Lee Nunn
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The Effects of Dietary Fat Intake on the Composition and Function of HDL in Relation to Cardiovascular Health

Abstract

Background

The extensive use of extra virgin olive oil (EVOO) in the Mediterranean diet (MD) has been positively associated with reduced cardiovascular risk (CVR) and cardiovascular disease (CVD). Various modifications to HDL in an antioxidant environment are thought to be responsible for this effect.

Objective

This review paper seeks to demonstrate the relationship between dietary fat consumption on the composition and functionality of HDL as it relates to reverse cholesterol transport (RCT), cholesterol efflux capacity (CEC), and endothelial cell (EC) repair mechanisms.

Methods

Journal articles were chosen from September 2021 through October 2021 using peer-reviewed literature from the PubMed database. Inclusion criteria were being published in the English language within the last five years. Various search terms used for the paper included extra virgin olive oil, Mediterranean diet, HDL, cardiovascular disease, composition, dysfunction, reverse cholesterol transport, atherosclerosis, and endothelial cell repair. Reviews were excluded.

Results

Dietary fat intake has direct effects on the composition of HDL which then alters its ability for RCT, CEC, and EC repair, essential mechanisms required for the prevention of atherosclerosis and CVD. EVOO, as part of an antioxidant MD, promotes a more functional HDL composition which improves these mechanisms and leads to overall reduced CVR and CVD.

Conclusions

High intake of EVOO, in the context of a MD, positively affects the composition of HDL promoting cholesterol hemostasis and decreasing CVR and atherosclerotic outcomes. Dietitians, through nutrition education and counseling, can apply these findings at the clinic level directly aiding patients in their health journey.

Introduction

Prominent nutritionist and physiologist Ancel Keys initiated the epidemiological Seven Countries Studies (SCS) in the late 1950's leading to a series of ongoing publications relating diet to cardiovascular disease (CVD).[1-4] The study was the first systematic study of diet tracking disease-specific risk and death rates between and within populations with contrasting traditional

diets.[5] It compared Mediterranean style diets of southern Europe rich in monounsaturated fats (MUFAs) from olive oil (OO), to northern European style diets, like that of the U.S, that were higher in saturated fats (SFAs).[3] This ultimately led to the diet-heart hypothesis which causally relates dietary intake of SFAs to increased serum cholesterol and incidence of CVD.[6] This hypothesis was adopted, and subsequently engrained, in the Dietary Goals of the United States in 1977 and has continued to guide dietary recommendations for Americans since.[7]

The Mediterranean diet (MD) is high in plant-based foods and MUFAs in the form of OO, and includes fruits, vegetables, whole grains, nuts, seeds, fish, and poultry, with minimal red meat and dairy and low to moderate red wine consumption.[8] The MUFA content of this diet plays a crucial role in its cardioprotective effects. Specifically, extra virgin olive oil (EVOO) rich in high phenolic content is linked to protecting the oxidation status of lipoproteins in circulation leading to reduced cardiovascular risk (CVR).[9-12]

Lipoproteins are carriers of molecules that cannot travel in aqueous blood by themselves. Their cargo is composed of cholesterol, cholesterol esters, triglycerides, fat soluble vitamins, phospholipids and other components that are hydrophobic. Low-density lipoproteins (LDLs) are cholesterol ester (CE) ladened and carryout Forward Cholesterol Transport (FCT), the mechanism by which cholesterol is transported from the liver throughout the circulatory system and delivered to tissues.[13] LDL is primarily responsible for this due to its half-life of approximately 3 days in persons without Familial Hypercholesterolemia.[14] This is in contrast to reverse cholesterol transport (RCT) where HDL returns excess cholesterol from the tissues to the liver for uptake and further processing.[15]

Cholesterol efflux capacity (CEC) is the efficiency and ability of HDL to remove excess cholesterol from tissues, such as endothelial cells (ECs) lining an artery, and is a marker for endothelial repair, CVR and CVD.[16-18] ECs line all blood vessels and are the interface between blood, lymph and tissues facilitating exchange including influx and efflux of cholesterol via LDL and HDL.[19] In a pro-inflammatory condition, ECs become burdened with oxidative stress, permeable and encourage monocyte adhesion and transmigration from the blood to the subendothelial layer of the arterial wall intima.[20,21] Accumulation of cholesterol laden foam cells ultimately leads to fatty streaks, plaques and atherosclerosis, the end result of excessive cholesterol deposits in the intima of artery walls. HDL can transverse the EC lining and play a critical role in removing cholesterol from overly burdened foam cells facilitating repair of the blood vessel wall and preventing atherosclerosis.[22,23]

