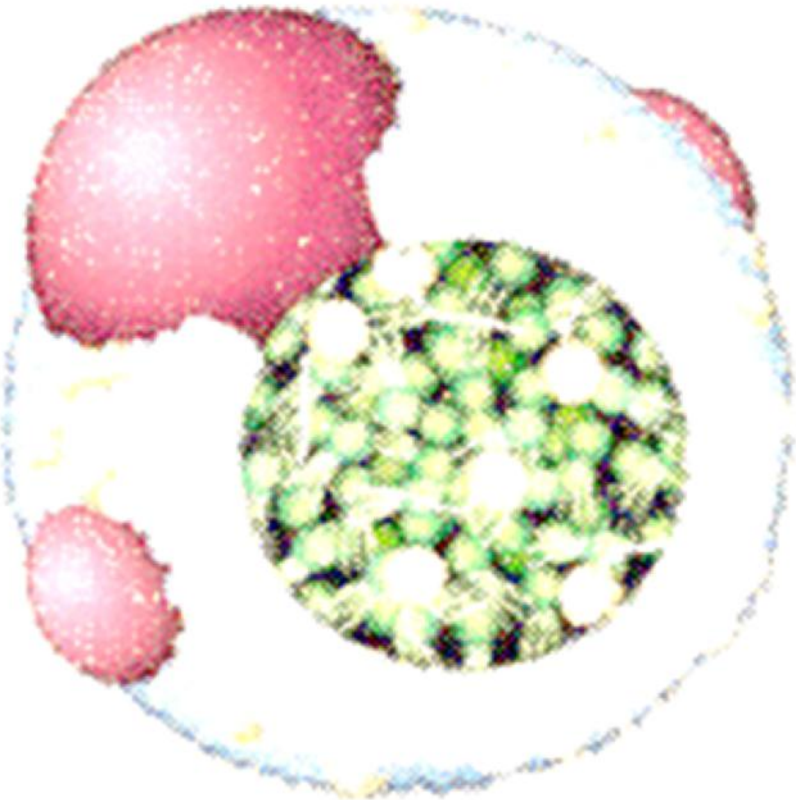
(Capurso C Vascul Pharmacol 2014)

- **stimulate smooth muscle relaxation**
- platelet activation
- suppress inflammation process

Membrane and monolayer composition

La composició lipídica-proteïna de monocapes de lipoproteïnes i bicapes de membranes cel·lulars s'associen íntimament al consum d'àcids grassos i altres nutrients.

La composició d'àcids grassos de les monocapes de lipoproteïnes i les membranes cel·lulars estan altament relacionats amb la fluïdesa de la mono- o bi-capa, i per tant a la viabilitat cel·lular final



Change in expression of cholesterol efflux-related genes was reported after a moderate (25 mL/day) and regular 3-week intake of olive oil in pre- and hypertensive subjects

(*Farràs M et al., J Nutr Biochem 2013*)

In addition, we recently have identified 6 potential loci associated with HDL functionality in

HOXA3, *PEX5*, *PER3, CMIP*, and *GABRR1*

through a epigenome-wide association study and candidate gene approach

(Sayols-Baixeras S *et al. ATVB 2017*).

One of the most highlight messages is that the main antioxidant of lipoproteins, vitamin E content in HDL, is preserved with the functional olive oil intervention.

This liposoluble vitamin is the main chain-breaking antioxidant in the organism and its protection is a key indicator of the HDL state

(*Farràs M et al. JBN under review*)

The reduction in plasma LDL-C concentrations

with the MedDiet even in the absence of weight loss appears

to be primarily due to an increased LDL clearance combined

with reduced intestinal cholesterol absorption, rather than

to any change in endogenous cholesterol synthesis.

