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Are we seeing the light at the end of the tunnel for high Lipoprotein(a)?

🗓 Meral Kayikcioglu¹*, Hasan Selcuk Ozkan², 🗓 Lale Tokgozoglu³*

¹Ege University Medical Faculty, Department of Cardiology, Izmir, Turkey

ABSTRACT

Keywords

Lipoprotein(a); Lipid lowering therapy; Apheresis; Olpasiran; Pelacarsen; Atherosclerotic cardiovascular disease



Lipoprotein(a) (Lp(a)) attests to be of interest as a new lipoprotein target. However, Lp(a) was discovered in 1963 and since then was recognized as a low-density lipoprotein (LDL)-like lipoprotein with a structurally similar domain to plasminogen. We are increasingly recognizing the importance of Lp(a) and cardiovascular pathologies including atherosclerotic cardiovascular disease, aortic valve stenosis, heart failure, and atrial fibrillation. However, we neither have a standardized measurement method nor an appropriate agent to intervene with this old threat that we have recognized for 60 years. Herein, we present an up-to-date review of our knowledge about Lp(a) covering measurement methods, its associates, and summary of the currently available therapies and emerging therapeutic agents for the

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Introduction

With the improvement of the novel drug technologies, lipoprotein(a) (Lp(a)) attests to be of interest as a new lipoprotein target. Lp(a) was discovered in 1963 and since then was recognized as a low-density lipoprotein (LDL)-like lipoprotein with a structurally similar domain to plasminogen (1-3).

For almost 50 years, Lp(a) could not find a place in clinical practice due to the lack of standardized method of measurement and lack of effective treatment. During the last decade, a large amount of information has accumulated about its role in atherosclerotic cardiovascular disease (ASCVD) and aortic valve stenosis. However, there are still many unknowns and discrepancy between the consensus of societies. The purpose of this review is to provide insight to the possible clinical use of Lp(a) and the currently available therapies and emerging therapeutic agents for the management of patients in the light of recent evidence and guideline recommendations.

What is Lp(a)?

management of high Lp(a) in the light of recent evidence and guideline recommendations.

Lp(a) is a unique liver-derived lipoprotein, consisting of an LDL-like particle and apolipoprotein (apo)(a) which is disulfide-linked to the apo-B100. Apo(a) is a homologue of plasminogen, containing multiple copies of plasminogen kringle IV, a single copy of plasminogen kringle V, and an inactive protease domain (4, 5). The similarity between Lp(a) and plasminogen allows Lp(a) to interfere with tissue plasminogen activator (tPA) – mediated plasminogen activation, as a result, possibly contributing to hypercoagulability (6). Interestingly, observational human studies showed only a slightly increased risk for venous thromboembolism at very high Lp(a) levels (>95th percentile) (7), whereas Mendelian randomization studies did not support any causality for thrombotic events (7, 8).

Lp(a) has a close association with inflammation due to the interleukin-6 (IL-6), an acute-phase cytokine, response element located on *LPA gene* (9). Lp(a) activates endothelial cells by enhancement

Corresponding Authors

Meral Kayikcioglu: meral.kayikcioglu@gmail.com

²Ege University Medical Faculty, Izmir, Turkey

³Hacettepe University Medical Faculty, Department of Cardiology, Ankara, Turkey

^{*}These authors equally contributed to this work

of PFKFB3-mediated glycolysis, which can be reversed by inhibition of glycolysis. An increase in vascular glycolysis, then, facilitates inflammation through the promotion of trans-endothelial migration of monocytes (10), a phenomenon seen mostly in Lp(a) concentrations over >50 mg/dL (>125 nmol/L) (9). Lp(a) as an opsonin, can also alone enhance the phagocytic function of macrophages, as proven against the encapsulated bacterium *Haemophilus influenzae*, a function dependent on scavenger receptor CD36 (11).

As suggested by the Mendelian Randomization studies, absolute changes in Lp(a) plasma levels modify the risk of ASCVD (2). Lp(a) levels are strongly determined through genetic variants in the *LPA* gene, particularly by a size polymorphism in apolipoprotein(a) [apo(a)] (12).

The *LPA gene* locus on chromosome 6q explains up to 90% of Lp(a) variance. While the *LPA gene* locus controls the synthesis of Lp(a), 30-70% of Lp(a) concentration is attributed to apo(a) isoform size, which is determined by the number of Kringle IV repeats. Kringle IV repeats in the *LPA gene* results in polymorphisms leading to apo(a) varying in size (5). All these apo(a) variants account for apo(a) isoforms. There is an inverse relationship between the number of Kringle IV repeats and Lp(a) concentration. Median Lp(a) concentrations are 4-5 times higher in individuals with small apo (a) isoforms, i.e., with low number of Kringle IV repeats (< 22 repeats) compared to those carrying only large isoforms (> 22 repeats). This inverse correlation is caused by more efficient maturation of smaller apo(a) isoforms in the endoplasmic reticulum (3, 12). Lp(a) is also assembled out of the hepatocyte membrane. Individuals carrying the

same isoforms of Lp(a) may still have varying plasma concentrations since the plasma concentration is not only dependent on precursor size but also the rate of the production (13).

Of all Kringle IV types and the number of Kringle IV type 2 domains are associated independently with the risk of ASCVD along with plasma Lp(a) concentrations. On the other hand, significant difference in average Lp(a) concentration among different populations independent of apo(a) allele frequency is also noted (14). In addition to the wide *LPA gene, APOE, CETP*, and a novel variant of *APOH* coding beta2-glycoprotein I (β 2GPI) are associated with Lp(a) levels (3, 15).

What conditions other than genetics affect the Lp(a) levels?

Though Lp(a) concentration is mainly determined by genetics (>90%), several physiologic and pathologic factors have been suggested to influence Lp(a) levels. **Table 1** depicts these factors including ethnicity, sex, hormones, and chronic pathologies such as hepatic and renal diseases.