Researchers recently discovered that HDL concentration (HDL-C) is not the primary driver of HDL function, but rather the constituent subtypes of HDL and its oxidative state, known as HDL quality, that confers efficient CEC.[22] This paper will seek to review some major findings related to how lipids in the diet, specifically the MD, affect the composition and function of HDL, how age plays a factor in HDL quality and how these changes, along with a pro-inflammatory environment, impact HDLs ability to participate in CEC and aid in repairing the endothelial lining.

Methods

This review paper was written using peer-reviewed English language literature from the PubMed database published within the last five years. Searches were conducted from August 2021 to September 2021. Various sets of key terms were used for each section of the research. In *Subsection 1* key word searches used were extra virgin olive oil, Mediterranean diet, HDL, and cardiovascular disease. Papers were selected based upon a diet intervention, namely the

Mediterranean Diet, and how that affected cardiovascular outcomes. In *Subsection 2*, articles focused on how HDL had been affected in its composition with relation to diet and age. Keyword searches used were combinations of HDL, dysfunction, composition, quality, and Reverse Cholesterol Transport. In *Subsection 3*, more mechanistic focused research was required so keyword searches used were reverse cholesterol transport, atherosclerosis, endothelial cell repair and HDL dysfunction. All different study methodologies were permissible for the results section, with the exception of review papers.

Results

Demographics

Of the 6 papers selected, 3 were variations of cross-sectional studies, one was an observational prospective study, another was a "quasi before and after" study and another was *in vitro*. Populations from Spain, Canada, Japan, and Iran were represented. Spain had the largest number of participants, 7,216 for the Guasch-Ferre *et al*. PREvención con DIeta MEDiterránea (PREDIMED) study, 502 for the Girona et al. study, and an additional 296 for the Hernaez et al PREDIMED study. Otrante *et al*. had 84 participants for the age-related research and Variji et al had 174 participants in the Coronary Artery Disease (CAD) study. Kameda *et al*. had the smallest number of participants, 7 for the *in vitro* Human Umbilical Vein Endothelial Repair (HUVEC) model study. In all human-subject studies, men and women were represented ranging in age from 23 to 85 years old. Most of the participants were at high CVR or had CVD while others were being tracked for CVD outcomes.

When studying the effects of dietary fat intake in the context of the MD, data from the PREDIMED study, which was conducted through a multicenter, randomized, controlled, clinical trial, cohort study analogous to NHANES in design, was used in 2 studies related to CVR and CVD outcomes. The 2 studies obtained specific sets of data from the PREDIMED cohort to investigate the effect of CVR, CVR scores and CVD outcomes.

Subsection 1 - The impact of EVOO, OO and the MD on CVD outcomes and HDL composition related to age after an EVOO intervention

Guasch-Ferre *et al*. found, after a median follow-up of 4.8 years, that of the 7,216 PREDIMED cohort participants 277 had cardiovascular events (CVE) and 323 deaths had occurred. In the highest energy-adjusted tertile, OO and EVOO consumption had 35% (HR; 0.65; 95% CI; 0.47 to 0.89) and 39% (HR: 0.61: 95% CI; 0.44 to 0.85) CVD risk reduction in an at-risk aged population, respectively. Consuming an additional 10g/d of EVOO decreased risk of CVD and mortality by 10% and 7%, respectively. Cancer and all-cause mortality were not affected by any increased consumption.[24] In an age-comparison study (n = 84), Otrante *et al*. found that age-related shifts in HDL subclass distribution at baseline affected CEC. The older age group, (mean age: 70.72 ± 5.6 years) had lower levels of CEC at 11.12% ($p<0.001$) than the younger age group, (mean age: 31.81 ± 6.79 years). After 12 weeks of consuming 25ml/d of EVOO, the CEC from the elderly subjects increased by approximately 8% ($p < 0.02$). However, after the intervention, the CEC was significantly improved for all participants by 7.12% ($p < 0.03$) compared to baseline values. For the elderly group CEC still remained significantly lower (6.91%, $p < 0.03$) than the younger age group. A Pearson's correlation analysis showed that the CEC at baseline was significantly and negatively associated with the age of subjects ($r = -0.28$ and $p < 0.003$), but this correlation disappeared after 12 weeks of intervention ($r = -0.012$ and $p < 0.24$). After HDL subclass analysis, HDL from the older age group was found to have lower