Richard C, Br J Nutr 2012

Phenolic compounds can selectively stimulate the growth of beneficial bacteria, such as ***Lactobacillus*** which can participate in lowering cholesterol levels

(Landete, J. M. *Food Chem.* 2008)

Intestinal **lactobacilli** encodes bile-salt hydrolase which **deconjugates bile acids**, this prevents their reabsorption, and promotes the **excretion of larger amounts of free bile acids in faeces**

(De Smet, Ecol Health Dis 1994)

As synthesis of new bile acids rises in compensation, blood cholesterol levels fall

As a consequence, **the uptake of low density lipoprotein by hepatic apo B:E receptors** is upregulated and **blood cholesterol concentration decrease**s

(Brown MS, Annu Rev Biochem 1983)

Virgin olive oil, Mediterranean Diet and LDL atherogenicity: state of the art

Virgin olive oil and LDL atherogenicity

- MUFAs → ↓ LDL-C levels and ↓ LDL atherogenic traits
  Schwab U et al, *Food Nutr Res,* 2014
  Ashton EL et al, *J Am Coll Nutr,* 2001
- VOO → ↓ Circulating levels of oxidized LDL
  Covas MI et al, *Ann Intern Med,* 2006
- VOO → ↑ Antioxidant content in LDLs
  Gimeno E et al, *Eur J Clin Nutr*, 2002

Mediterranean Diet and LDL atherogenicity

- Mediterranean Diet → ↓ LDL-C/HDL-C
  Estruch R et al, Ann Intern Med, 2006
- Mediterranean Diet → ↓ LDL atherogenic traits
  Fitó M et al, *Arch Intern Med*, 2007
  Damasceno NRT et al, *Atherosclerosis*, 2013

Virgin olive oil, Mediterranean Diet and HDL functionality: state of the art

Virgin olive oil and HDL functionality

- VOO → ↑ HDL-C levels
  Covas MI et al, *Ann Intern Med,* 2006
- MUFAs → ↑ HDL functions (in non-controlled, non-randomized trials)
  Solà R et al, *Arterioscler Thromb,* 1993
  Solà R et al, *Free Radic Biol Med,* 1997
- VOO → ↑ HDL functions (in non-controlled, non-randomized trials)
  Helal O et al, *Br J Nutr*, 2013
  Loued S, *Br J Nutr*, 2013
- VOO → Improvement in the gene expression related to HDL function
  Farràs M et al, *J Nutr Biochem*, 2013
- VOO → Improvement in the endothelial function
  Valls RM et al,. xxx

Mediterranean Diet and HDL functionality

- Mediterranean Diet → Better HDL-related lipid profile
  Estruch R et al, Ann Intern Med, 2006
  Solà R et al, *Atherosclerosis,* 2011
- Antioxidant-rich dietary interventions → ↑ HDL functions
  Qin Y et al, *Am J Clin Nutr,* 2009
  Zhu Y et al, *J Clin Endocrinol Metab,* 2014
  McEneny J et al, *J Nutr Biochem*, 2013
  Daniels JA et al, *Cardiovasc Diabetol*, 2014

Differences between post- and pre-intervention values in the dietary profile of the volunteers in the three interventions of the study.