ARIC and Dallas Heart studies have shown that Lp(a) levels are increased and less skewed in Black individuals compared to other ethnicities (3, 16, 17). UK Biobank data revealed that median Lp(a) levels are highest in Black individuals and sequentially decreasing in South Asian, White, and Chinese individuals (75, 31, 19 and 16 nmol/L, respectively) (3, 18).

Table 1 | Summary of conditions that affect Lp(a) levels.

Conditions/ interventions		Effect on Lp(a) levels		
Genetics	More than 90% of the levels of Lp(a) are determined genetically. <i>LPA</i> is the major gene regulating Lp(a). Other genes including <i>APOE</i> , <i>CETP</i> , and a novel variant of <i>APOH</i> may have some influence.			
Ethnicity	Ethnicity differs Lp(a) concentration. Lp(a highest in Black individuals	Ethnicity differs Lp(a) concentration. Lp(a) levels are sequentially increased in Chinese, White, South Asian, and highest in Black individuals		
Fasting	No change			
Lifestyle	Diet	Replacement of dietary saturated fat with carbohydrate or unsaturated fat is associted with ~10%–15% \uparrow		
		Low carbohydrate diet high in saturated fat ~15% \downarrow		
	Alcohol consumption	No association or minor ↓		
	Physical activity	No or minor change		
Sex hormones	Sex	No change or minor in women compared to men		
	Endogenous sex hormones	No or minor change		
	Menopause	No change or minimal †		
	Postmenopausal HRT	Almost 20 – $25\% \downarrow$; Decrease is greater with oral vs transdermal estrogen. No difference between continuous vs cyclic HRT		
	Surgical or biochemical castration in males	Small ↑		
	Ovariectomy, estrogen receptor antagonist	Small ↑		
	Pregnancy	2-fold ↑		

Continue >>>

Conditions/ interventions		Effect on Lp(a) levels		
Hormones and	Hyperthyroidism	\downarrow and Treatment of hyperthyroidism results in 20–25% \uparrow		
related conditions	Hypothyroidism	\uparrow and Treatment of Hypothyroidism is associated with 5%–20% \downarrow		
	Growth hormone replacement therapy	2-fold ↑		
Chronic kidney disease	Chronic kidney disease and hemodialysis	†; an inverse association with kidney function; a 2–4-fold higher level only in patients with large size apo(a) vs controls		
	Nephrotic syndrome	Almost 3–5-fold ↑		
	Peritoneal dialysis patients	2-fold ↑		
•	Kidney transplantation	Significant ↓ or normalization		
Hepatic impairment	Hepatocellular damage	Decreased in parallel with the disease progression >40% reduction in hepatitis; a 2-fold increase with antiviral treatment		
	Liver transplantation	Changes of apo(a) isoform to that of the donor, with corresponding changes in Lp(a) levels		
	Non-alcoholic fatty liver disease	Inconsistent association across population groups		
Inflammation and	Severe, life-threatening acute-phase conditions	In sepsis, severe burns ↓		
related conditions	Several inflammatory conditions	1		
•	Tocilizumab (IL-6 inhibitor)	Almost 30%–40% ↓		
	Protease inhibitors or antiretroviral therapy	1		
	Statins	May slightly increase Lp(a) (but reports are heterogeneous)		
,	Air pollution	Slight ↑		

HRT: hormone replacement therapy; IL: interleukin, Lp(a): Lipoprotein(a).

Sex hormones may also affect Lp(a) levels. Women generally have 5–10% higher Lp(a) levels than men (17,19). There are reports of elevated Lp(a) in pregnant women that return to baseline postpartum. Lp(a) levels also increase at menopause but remain relatively constant in men (20). Moreover, exogenous androgens and estrogen reduce Lp(a) level. Other hormones, particularly those affecting lipoprotein metabolism, may influence Lp(a) concentrations. Thyroid dysfunctions modestly affect Lp(a) levels, and treatment of overt or subtle hypothyroidism decreases Lp(a).

Research on the interaction between lifestyle and Lp(a) is expanding. Physical activity seems to have no or minimal effect on Lp(a) levels, with conflicting variations in younger or diabetic populations (3, 18). Though diet was initially suggested not to affect Lp(a) levels, recent studies denote some interaction between eating pattern and Lp(a). Replacement of dietary saturated fat with unsaturated fat or protein may increase Lp(a) up to 10-15%. A diet regimen enriched with walnuts ($41-56\ g/day$) or pecans ($72\ g/day$) can only result in a minimal (6-15%) reduction of Lp(a) (18).

Both liver and kidney diseases affect Lp(a) levels. Hepatic damage is associated with Lp(a) decrease in parallel with the disease progression (3, 18). Contrary, decreasing glomerular filtration rate results in elevation of Lp(a) levels (21). In chronic kidney disease, the catabolism of Lp(a) is reduced, leading to elevation of larger apo(a) isoforms. Meanwhile, in patients with nephrotic syndrome, Lp(a) synthesis is increased with subsequent elevation of both large

and small sized apo(a)s due to urinary protein loss (3, 18, 21). Lp(a) levels are highest in patients with nephrotic syndrome or in those treated by peritoneal dialysis (22). Following renal transplantation, Lp(a) concentration reduces independent of the type of immunosuppressive therapy (21-23).