levels of large HDL (L-HDL) ($p<0.03$) and higher levels of small HDL (S-HDL) ($p<0.002$) compared to the younger group. After multiple linear regression analysis, a positive correlation was found between CEC and L-HDL levels ($r=0.35$, $p<0.001$) and an inverse correlation between CEC and S-HDL ($r=-0.27$, $p<0.01$). These results were maintained after adjusting for variables such as age, sex, BMI, SBP. DBP, TG, LDL-C, HDL-C, and blood glucose.[25]

Subsection 2 - HDL composition in high CVR patients and HDL-TG as a CVR biomarker

Hernaez *et al*. found that, in their high CVR HDL functionality group (n = 296, median age 65.9) high CVR was associated with low HDL cholesterol (HDL-C) and ApoA-I levels, low cholesterol efflux values, high HDL oxidation (HDL-ox), high content of triglycerides in the HDL core ($P<0.001$ in the five previous cases), and low values of the HDL2/HDL3 ratio (smaller HDL size) ($P = 0.002$).[26] In the cross-sectional study (n = 502, median age 61 years old) focusing on HDL-TG, subjects with type 2 diabetes mellitus (T2DM), metabolic syndrome (Met-S), dyslipidemia or any combination, Girona *et al*. found that HDL-TG was positively correlated with total TG (0.652, $p<0.0001$) and cholesterol ester transfer protein (CETP) (0.264, $p<0.001$) and negatively correlated with HDL-C (-0.135, $p<0.002$) and HDL particle number (HDL-P). As the number of metabolic syndrome factors increased, HDL-TG was higher. Patients with carotid plaques (32.8%) also showed higher HDL-TG ($p<0.05$) but was negatively correlated with HDL-C ($p=0.05$). Unlike HDL-C, HDL-TG is directly associated with metabolism and arteriosclerosis vascular alterations.[27]

Subsection 3 - How enzyme modification of HDL affect migration and wound healing and enzymes are predictive biomarkers for CVD

Kameda *et al*. used control HDL (normal), myeloperoxidase-oxidized (MPO-oxidized) HDL, *N*-Homocysteinylated (*N*-Hcy) HDL and plasma samples from 7 patients with varying levels of troponin I (after acute myocardial infarction) and looked for effects on migration using a HUVEC *in vitro* model. HDL increased HUVEC migration to 123.4 ± 15.4% of the control value ($p = 0.007$), while MPO-oxidized HDL decreased migration to 97.8 ± 17.5% compared to control ($p = 0.003$). MPO-oxidized HDL decreased migration to 78.9 + 12.3% of normal HDL. Treatment with *N*-Hcy HDL significantly increased HUVEC migration relative to control at both 1 and 10 mM by 144.8 ± 38.8% and 129.1 ± 25.5%, respectively, while normal HDL induced 162.0 ± 76.9% migration, compared to control. *N*-Hcy was also detected in all patient plasma samples and was significantly higher in the patient group than in healthy subjects (19.1 ± 3.8% vs. 6.9 ± 4.2%, $p < 0.001$). HDL from healthy subjects increased HUVEC migration to 120.9 ± 9.6% of the control value, while HDL from patients increased migration to 103.1 ± 12.1% compared to control ($p = 0.052$ vs. healthy subjects).[28] MPO can also be used in the prediction of CAD when coupled with paraoxonase 1 (PON1). Varjil *et al*. noted that, in the Iranian sample set, PON1 activity was significantly lower in CAD patients ($p = 0.007$). In addition, patients with CAD had a significantly higher MPO/PON1 ratio than non-CAD subjects ($p = 0.02$). ROC curve analysis showed that PON1 (AUC = 61%, $p = 0.003$) and MPO/PON1 (AUC = 60%, $p = 0.01$) have a better diagnostic predictive value than MPO alone (AUC = 50%, $p = 0.42$) in detecting patients with CAD. For the age range ≥52 and < 60 years, PON1 and MPO/PON1 were found to have significantly stronger discriminatory power (AUC = 69%, $p = 0.008$ for PON1; AUC = 66%, $p = 0.022$ for MPO/PON1). Multivariate analysis revealed PON1 as an independent variable was a predictor of CAD [adjusted OR = 0.98 (0.97–0.99) $p = 0.024$] and also multi-vessel CAD [odds ratio (OR) = 0.98; $p = 0.017$].[29]

Discussion

Study Comparisons

The studies of Guasch-Ferre *et al*. and Hernaez *et al*. had similar baseline data due to their PREDIMED data source. Each used high CVR patients.