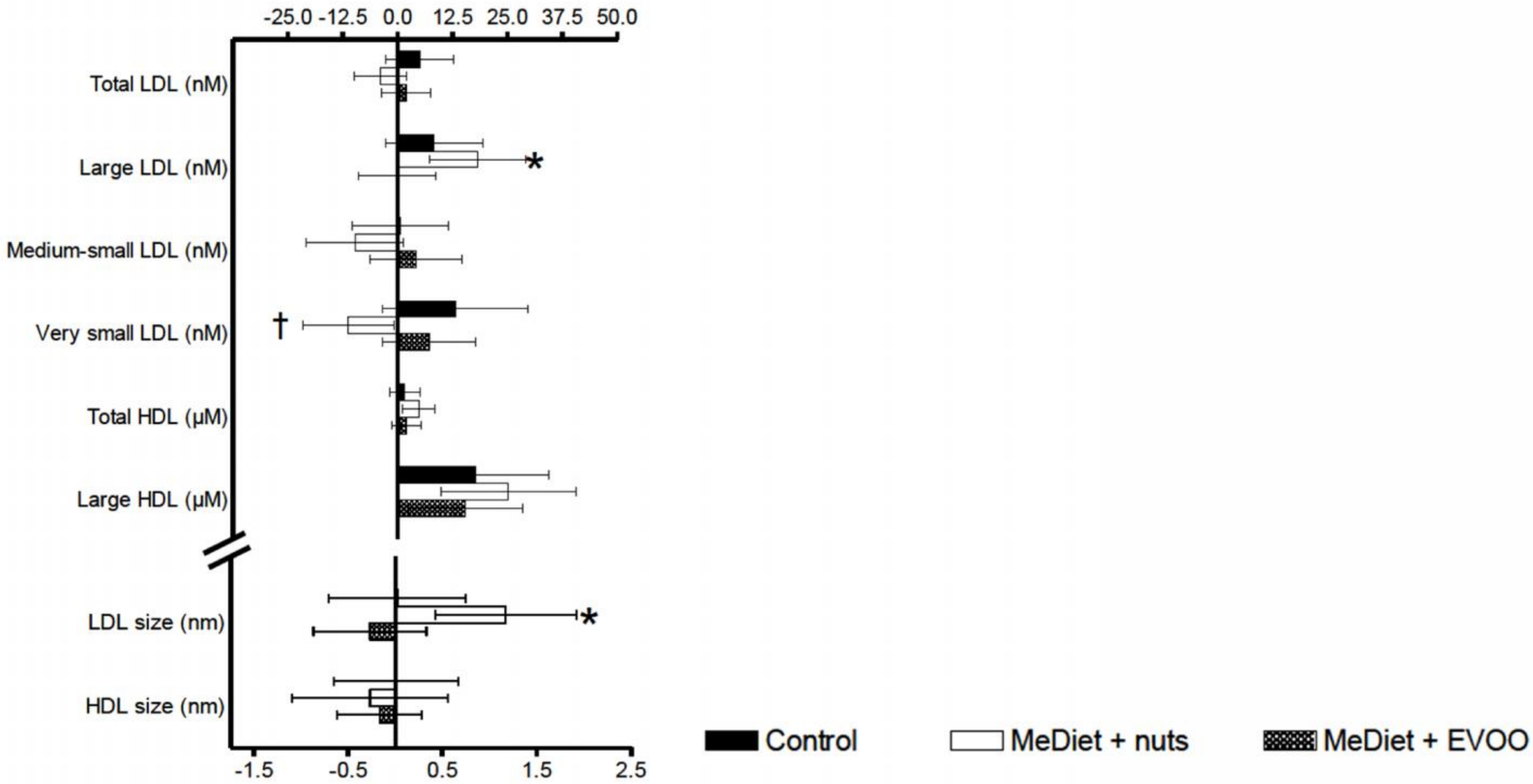
	TMD-VOO		TMD-Nuts		Low-fat control diet	
VARIABLES	**Difference**	***P*-value**	**Difference**	***P*-value**	**Difference**	***P*-value**
Adherence to TMD (score)	1.53 (1.82)*	<0.001	1.24 (1.91)	<0.001	-0.031 (1.99)	0.878
Total energy intake (kcal/day)	-7.95 (545)	0.884	57.6 (600)	0.344	-55.6 (661)	0.412
Carbohydrates (g/day)	-10.6 (79.3)	0.186	-6.18 (80.1)	0.447	4.95 (88.6)	0.585
Proteins (g/day)	2.01 (23.3)	0.391	-0.13 (22.0)	0.952	-4.30 (25.7)	0.105
Total fats (g/day)	3.26 (24.9)	0.194	10.0 (28.9)	<0.001	-5.83 (30.9)	0.069
MUFAs (g/day)	4.14 (13.0)	0.002	6.07 (15.3)	<0.001	-2.73 (16.6)	0.112
SFAs (g/day)	-1.58 (7.85)	0.046	-1.04 (7.63)	0.181	-3.20 (9.73)	0.002
PUFAs (g/day)	0.086 (6.96)	0.901	3.92 (7.50)	<0.001	-0.78 (7.07)	0.285
Fiber (g/day)	-0.50 (9.30)	0.591	2.40 (9.28)	0.012	0.18 (10.4)	0.863
Total olive oil (g/day)	11.0 (15.2)	<0.001	8.21 (18.1)	<0.001	1.06 (18.1)	0.566
Virgin olive oil (g/day)	32.4 (21.3)	<0.001	9.01 (22.4)	<0.001	5.37 (23.7)	0.029
Refined olive oil (g/day)	-21.4 (19.5)	<0.001	-0.76 (22.5)	0.740	-4.83 (19.9)	0.020

Differences between post- and pre-intervention values in the biochemical profile of the volunteers in the three interventions of the study.