Lp(a) levels show association with both acute and chronic inflammatory states (9). Lp(a) reduction is reported in severe, life-threatening acute phase conditions such as sepsis etc, whereas increased levels are reported in several acute and chronic inflammatory conditions (18, 21). As an example in patients with systemic lupus erythematosus, Lp(a) higher than >125 nmol/L is associated with lupus proteinuria, reduced glomerular filtration rate, and elevated erythrocyte sedimentation rate (24). Moreover, moderately increases of Lp(a) are related to IL-6 levels and may reduce with IL-6 blockade through tocilizumab therapy (25). This effect is not observed with TNF-α blockade (25, 26). The clinical impact of inflammation on Lp(a) concentration is small in population studies (1,18). In human immunodeficiency virus infected patients with baseline Lp(a) concentrations >20-30 mg/dL, treatment with protease inhibitors or antiretroviral therapy is associated with Lp(a) increase (27). Lp(a) levels are increased in patients with coronavirus disease 2019 (COVID-19) and correlate positively with the disease severity, which includes acute kidney damage, and also positively associated with IL-8, fibrinogen, and creatinine levels (28). COVID-19 alone is known to cause five-times higher frequency of thromboembolic

events. Though elevated D-dimer levels are more common in COV-ID-19 patients with elevated Lp(a) (29), the thromboembolic risk is not influenced by Lp(a) levels. However, the risk for ischemic heart disease (IHD) is higher in patients with COVID-19, if elevated Lp(a) levels are additionally present (8). Air pollution as an inflammatory trigger is associated with elevated Lp(a) levels. Long term exposure to fine particles (PM2.5) has the strongest effect on Lp(a)- which is stronger than for any other lipoproteins (18).

Clinical implications of high Lp(a)

Observational and genetic studies consistently recognize the causal association between high Lp(a) and ASCVD, aortic valve stenosis and mortality (cardiovascular and all-cause) in both sexes and across ethnic groups (3). Extreme Lp(a) elevation is one of the few conditions which faces affected individuals with high or very high cardiovascular risk category without additional risk scoring, along with familial hypercholesterolemia (FH), documented ASCVD, long-standing diabetes mellitus, and chronic kidney disease (2, 3). Therefore, current guidelines recommend the Lp(a) measurement for every adult to identify Lp(a) levels >180 mg/dL (>430 nmol/L) (2). An elevation of Lp(a) may increase the risk for an incident CAD without familial risk factors or even in those with LDL-C<70 mg/dL (30). ACCELERATE trial has shown that high Lp(a) levels are associated with an increased risk of major adverse ASCVD events at low levels of LDL-C (<80 mg/dL) (8). Also, smaller apo(a) accompanying high Lp(a) concentration is associated with higher risk of ASCVD (3). Elevated Lp(a) levels may activate macrophages and coronary artery smooth muscle cells and result in coronary artery spasm by upregulating α7-nAChR/IL-6/p38 MAPK signaling, resulting in secretion of proinflammatory IL-6 and reduction of the inducible nitric oxide synthase expression through M1 polarization of macrophages, which are not triggered by LDL-C (31).

Many different ASCVD presentations have been shown to be associated with high Lp(a) levels. Men with Lp(a) levels above 95th percentile have a 4-fold increased risk of severe angina, likewise women have 2-fold increased risk for IHD with same Lp(a) levels (32). Copenhagen City Heart Study proved a continuous relation between Lp(a) levels and risk of IHD and myocardial infarction (MI), and in the general population, where levels exceeding the 95th percentile predict a 3-4 fold increased risk of MI (33). Higher Lp(a) levels are reported as a culprit for premature CAD (34). It also correlates with the CAD severity (35) and the volume and progression of the coronary atheroma (36). High Lp(a) levels are associated in men aged >45 years with higher coronary artery calcium (CAC) scores, which has a positive correlation with traditional cardiovascular risk factors (37). High Lp(a) levels are also associated with the progression of CAC (38). Furthermore, in individuals with established ST-elevation MI, very high (>135 mg/dL) levels of Lp(a) can predict the worse long-term outcomes as proven by the prospective cohort studies, highlighting the need for Lp(a)-based risk stratification in these patients (39). Combining Lp(a) with other risk estimates such as fibringen, Syntax Score etc have been shown to increase the prediction of cardiovascular events in acute or chronic ASCVD states (40, 41). Even slightly elevated Lp(a) levels may result in early loss of vein grafts in the first year of the coronary artery bypass grafting surgery (42) Lp(a) levels have been shown predict mid and long term ASCVD events in patients undergoing percutaneous coronary intervention (PCI) (43). Lp(a) levels have been also suggested to predict contrast induced kidney injury following emergent PCI (44).

High Lp(a) also attenuates the ASCVD risk in patients with CKD. In patients with impaired renal functions, Lp(a) at levels of

30-50 mg/dL is associated with acute coronary syndrome related adverse outcomes, whereas in patients with normal kidney functions, Lp(a)>50 mg/dL is usually required for such a risk level (45). Similarly, in both pre-diabetic and type 2 diabetic patients, recurrence of ASCVD events is more prominent in those with elevated Lp(a) (46).

Apart from being a risk factor for CAD, elevated Lp(a) levels (> 30-50 mg/dL) are associated with calcific aortic valve stenosis, especially the LPA single nucleotide polymorphism rs10455872 is responsible for a 2-fold increased risk of aortic-valve calcification (47). Additionally, Lp(a) levels >90 mg/dL is associated with a 3-fold increase in the risk of aortic-valve calcification (48). The Copenhagen General Population Study also presented that elevated Lp(a) is not only associated with the calcification of the aortic valve but also of the mitral valve (OR 1.53) (49). The risk of aortic involvement with high Lp(a) levels is especially important in patients with FH who already have smaller aortic valve areas and increased inflammatory markers, therefore susceptible to accelerated valvular dysfunction even within the normal range of Lp(a) levels (50). Lp(a) is of use, along with hypertension and LDL-C, for predicting the need of aortic valve replacement in FH. However, the effect of Lp(a) reduction on the progression of aortic valve stenosis is currently a mystery requiring clinical trials to ascertain (51). High levels of Lp(a) in the upper 4th quartile also have been shown to result in aortic dissection, a correlation independent from any other risk factor (52).