Guasch-Ferre *et a*l. implemented an intervention using EVOO, OO and nuts (results not reported) and followed patients for a median of 4.8 years before looking at CVD outcomes. Otrante *et al*. specifically looked at how HDL subclass distribution changes when treated with an EVOO intervention among young and old age groups. Hernaez *et al*. assessed CVR score by measuring atherogenic particles and CEC and Girona *et al*. looked at classical biomarkers of metabolic disease in subjects that had plaque formation, a marker for CAD. Both Kameda *et al*. and Variji *et al*. focused on the enzymatic components related to CVR and CVD prediction. Kameda *et al*. only used known AMI patient samples (n=7) for one of the experiments. All of this work pivoted around an *in-vitro* endothelial cell repair HUVEC model, and the components of which were thoroughly tested (results not reported here). Variji *et al*. used plasma from CVD patients to measure predictive biomarkers for CVD. Sample sizes were ample to power the statistical conclusions in the cross-sectional and observational studies.

Major Findings

This review provides compelling evidence for the hypothesis that type of fat intake has a compositional effect on HDL which is correlated with HDL function, RCT, and EC repair.[15,23,27] In essence, HDLs properties change depending upon the type of dietary fat intake. Increasing MUFAs from EVOO promote HDLs anti-atherogenic effects and lead to endothelial cell repair and efficient CEC.[9,11,16,24,26] EVOO also promotes compositional changes seen in HDL from the natural ageing process reversing its composition to that seen in younger healthy adults.[25] Oxidative environments, which are typically associated with diets high in saturated fat intake and processed foods, diminishes HDLs anti-atherogenic efficiency and leads to a lack of EC repair and eventual plaque formation.[21,28] These findings emphasize that dietary modifications can minimize endothelial stress and atherosclerotic burden. Diet has a preventative role in minimizing CVR and possibly preventing CVD if adopted on a routine basis, an exciting finding considering CVD is the number one cause of death worldwide.

Though the diet-heart hypothesis postulated by Keys 70 years ago still has relevance for its ground-breaking work connecting diet, fat intake, and CVD, this hypothesis should be reframed around a specific type of fat intake rather than the total amount of fat and cholesterol.[6] Different dietary lipids alter the quality of HDL leading to impaired function.[8] Cholesterol, specifically exogenous cholesterol, has much less significance to serum cholesterol values which are internally regulated. However, consumption of SFAs is known to increase LDL concentration because this is the transport mechanism by which cholesterol, TG and other hydrophobic components are trafficked to tissues throughout the body. The ratio of FCT and RCT, CEC, and the oxidative environment plays a significant role in determining cholesterols fate, either deposition into artery walls or simply trafficked to tissues with any excess being returned to the liver for processing.[17] In general, serum HDL-C appears to be a secondary consideration compared to HDL composition which determines functionality, the overriding factor in protecting and reversing atherosclerotic development.[22,] HDL has been merely thought of as the main mediator of RCT, but HDL performs many functions including protecting ECs

from oxidative stress, damage, and preventing foam cell development.[18,28,29,] Maintaining high HDL quality is paramount in preventing atherosclerosis and CV-related diseases.

Applications of Findings

Given that components of the MD are readily available year-round in most developed countries, this presents an opportunity to have a meaningful impact and exercise some control over the inevitable outcome of CVD related mortality. Simply replacing your dietary fat components from SFA and highly processed and refined seed oils with unprocessed cold-pressed EVOO is a pivotal step in maintaining HDLs anti-atherogenic properties.[9,12,24,25] Furthermore, a MD style diet that minimizes processed foods while emphasizing plant based whole foods will provide an anti-oxidative environment that protects both LDL and HDL, allowing these lipoproteins to maximally function in their respective roles to minimize CVR by returning excess cholesterol to the liver in an efficient manner.[9,12] Unfortunately, to date, the U.S. adoption of the U.S. Dietary Guidelines report over 50 years ago has steered dietary recommendations in the direction of demonizing all fats and cholesterol with the unintended consequence of replacing fats with refined carbohydrates leading to an increase in obesity and CVD. As in most cases, a nuanced approach based on the most up-to-date science without a political agenda should be a goal we can all strive for. Implementing the MD style diet leads to maximum cardioprotective benefits with no negative effects.