	TMD-VOO		TMD-Nuts		Low-fat control diet	
VARIABLES	**Difference**	***P*-value**	**Difference**	***P*-value**	**Difference**	***P*-value**
Glucose (mg/dL)*	-0.037 (0.22)†	0.092	-0.003 (0.23)	0.916	-0.013 (0.24)	0.616
Triglycerides (mg/dL)*	0.011 (0.35)	0.762	0.047 (0.35)	0.193	0.045 (0.41)	0.297
Total cholesterol (mg/dL)	-0.35 (25.7)	0.896	-0.46 (26.5)	0.865	-7.72 (35.6)	0.039
HDL cholesterol (mg/dL)	-1.12 (6.58)	0.092	-0.99 (6.95)	0.169	-0.18 (8.88)	0.845
LDL cholesterol (mg/dL)	2.45 (20.9)	0.275	-0.24 (21.5)	0.916	-7.22 (28.7)	0.019
Apolipoprotein A-I (mg/dL)	0.38 (12.7)	0.814	1.88 (12.9)	0.196	2.08 (11.9)	0.135
Apolipoprotein B (mg/dL)	-0.78 (14.5)	0.669	0.98 (14.5)	0.550	-0.66 (18.2)	0.748
HDL cholesterol/Apolipoprotein A-I (unitless ratio)	-0.005 (0.020)	0.031	-0.010 (0.025)	<0.001	-0.005 (0.026)	0.129
Apolipoprotein B/Apolipoprotein A-I (unitless ratio)	-0.007 (0.13)	0.668	-0.004 (0.10)	0.745	-0.017 (0.15)	0.345

*: Log-transformed variables. †: Mean (SD). *TMD-VOO*: Traditional Mediterranean Diet enriched with virgin olive oil. *TMD-Nuts*: Traditional Mediterranean Diet enriched with mixed nuts

Figure 1. Percent changes from baseline in selected LDL and HDL subfractions.



Values are means (95% CI). MeDiet, Mediterranean diet; EVOO, extra-virgin olive oil.

* $p < 0.05$ compared to the MeDiet with EVOO group; † $p < 0.05$ compared to the control diet group, by ANOVA with Bonferroni post-hoc test.

Despite regional variations,
the use of these 3 products
is common in all Mediterranean countries.