Elevated Lp(a) levels are also associated with the risk of heart failure, peripheral arterial disease (PAD) or stroke but higher Lp(a) concentrations are required than those associated with the risk of coronary or aortic diseases (1). The increased risk of heart failure associated with high Lp(a) levels is attributed to ASCVD and calcific aortic valve stenosis (53). Likewise, elevated Lp(a) (\geq 30 mg/dL) is associated with higher serum N-terminal pro-B natriuretic peptide levels and lower left ventricular ejection fraction at follow-up (54). Increased cardiovascular mortality and hospitalization rates are reported within the first year of the diagnosis of heart failure in those with high Lp(a) (\geq 30 mg/dL) (55).

Different studies suggest that patients within the highest quartiles for Lp(a) are at increased risk for PAD, an association generally not affected by LDL-C levels. In patients with established PAD, extremity amputations are more common in those with elevated Lp(a). However, it's important to note that the predictive value of Lp(a) levels in PAD is higher than CAD (56). The strongest genetic predictor of PAD is the *LPA gene* locus (57). Lp(a) is also a non-traditional risk factor for premature lower extremity PAD (57). High Lp(a) may not only result in a worse long-term overall prognosis including ASCVD related mortality in patients with PAD, but also with an increased risk for major adverse limb events after iliofemoral endarterectomy (58).

Increased Lp(a) levels are associated with cerebrovascular atherothrombotic events. High Lp(a) is reported to be associated with the presence of carotid atherosclerosis independent of the conventional risk factors including LDL-C level (59). Interestingly elevated luminal levels of Lp(a) in the aneurysm sack is associated with enlargement of the aneurysm wall within the unruptured intracranial aneurysms (60) Additionally, elevated Lp(a) is not only responsible for large artery atherosclerosis, but also for recurrence of cerebrovascular events primarily in Caucasian individuals aged <60 years or with evident ASCVD (61). High Lp(a) (>137 nmol/L), also predicts major ASCVD events following the carotid endarterectomy (62).

The relationship between atrial fibrillation (AF) and Lp(a) levels is another popular topic. A recent study from the UK Biobank showed that each 50 nmol/L (23 mg/dL) increase in Lp(a) is associated with an increased risk of incident AF for both the measured and genetically predicted Lp(a) (63). Mendelian randomization analyses

also denoted similar results. Interestingly there was no evidence of risk-conferring effect from LDL-C or triglycerides, and only 39% of Lp(a) risk was mediated through ASCVD and aortic valve stenoses, suggesting that Lp(a) may partly influence AF independent of its known effects on ASCVD (64). Apart from these results, presence of left atrial thrombus or thromboembolic events are shown to be increased in patients with non-valvular chronic AF with Lp(a) levels $\geq 30\,\mathrm{mg/dL}$ (64).

Lp(a) seems to be an important marker in extra cardiovascular pathologies, too. In mildly preeclamptic patients, serum Lp(a) level > 40.5 mg/dL predicts the development of severe preeclampsia later during the pregnancy (65). Several neuro-vascular pathologies are also shown to be associated with high Lp(a) levels. For example, Lp(a) levels correlate with major extracranial arterial vessel size in patients with multiple sclerosis (66). Similarly, high Lp(a) levels are reported in several forms of early-onset vascular dementia and ceroid lipofuscinosis due to accelerated atherosclerotic disease (67). Meanwhile a weak association between elevated serum Lp(a) levels and worse motor symptoms in Parkinson disease is noted (68). Serum Lp(a) levels are also associated with the severity of diabetic retinopathy and primary angle-closure glaucoma and resultant neuropathy (69, 70).

In addition, Lp(a) has some special implications for FH patients, as it has been shown as a culprit for aortic valve calcification in these patients. Furthermore, high Lp(a) may interfere with the diagnostic accuracy of the clinical diagnosis of FH with Dutch Lipid Clinic Network (DLCN) criteria. Lp(a)-adjusted LDL-C level (LDL-C $-0.3 \times$ Lp(a)) might be helpful, leading to the differences during the placement of patients into different risk categories after DLCN criteria scores. In patients with already diagnosed FH, a Lp(a) level ≥40 mg/ dL has resulted in FH re-diagnosing with a sensitivity of 63% and specificity of 78% (area under the curve = 0.7, 95% CI 0.7–0.8, p < 0.001) after adjustment according to the proposed formula (71). In the same patient group with $Lp(a) \ge 40 \text{ mg/dL}$, 51% were reclassified after DLCN criteria score and 34% reclassified in means of diagnosis. The rates of reclassification after DLCN criteria score and reclassification in means of diagnosis in FH patients with Lp(a) <40 mg/dL were only 15% and 11%, respectively (71, 72).

Low Lp(a) levels

The implications of low Lp(a) levels are not well known. Certain associations with very low Lp(a) levels are noticed. For example, low circulating levels of Lp(a), along with transaminases may serve as a mean to noninvasively measure the severity of Non-alcoholic fatty liver disease (NAFLD), as Lp(a)-synthesis also depends on hepatocyte function (73). Patients with Child-Pugh class B and C levels have significantly lower levels of Lp(a) levels than those in Child-Pugh class A, with lower levels of total cholesterol and Lp(a) relating with de-

compensatory events in cirrhotic patients (74).

Lower concentrations of Lp(a), bottom 10%, are associated with increased susceptibility to incident type 2 diabetes, another cause of vascular glycolysis leading to endothelial dysfunction (32) Meta-analysis of all available studies showed a 38% (95% CI 29-48%, P < 0.0001) higher risk for the lowest quintile compared to highest quintile of the Lp(a) levels (3). Likewise, an observational cross-sectional study demonstrated an association between low Lp(a) and increased risk of pre-diabetes, insulin resistance, and hyperinsulinemia (3, 75). The mechanisms underlying this association are still unclear and not explained by recognized risk factors or known variants of diabetes (3). Furthermore, we do not know if aggressive Lp(a) lowering may exacerbate diabetes.