Limitations and Strengths

This review has certain limitations since it was based only on 6 papers which does not fully represent the literature on the topic. However, within the limited number of studies, we demonstrated the connections between type of fat consumed, quality of HDL and mechanisms involved. The majority of papers used for contextual information appear to support the main hypothesis that simply focusing on all fats, cholesterol, LDL-C and HDL-C without regard to HDL quality and the oxidative environment, which is influenced by the diet, oversimplifies HDLs role and the diet's impact on its function, a critical part in the prevention of atherosclerosis

Further Research

As with all complex systems, further research is needed to elucidate the exact role of dietary fat composition on the long-term outcome of CVD with respect to HDLs function, CEC and how that relates to EC repair mechanisms. Specifically, research that takes into consideration LDL-C, LDL-particle number (LDL-P), and its oxidative state and how that affects the anti-atherogenic role of HDL would be beneficial. Suggested topics for future studies are looking at the impact of high LDL-P on the effectiveness of fully functional HDL and how the oxidative state of LDL can create a more pro-atherogenic environment for HDLs that are participating in RCT and EC repair. The current research does point toward an antioxidant environment being supportive of anti-atherogenic activity by both LDL and HDL.

Final Thoughts

There is ample evidence to suggest that dietary fat composition and dietary patterns impact CVD outcomes, but there are details that need to be further researched. Making healthier food choices, especially those concerning dietary fats and minimally processed foods as emphasized in the MD, are steps that individuals can take to minimize CVR and maximize health span. We can control what we eat so we can impact our future risk of CVD.

1. Keys A, Aravanis C, Blackburn HW, et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. *Acta Med Scand Suppl*. 1966;460:1-392.
2. Menotti A, Puddu PE. Can we still learn from the Seven Countries Study? *Curr Opin Lipidol*. Aug 2018;29(4):313-317. doi:10.1097/mol.0000000000000520
3. Menotti A, Puddu PE. How the Seven Countries Study contributed to the definition and development of the Mediterranean diet concept: a 50-year journey. *Nutr Metab Cardiovasc Dis*. Mar 2015;25(3):245-52. doi:10.1016/j.numecd.2014.12.001
4. Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the seven countries study. *Am J Epidemiol*. Dec 1986;124(6):903-15. doi:10.1093/oxfordjournals.aje.a114480
5. Blackburn H. Invited Commentary: 30-Year Perspective on the Seven Countries Study. *Am J Epidemiol*. Jun 1 2017;185(11):1143-1147. doi:10.1093/aje/kwx071
6. Forouhi NG, Krauss RM, Taubes G, Willett W. Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. *Bmj*. Jun 13 2018;361:k2139. doi:10.1136/bmj.k2139
7. States U, Congress, Senate, Needs SCoNaH. Dietary goals for the United States. Article. 0000 1977;Other US:xli, 83 p. : ill. ; 24 cm.
8. Rees K, Takeda A, Martin N, et al. Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. Mar 13 2019;3(3):Cd009825. doi:10.1002/14651858.CD009825.pub3
9. Katsiki N, Pérez-Martínez P, Lopez-Miranda J. Olive Oil Intake and Cardiovascular Disease Prevention: "Seek and You Shall Find". *Curr Cardiol Rep*. May 7 2021;23(6):64. doi:10.1007/s11886-021-01496-1
10. Tung WC, Rizzo B, Dabbagh Y, et al. Polyphenols bind to low density lipoprotein at biologically relevant concentrations that are protective for heart disease. *Arch Biochem Biophys*. Nov 15 2020;694:108589. doi:10.1016/j.abb.2020.108589
11. Bucciantini M, Leri M, Nardiello P, Casamenti F, Stefani M. Olive Polyphenols: Antioxidant and Anti-Inflammatory Properties. *Antioxidants (Basel)*. Jun 29 2021;10(7)doi:10.3390/antiox10071044
12. Gantenbein KV, Kanaka-Gantenbein C. Mediterranean Diet as an Antioxidant: The Impact on Metabolic Health and Overall Wellbeing. *Nutrients*. Jun 6 2021;13(6)doi:10.3390/nu13061951
13. Lee CK, Liao CW, Meng SW, Wu WK, Chiang JY, Wu MS. Lipids and Lipoproteins in Health and Disease: Focus on Targeting Atherosclerosis. *Biomedicines*. Aug 9 2021;9(8)doi:10.3390/biomedicines9080985
14. Langer T, Strober W, Levy RI. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J Clin Invest*. Jun 1972;51(6):1528-36. doi:10.1172/jci106949
15. Papotti B, Escolà-Gil JC, Julve J, Potì F, Zanotti I. Impact of Dietary Lipids on the Reverse Cholesterol Transport: What We Learned from Animal Studies. *Nutrients*. Jul 30 2021;13(8)doi:10.3390/nu13082643
16. Anastasius M, Kockx M, Jessup W, Sullivan D, Rye KA, Kritharides L. Cholesterol efflux capacity: An introduction for clinicians. *Am Heart J*. Oct 2016;180:54-63. doi:10.1016/j.ahj.2016.07.005
17. Qiu C, Zhao X, Zhou Q, Zhang Z. High-density lipoprotein cholesterol efflux capacity is inversely associated with cardiovascular risk: a systematic review and meta-analysis. *Lipids Health Dis*. Nov 10 2017;16(1):212. doi:10.1186/s12944-017-0604-5
18. Daniels TF, Killinger KM, Michal JJ, Wright RW, Jr., Jiang Z. Lipoproteins, cholesterol homeostasis and cardiac health. *Int J Biol Sci*. Jun 29 2009;5(5):474-88. doi:10.7150/ijbs.5.474
19. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. *Am J Physiol Cell Physiol*. Apr 2001;280(4):C719-41. doi:10.1152/ajpcell.2001.280.4.C719

20. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and Their Role in Atherosclerosis: Pathophysiology and Transcriptome Analysis. *Biomed Res Int*. 2016;2016:9582430. doi:10.1155/2016/9582430
21. Millan-Orge M, Torres-Peña JD, Arenas-Larriva A, et al. Influence of dietary intervention on microvascular endothelial function in coronary patients and atherothrombotic risk of recurrence. *Sci Rep*. Oct 13 2021;11(1):20301. doi:10.1038/s41598-021-99514-3
22. Zanotti I, Potì F, Cuchel M. HDL AND REVERSE CHOLESTEROL TRANSPORT IN HUMANS AND ANIMALS: LESSONS FROM PRE-CLINICAL MODELS AND CLINICAL STUDIES. *Biochim Biophys Acta Mol Cell Biol Lipids*. Oct 9 2021:159065. doi:10.1016/j.bbalip.2021.159065
23. Tran-Dinh A, Diallo D, Delbosc S, et al. HDL and endothelial protection. *Br J Pharmacol*. Jun 2013;169(3):493-511. doi:10.1111/bph.12174
24. Guasch-Ferré M, Hu FB, Martínez-González MA, et al. Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Med*. May 13 2014;12:78. doi:10.1186/1741-7015-12-78
25. Otrante A, Trigui A, Walha R, Berrougui H, Fulop T, Khalil A. Extra Virgin Olive Oil Prevents the Age-Related Shifts of the Distribution of HDL Subclasses and Improves Their Functionality. *Nutrients*. Jun 29 2021;13(7)doi:10.3390/nu13072235
26. Hernáez Á, Soria-Florido MT, Schröder H, et al. Role of HDL function and LDL atherogenicity on cardiovascular risk: A comprehensive examination. *PLoS One*. 2019;14(6):e0218533. doi:10.1371/journal.pone.0218533
27. Girona J, Amigó N, Ibarretxe D, et al. HDL Triglycerides: A New Marker of Metabolic and Cardiovascular Risk. *Int J Mol Sci*. Jun 27 2019;20(13)doi:10.3390/ijms20133151
28. Kameda T, Horiuchi Y, Shimano S, et al. Effect of myeloperoxidase oxidation and N-homocysteinylation of high-density lipoprotein on endothelial repair function. *Biol Chem*. Aug 25 2021;doi:10.1515/hsz-2021-0247
29. Variji A, Shokri Y, Fallahpour S, et al. The combined utility of myeloperoxidase (MPO) and paraoxonase 1 (PON1) as two important HDL-associated enzymes in coronary artery disease: Which has a stronger predictive role? *Atherosclerosis*. Jan 2019;280:7-13. doi:10.1016/j.atherosclerosis.2018.11.004