Wheat

Olive tree

Vineyard

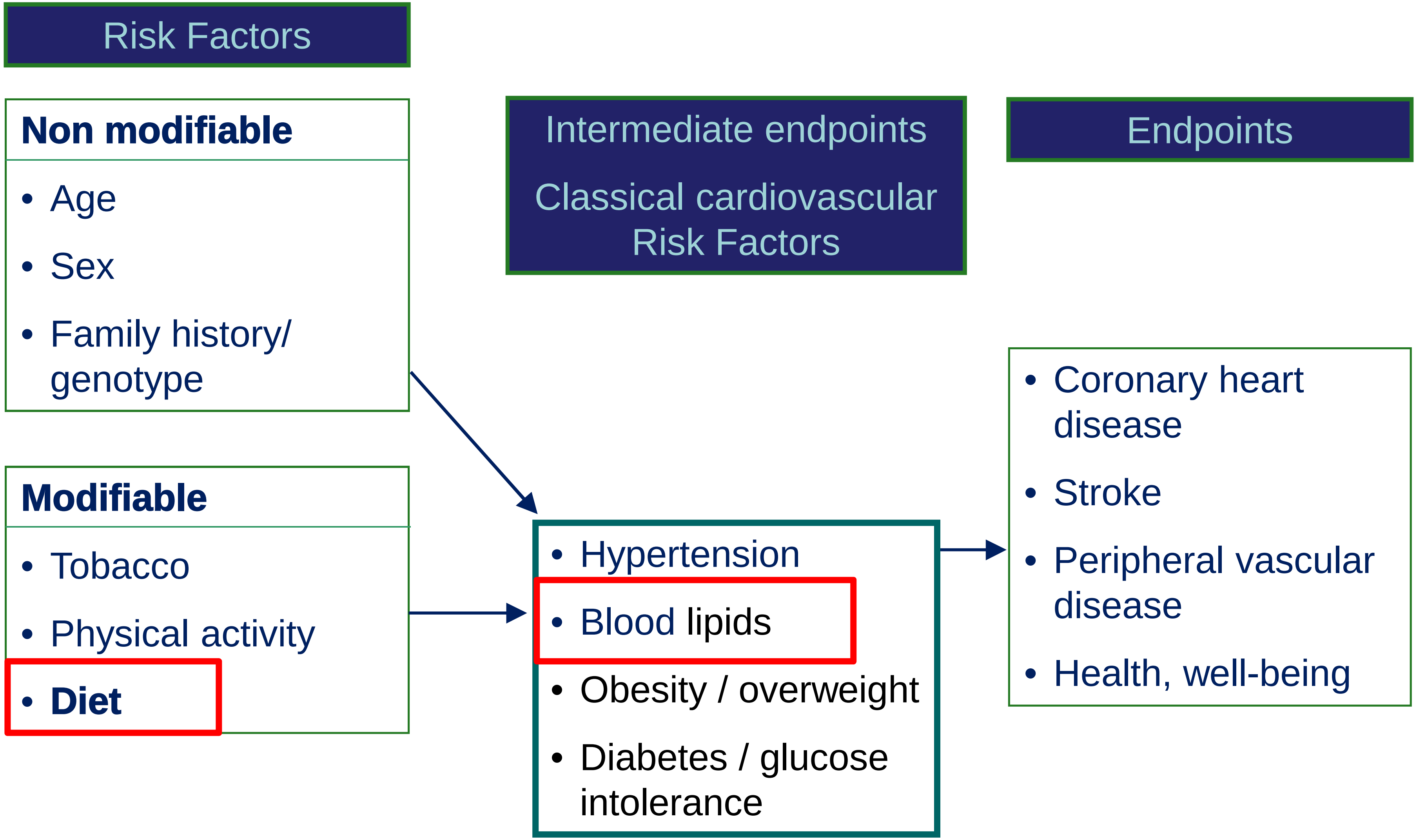
The Mediterranean diet trilogy


Relevance of overall high-quality food patterns.
Synergistic interactions and cumulative effects

The underlying mechanisms by which a healthy diet can exert its beneficial effects on CVD are not fully understood.

The benefits of the Mediterranean diet have been traditionally attributed to its richness in antioxidants, due to a high vegetal food intake.

De Sosa et al.
Curr Vasc Pharmacol 2010.