Copenhagen General Population Study proved recently that low Lp(a) levels do not correlate with any cancer or infectious disease (76). Despite that, in breast cancer patients, when compared to healthy controls, higher levels of Lp(a) are prominent in the Han Chinese population (77) Moreover, receptor tyrosine-protein kinase erbB-2 proto-oncogene expression is inversely associated with serum Lp(a) levels in these patients, which is of clinical importance as the expression of this growth factor receptor may change the treatment regimen and prognosis.

The big challenge - How to measure Lp(a) levels?

Lp(a) plasma or serum levels can be measured using several immunochemical methods including enzyme-linked immunosorbent assay (ELISA), immunoturbidimetry, nephelometry, and dissociation-enhanced lanthanide fluorescent immunoassay (78) Although measurements with these immunoassays are made with polyclonal antibodies against apo(a), there are studies regarding a monoclonal antibody binding on a single-site on Lp(a) (79).

As there is no standardized assay to measure Lp(a) in serum or plasma, immunochemical methods are also divided into mass dependent and independent assays (Table 2). Mass-independent assays use antibodies against non-repeating kringles of Lp(a) therefore measure the actual particle number, i.e., each apo(a) molecule is only recognized once. These assays are reported in nanomoles per liter (nmol/L). Contrary, mass-dependent assays calculate the entire molecular components of the Lp(a) including proteins, lipids, and carbohydrates, and are reported in milligrams per liter (mg/ dL). As only the total amount of Kringle domains are recognized and distinguishment of one Kringle domain from the others is not made; detected Lp(a) levels may be mistaken in patients with smaller or larger apo(a) isoforms, resulting in underestimated or overestimated Lp(a) levels, respectively (80). In a systematic comparison of apo(a) isoform dependent and independent assays, Lp(a) was underestimated by ~10% in patients with smaller isoforms (associated

Table 2 | Methods of Lp(a) Measurements.

Lp(a): Lipoprotein(a)	Mass-Dependent	Mass-Independent
Measured component	The entire molecular components of the Lp(a) including proteins, lipids, and carbohydrates	Antibodies against non-repeating kringles of Lp(a)
Reported in units	Milligrams per liter (mg/dL)	Nanomoles per liter (nmol/L)
Disadvantages	Isoform size can alter the measured levels of Lp(a)	Measurements are made with polyclonal antibodies against $\operatorname{Lp}(a)$, there is no standardized immunoassay while different methods are in use

 $Lp(a) \colon Lipoprotein(a) \, .$

with high Lp(a) levels and high ASCVD risk) and overestimated by up to 35% in those with large isoforms (associated with low Lp(a) concentrations and low ASCVD risk) (3, 81). Such a variance might be interpreted as an average absolute bias of ± 10 nmol/L (or 4 mg/dL) which might be clinically ignored as will not result in a major alteration of the risk classification. However, several studies showed biases varying between -25% and +35% (3, 81).

Another important problem in clinical practice is the conversion of Lp(a) concentrations from mass unit to molar units. As assays vary extensively, using a standard converting factor between mg/dL and nmol/L values of Lp(a) is not recommended (1). Some investigators who use both units in clinical practice suggest conversion factor of 2-2.5 as 'best guess' from mg/dL to nmol/L (3, 81, 82). In anyway, apo(a) isoform-sensitive assays are not reliable and manufacturers are suggested to provide an appropriate conversion factor if both measures are given (3, 81). Guidelines and consensus statements also define clinical assays using an antibody for a unique non-repetitive epitope in apo(a), i.e. recognizing each Lp(a) particle once that report in molar units as ideal for Lp(a) measurements (3). However, generating such antibodies is difficult and assays mostly use polyclonal antibodies which recognize different epitopes leading to inaccurate Lp(a) measurements (81). It's also suggested that integrating multiple calibrators spanning a range of sizes in the assay can at least partly address this issue (3). But further standardization of Lp(a) measurement is warranted. Meanwhile, earlier studies have failed to detect the association between Lp(a) and IHD, due to the use of fresh-frozen plasma samples stored for years, which resulted in false lower concentrations of Lp(a) (83).

Prevalence of high Lp(a)

Distribution of Lp(a) in the general population, as determined by the Copenhagen General Population Study, is very positively skewed direkt kaldırabiliriz bu ifadeyi with 80% of all the subjects having Lp(a) levels <50 mg/dL. The rest, 20%, having levels $\ge50 \text{ mg/dL}$, proving that elevated Lp(a) is not a rarity (84).

Though Lp(a) is proven to be associated with many pathologies, we still do not have enough epidemiological data providing the prevalence of high Lp(a) levels in many countries. INTERHEART study has evaluated variations in Lp(a) concentrations and isoform sizes in multiple ethnicities including African, Arab, Chinese, European, Latin American, South Asian, and Southeast Asians by using an immunoassay (83). According to the INTERHEART investigators, Lp(a) > 50 mg/dL was not associated with increased risk of MI in Africans and Arabs, contrarily, South Asians and Latin Americans had higher population attributable risks. Additionally, mean Lp(a) concentration was 27.2 mg/dL in Africans and only 7.8 mg/dL in Chinese individuals with the lowest concentration among the studied populations. Also, Chinese and South Asians had greater isoform sizes when compared to other populations.

The investigators of the Copenhagen Heart Study described different median Lp(a) concentrations under 3 categories for their cohort (33). In women with no history of ASCVD event, the mean Lp(a) concentration was 18 mg/dL, in women with a previous history of MI, it was 24 mg/dL and finally, in women with IHD history was 22 mg/dL. In men, the median values were 15 mg/dL for the no event group and 17 mg/dL for both MI and IHD groups. Like the INTERHEART study, Copenhagen Heart Study used polyclonal antibody-based immunoassay (33, 83).

Another study published by Varvel et al. in 2016, in which 532,359 subjects whose records were in the databases in the United States were evaluated, the mean Lp(a) for this group was 34~mg/dL, with

the median of 17 mg/dL (19). Being in rapport with the Copenhagen Heart Study findings, Varvel et al. found that females had a higher mean Lp(a) when compared with the males (with 37 mg/dL vs 30.7 mg/dL, respectively (19, 33).

In whom should we have Lp(a) measurement?

Current guidelines recommend that Lp(a) should be measured at least once in an adult's lifetime, preferably in the first lipid profile, to identify those with high ASCVD risk (2, 3). Lp(a) measurement is more valuable especially in patients with premature ASCVD (men <55 years, women <60 years), family history of premature ASCVD, FH, or recurrent ASCVD even on optimal statin therapy to determine ASCVD risk and characterization of dyslipidemias (2). Incorporation of Lp(a) level in risk assessment has been shown to recuperate risk stratification, especially for those with very high levels of Lp(a) (>99th percentile) in 31%-63% of whom were reclassified from moderate to higher risk (3, 85). Guidelines also recommend its measurement in patients with aortic valve stenosis (3).

For children, Lp(a) screening is recommended in only cases with a history of ischemic stroke or a family history of premature ASCVD or elevated Lp(a) with no other identifiable risk factors (3, 86). In the setting of FH, family history of very high Lp(a), and personal or family history of early ASCVD, systematic or opportunistic screening, especially the cascade screening is also suggested (3). As Lp(a) levels may increase until adulthood repeated testing may be required (3, 15).

For adults it is not necessary to include Lp(a) in the traditional lipid profile in repeated measurements, as Lp(a) serum and plasma concentrations do not exhibit significant variations over time and in response to food intake (3, 18). Some instances, in which variations can be expected are transition to menopause, oral contraceptives, pregnancy, hypothyroidism, chronic kidney disease, nephrotic syndrome, growth hormone treatment, and specific Lp(a)-lowering treatment. Inflammation can also cause mild increases in circulating Lp(a) levels (18). Another cause of significantly increased Lp(a) levels is bariatric surgery, especially within the 12 months following the surgery, however followed by an overall decrease in Lp(a) levels (87).

What should be the goal for Lp(a) reduction?

Though the association between Lp(a) and ASCVD outcomes is linear, determining a threshold level of Lp(a) which can be applied to every individual is a challenge, as risks associated with Lp(a) plasma concentrations are affected by many factors including the structural differences of Lp(a), LDL-C levels, underlying cardiovascular or metabolic disease (3, 18) etc. However, many centers measuring with mass-dependent methods uses 30 mg/dL, while many others using mass-independent methods use 72 nmol/L as a threshold for increased atherosclerotic risk (3, 18, 33). Contrarily, it is known that plasma Lp(a) levels greater than 24 mg/dL puts the individuals at an increased risk for CAD (88), so using 30 mg/dL threshold will eventually result in overseeing the risk of CAD for individuals who has Lp(a) plasma levels greater than 24 mg/dL but lesser than 30 mg/ dL. As INTERHEART study proved Lp(a) > 50 mg/dL, on the other hand, is not associated with increased risk of MI in African and Arab individuals, yet, may put a South Asian or Latin American individual at a great risk, so when setting a threshold for Lp(a), population difference is another issue to be considered (3, 83, 88). Additionally, baseline and on-statin elevated levels of Lp(a) in Chinese patients with heterozygous FH are associated with cardiovascular events (89).

A recent Mendelian randomization analysis estimated that the same effect size achieved by a 38.67 mg/dL lowering of LDL-C can

be achieved with a Lp(a) reduction of 65.7 mg/dL (90). Additionally, it is already known that the contribution of Lp(a) to the risk attributed to the LDL-C is less relevant with lower Lp(a), however, when Lp(a)>50 mg/dL, 14% of the risk attributed to LDL-C is due to Lp(a), with Lp(a)>100 mg/dL, the risk attributed is 28%. This attribution is especially important when treating high-risk patients with elevated Lp(a) and trying to achieve the LDL-C targets of <70 mg/dL while the measured LDL-C levels might be higher than the exact LDL-C, due to the contribution of Lp(a) (91, 92). As cholesterol enclosed in Lp(a) and LDL-C particles cannot be separated, reported collectively as LDL-C concentration. Analyses of isolated Lp(a) particles denoted that cholesterol accounts for 30%-45% of Lp(a) mass concentration (3, 93, 94). Therefore, Lp(a)-cholesterol is estimated by multiplying Lp(a) mass (mg/dL) by 0.3 and used to correct LDL-C with the formula (=Lp(a)-cholesterol-corrected LDL-C) (3). However, estimated Lp(a)-cholesterol shows 6% to 60 % variation from the direct measured Lp(a)-cholesterol (95), thus routine correction of LDL-C for Lp(a)- cholesterol is not recommended (3).

All considered, a stratified approach for the evaluation of individuals with elevated Lp(a) by risk factors, polymorphisms, ethnicity etc., as done for LDL-C in the 2019 EAS/ESC Guidelines on Management of Dyslipidemias, might be necessary, while a single threshold does not seem to be effective for determining the risk for everyone. To do so, studies targeting Lp(a) as a single risk factor wouldn't be enough but studies handling combined risk factors might prove to be useful (2, 3). EAS 2022 Lp(a) consensus panel accepts a pragmatic approach, with Lp(a) cut-offs to 'rule out' (<30 mg/dL or <75 nmo-1/L) and 'rule-in' (>50 mg/dL or >125 nmol/L) the ASCVD risk (3, 96). Panels also defines Lp(a) levels ranging 75-125 nmol/L (30-50 mg/dL) as a relevant grey zone, when considering Lp(a)-attributable risk in the presence of other risk factors in risk stratification (3, 96). And finally, panel highligths that Lp(a) plasma concentration is sufficient to estimate the Lp(a)-related risk without need for further analysis including genotyping, polygenic risk scores, or investigation of expressed apo(a) isoform sizes (97).