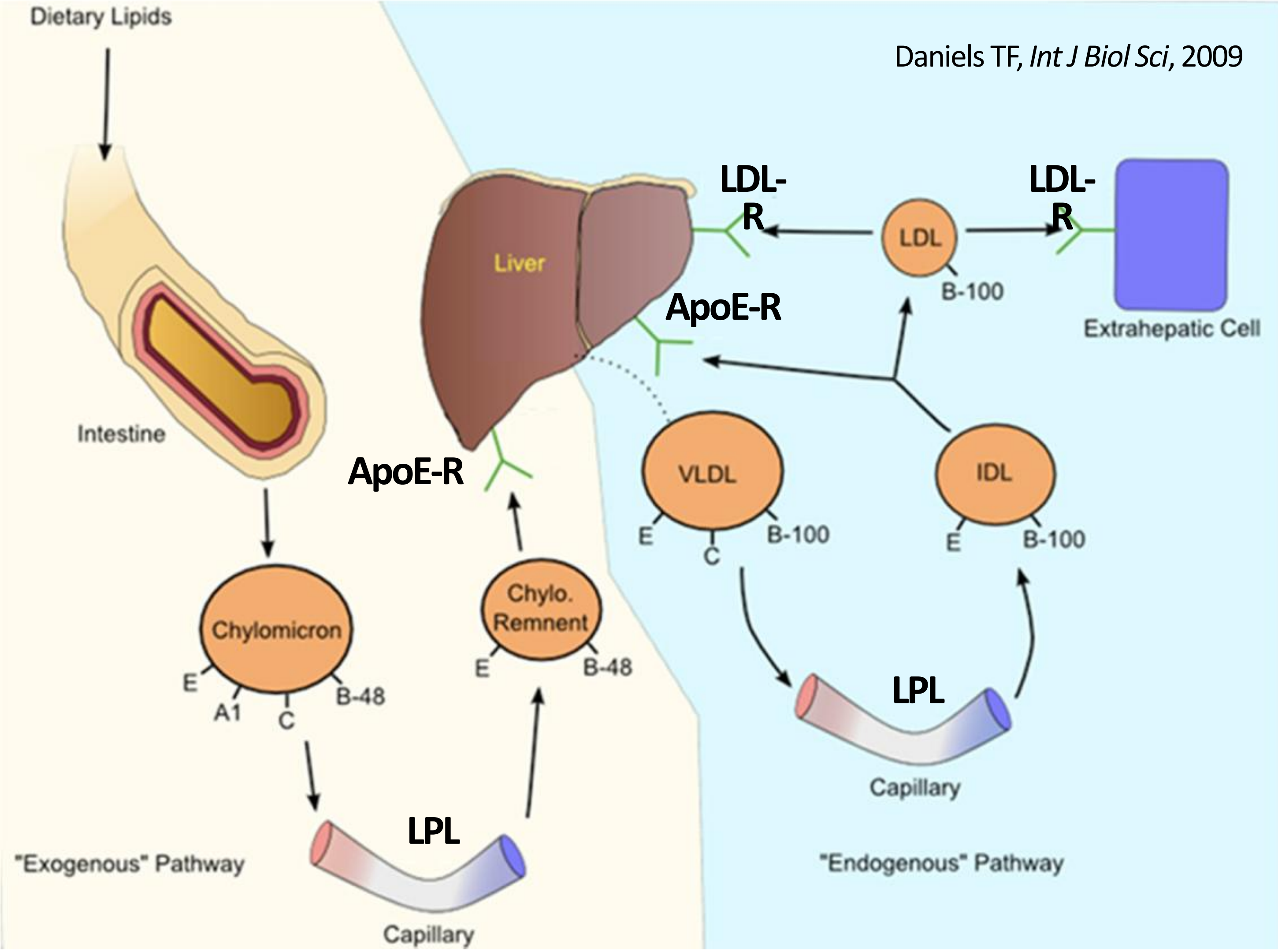


Cardiovascular Disease Risk Factors (Adapted from Ordovas, J.M. (2006) Am J Clin Nutr 83, 443-446)