Interventions to reduce Lp(a) levels

 $Lp(a) \ has \ not \ received \ much \ attention \ for \ many \ years \ as \ a \ lipid \ fraction \ without \ an \ effective \ therapeutic \ agent, \ meanwhile \ nicotinic \ acid \ and \ Lp(a)-apheresis \ were \ the \ only \ treatments \ available \ for \ high \ Lp(a) \ with \ limited \ use. \ Table \ 3 \ depicts \ the \ summary \ of \ lipid \ modifying \ therapies \ and \ their \ effect \ on \ Lp(a) \ levels.$

Effect of conventional LLT on Lp(a)

Conventional lipid-lowering therapies including statins, ezetimibe, and fibrates reduce ASCVD risk without affecting Lp(a) levels. Conversely, nicotinic acid reduces Lp(a) without substantially changing cardiovascular risk.

Statins. Clinical Statin trials denote varied effects on Lp(a) levels (3, 98). Rosuvastatin had no effect on median Lp(a) levels, and shifted the overall distribution of Lp(a) to higher percentiles in the JUPITER trial (98). Moreover, rosuvastatin showed similar cardiovascular benefits in those with higher and lower levels of Lp(a). Similarly, statin therapy did not significantly change Lp(a) levels in a meta-analysis covering 7 placebo-controlled statin trials (n=29,069 patients) (91) and there was a significant relationship between Lp(a) levels and ASCVD risk despite statin therapy. Another meta-analysis enrolling 6 statin trials with overall 5256 patients showed a significant increase of Lp(a) levels ranging from 8.5% to 19.6% in statin groups where the increase was more pronounced with atorvastatin therapy than the other statins. The investigators also showed that incubating

HepG2 hepatocytes with atorvastatin increased the expressions of *LPA* mRNA and apo(a) (99). Conversely, a meta-analysis of 39 placebo-controlled trials with various statins covering 24,448 subjects demonstrated a non-significant increase of 0.1% in Lp(a) levels with statin therapy compared to controls with no significant differences among individual statins. Intensities of statin therapies also did not differ regarding Lp(a) lowering effect (100, 101).

Nicotinic acid (Niacin). Nicotinic acid is an essential micronutrient, that has favorable effects on all lipid profile at pharmacologic doses. Nicotinic acid was so far the only known therapeutic agent able to lower Lp(a) levels (3). However, this drug could not find a place as a Lp(a) lowering agent in clinical practice due to low tolerability. Moreover, its effect on Lp(a) was prominent at only very high baseline levels which was in overall less than 30% reduction. A meta-analysis of 14 randomized placebo-controlled trials showed extended-release niacin reduced Lp(a) by a mean of 23% (102). The effect of niacin to lower Lp(a) concentration is likely due to decreased apo(a) production rate (3, 98). This Lp(a) lowering effect was only limited to lower molecular weight apo(a) containing isoforms (103).

Two placebo-controlled trials (AIM-HIGH and HPS2-THRIVE) have evaluated the effect of extended-release niacin (with or without the anti-flushing agent laropiprant) added to background simvastatin treatment on ASCVD events. Neither trial showed cardiovascular efficacy of niacin and HPS2-THRIVE, documented increase of non-cardiovascular adverse events. In AIM-HIGH, Niacin reduced Lp(a) by a placebo-corrected mean of 19.6%, with greater absolute Lp(a) reduction as baseline Lp(a) concentration increased (104). However, there was no interaction of baseline Lp(a) and treatment on cardiac adverse events. In HPS2-THRIVE Lp(a) was reduced with niacin/laropiprant to a similar extent as with niacin in AIM-HIGH (101). In the light of these data denoting no cardiovascular benefit, and high adverse events, niacin is no more recommended for Lp(a) lowering (3, 98). Meanwhile, niacin, is no longer in use as it didn't provide clinical evidence regarding its benefit as a substitute to statins over statins alone (3, 98).

Other agents. The effect of ezetimibe on Lp(a) is not clear (3, 98). A meta-analysis covering 10 trials of ezetimibe showed no effect on Lp(a) levels either with ezetimibe monotherapy or combined with a statin (105). However, ezetimibe monotherapy significantly decreased Lp(a) level by only 7.1% in a meta-analysis of 7 trials, but such a small reduction in Lpa(a) could be ignored to be clinically significant (106). Similarly, bile acid sequestrants have no significant effect on Lp(a) levels (3, 98). Interestingly an inverse relationship between plasma triglycerides and Lp(a) levels has been observed (98). Thus, fibrate therapy may be associated with an increase in Lp(a) in patients with severe hypertriglyceridemia. However, fibrates do not substantially change Lpa(a) therapy (107).

New lipid modulating agents and Lp(a)

PCSK9 inhibitors. Proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to LDL-receptor and promotes ingestion of LDL particles into the cell, which results in decreased LDL-C concentrations. The blockage of PCSK9 leads to an increased number of LDL-receptors on the cell surfaces to remove LDLs from circulation. Monoclonal PCSK9 inhibitors along with LDL-C, also decrease Lp(a) levels by 19-27% (108-112). The mechanisms of this decrease remain uncertain, but suggested to include increased receptor-mediated clearance, decreased production of apo(a), and/or decreased Lp(a) particle assembly due to reduced availability of apo-B (3, 98).

Monoclonal PCSK9 inhibitors comprise the only approved drug class that has been shown to reduce both the Lp(a) levels and the

Table 3 | Lp(a) targeting therapeutics, their mechanisms of action, and their effects on Lp(a) levels.