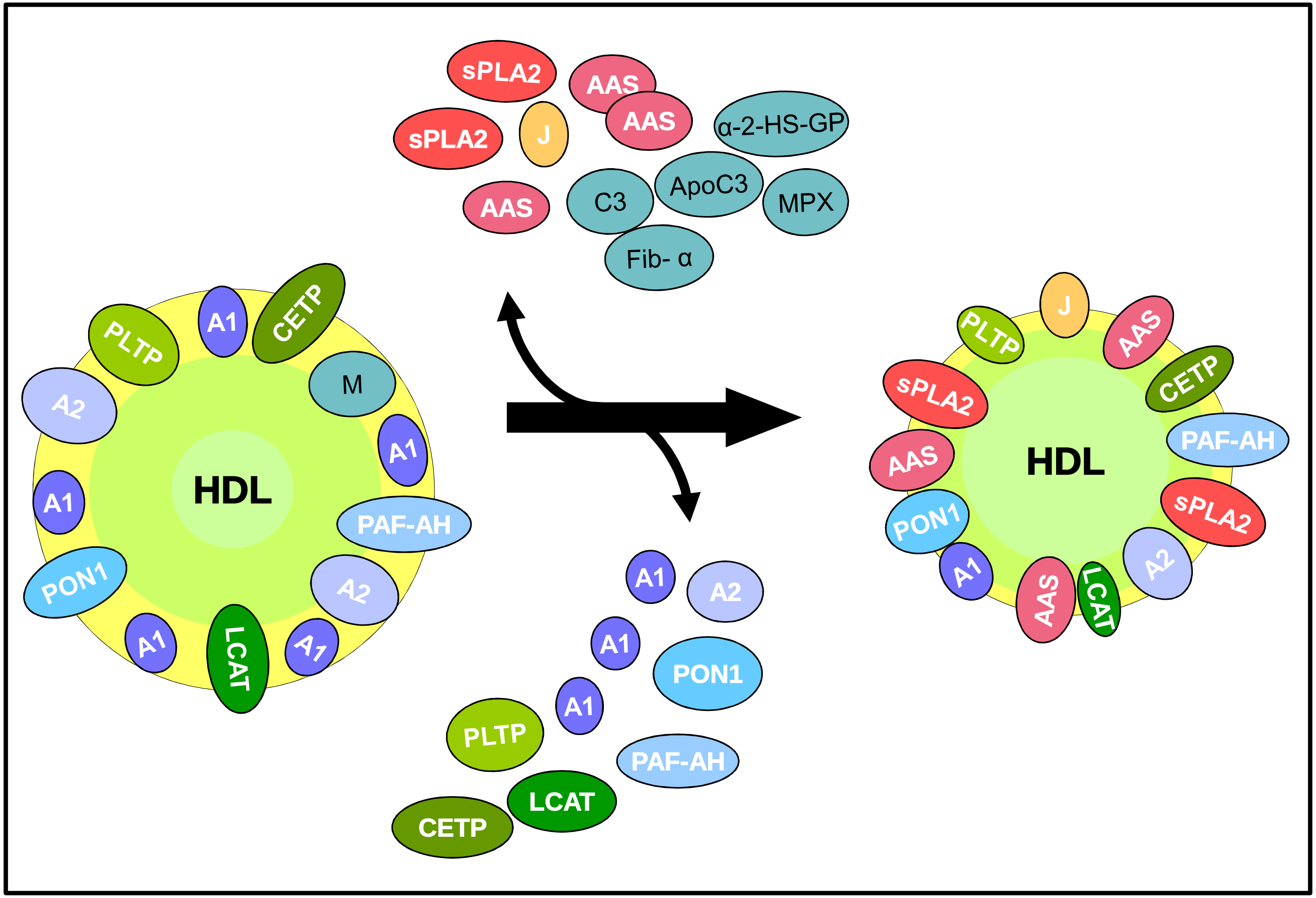

The Mediterranean diet pattern and virgin olive oil interventions has been shown to improve **lipid profile**.

Nevertheless, it is becoming increasingly more accepted that the information provided by **HDL functionality and LDL pro-atherogenic traits** can be more informative with respect to the unexplained cardiovascular risk of an individual.

Lipoproteins: delivery of lipids to peripheral cells

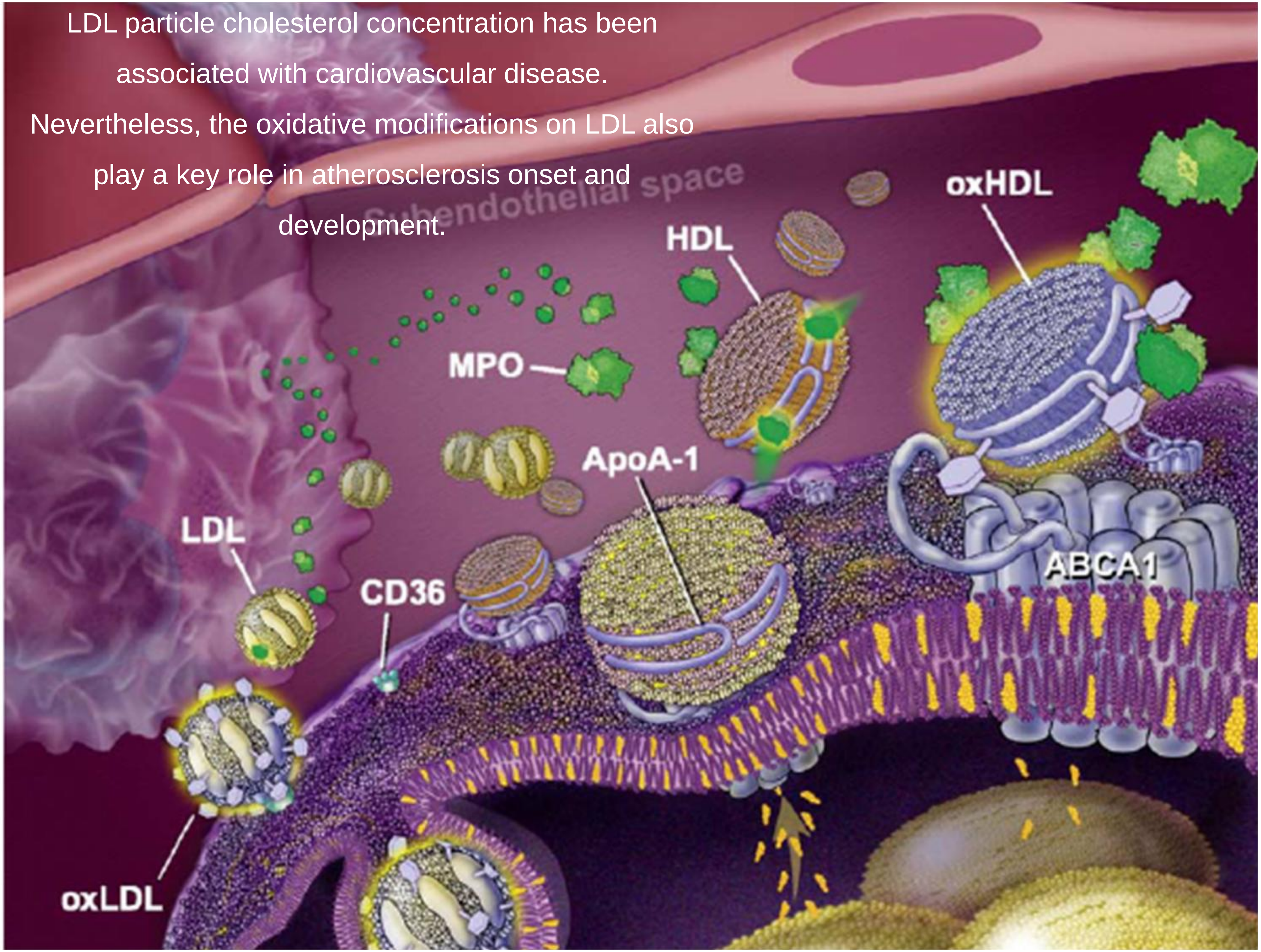


The dietary lipids are transported by the lipoproteins of intestinal synthesis, and the lipids of endogenous origin are transported by hepatic lipoproteins.
Given that cholesterol can be toxic for the cells when it is found at high levels, the uptake of lipid excess of from peripheral cells is performed by HDL.



During the systemic inflammatory processes, the serum amiloide A, among other acute phase proteins, replaces the ApoA1 and PON1 and produces a pro-inflammatory HDL

LDL particle cholesterol concentration has been associated with cardiovascular disease. Nevertheless, the oxidative modifications on LDL also play a key role in atherosclerosis onset and development.



HDL-related techniques

REVERSE CHOLESTEROL TRANSPORT	HDL ANTIOXIDANT PROPERTIES	HDL ENDOTHELIAL FUNCTION	HDL QUALITY-RELATED PROPERTIES
Cholesterol efflux capacity	Paraoxonase-1 activity	HDL vasodilatory capacity	HDL oxidative status
LCAT activity / HDL ability to esterify cholesterol	Total antioxidant effect on LDLs		HDL resistance against oxidation
CETP activity	HDL inflammatory index		HDL composition
			HDL size distribution

LDL-related techniques

LDL OXIDATION

- LDL resistance against oxidation
- LDL oxidative status

LDL LIPID/PROTEIN COMPOSITION

- Apolipoprotein B-100
- Proteins in LDL others than ApoB
- Triglycerides in isolated LDLs
- Cholesterol in isolated LDLs

LDL SIZE

- LDL-C/ApoB ratio

LDL *EX VIVO* CYTOTOXICITY

- **LDL cytotoxicity in macrophages**

Increased adherence to TMD after the TMD-VOO intervention was due to: increases in the consumption of **virgin olive oil, legumes, fish, white meat;** and decreases in the consumption of **precooked foods and industrial confectionary.**

Increased adherence to TMD after the TMD-Nuts intervention was due to increases in the consumption of **nuts, virgin olive oil (less than in the TMD-VOO intervention), fruit, vegetables, and oily fish**
and decreases in the consumption of **red and processed meat, precooked meals, and industrial confectionary.**

Last, adherence to a low-fat diet was observed as
a decrease in total fat intake (particularly saturated fats) resulting from decreases in the consumption of **high-fat dairy products, red and processed meat, precooked meals, and industrial confectionary.**