	Mechanism of Action	Effect on Lp(a) level	Effect on ASCVD risk
	Conventional Li ₁	bid Modifying Agents	
Statins	HMG-CoA reductase inhibition	Results in increase of Lp(a) levels in low molecular weight apo(a) containing isoforms, however resulted in no change in high molecular weight isoforms Possible increase, 0–10%	Reduced 20–30%
Ezetimibe	NPC1L1protein inhibition	Possible reduction, 0–7%	Reduced 6% when added to statin therapy
Bile acid sequestrants (Cholestyramine, Colestipol & Colesevelam)	Decrease reabsorption of bile acids Reduce cholesterol content in hepatocytes	No effect	Reduced 20%
Nicotinic acid (Niacin)	Hormone-sensitive lipase inhibition in adipose tissue	%20-28 only in low molecular weight apo(a) containing Lp(a), high molecular weight containing isoforms are not affected Overall reduction, 20%	Neutral
Fibrates	Minimal, possible increase in the setting of hypertriglyceridemia	Reduced 22% with gemfibrozil monotherapy, non-significant reduction with fenofibrate	Variable
	Novel Lipid	Modifying Agents	
Bempedoic acid	ACL inhibition	No effect	Under investigation
Alirocumab Evolocumab	Monoclonal PCSK9 inhibition	Reduction, 20–30%	Reduced by 15%
Inclirisan	SiRNA inhibiting the translation of PCSK9	15-26% reduction in standard deviations depending on the dosing regimen	
Lerodalcibep	PCSK9 inhibition	Ongoing Phase 3 studies comparing safety and efficacy with Evolocumab	Unknown yet
CETP inhibitors	Cholesteryl ester transfer protein inhibition	Reduction up to 25%	Favorable anacetrapib neutral dalcetrapib & evacetrapib; unfavorable torcetrapib
Lomitapide	MTP inhibition	15% reduction (in addition to the standart treatment regime in HoFH)	Reduced in HoFH
Evinacumab	Monoclonal antibody targeting ANGPTL3	$5.5{\pm}4.0~\%$ reduction from the baseline	
Mipomersen	ASO targeting Apo B100 synthesis	Reduction, 20–25%	Not determined
	Targeted L	PA therapeutics	
Pelacarsen (formerly AKCEA-APO(a)-L _{RX} , TQJ230)	ASO targeting LPA mRNA in liver	35-80% reduction depending on the dosing and timing of the regimen	Phase 3 outcomes RCT is ongoing-HORISON
Olpasiran (formerly AMG-890, ARO-LPA)	siRNA to apo(a)	Phase 1-2: reduction, 70–98%	Phase 2 ongoing Phase 3 underway (OCEANS)
SLN360	siRNA to apo(a)	Phase 1: reduction, $46-98\%$ Only patients with $Lp(a) \ge 150 \text{ nmol/L}$ is planned to be included in the study. The study is still in Phase 1.	Phase 2 planned

Continue >>>

	Mechanism of Action	Effect on Lp(a) level	Effect on ASCVD risk		
Lp(a) Apheresis	Removal of apo-B containing lipoproteins	50-75% reduction, depending on the method, with dextran sulphate immunoadsorption providing the most prominent decrease by 72%	Observational data suggest a substantial est clinical benefit		
Drugs other than lipid modifying agents					
Aspirin	Reduction of the expression of apo(a)	None to 30% reduction	Mortality reduction of 25% in high-risk patients		
HRT	Estrogens reduce the transcription of the <i>LPA</i> gene	Almost 20–25% reduction; Decrease is greater with oral vs transdermal estrogen. No difference between continuous vs cyclic HRT	None		

ACL: Adenosine triphosphate-citrate lyase; ANGPTL3, angiopoietin-like protein 3; Apo: Apolipoprotein; ASCVD: Atherosclerotic cardiovascular disease; ASO; Antisense oligonucleotide, FH, familial hypercholesterolemia, HRT: Hormone Replacement Therapy HoFH, homozygous FH; LDL: low-density lipoprotein, LDLR; low-density lipoprotein receptor, MTP; microsomal triglyceride transfer protein; NPC1L1; Niemann-Pick C1-like 1 protein PCSK9; pro-protein convertase subtilisin/ kexin 9. SiRNA; Small interfering RNAs,

risk of cardiovascular events (3). Two cardiovascular outcomes trials which evaluated the effects of PCSK9 monoclonal antibodies, added to statin therapy, resulted in 27% (FOURIER Trial) and 23% (OD-YSSEY OUTCOMES Trial) decrease in circulating Lp(a) levels (109, 112). Absolute Lp(a) reductions were directly related to baseline levels. Moreover, the reduction in ASCVD risk with PCSK9 inhibition was observed in patients with elevated Lp(a) levels, and was achieved with only 16-22% reduction of Lp(a) levels in the highest baseline Lp(a) quartile (3, 98, 109, 110, 112). Moreover, the reduced risk of major adverse limb events including acute limb ischemia, major amputation, or urgent limb revascularization for ischemia observed with PCSK9 inhibition was strongly associated with the baseline Lp(a) level but not with LDL-C levels in ODYSSEY OUTCOMES trial. Furthermore, a meta-analysis of the FOURIER and ODYSSEY OUTCOMES trials showed a consistent, favorable effect of PCSK9 inhibition on the incidence of venous thromboembolic events. Interestingly this benefit was evident when the baseline Lp(a) was higher than the median of 37 nmol/L despite the similar reductions in LDL-C with evolocumab in both Lp(a) categories (higher and lower than the median 37 nmol/L) (113).

Inclisiran is a small interfering RNA (SiRNA) reducing PCSK9 synthesis with the potential advantage of twice-yearly dosing (98). Inclisiran provides similar Lp(a) and LDL-C reductions as monoclonal PCSK9 inhibitors. Lp(a) levels were reduced by 25.6% and 18.6%, in the ORI-ON-10 and ORION-11 trials respectively (114). The ongoing ORION-4 trial will address the potential benefit of inclisiran on ASCVD events.

Bempedoic acid. Bempedoic acid is an oral adenosine triphosphate citrate lyase inhibitor decreasing synthesis of cholesterol in the liver, thereby upregulating the LDL-receptors. Bempedoic acid has been shown to safely decrease LDL-C and improve cardiovascular outcomes, however it has no shown definite effect on Lp(a). Similar to statins the phase 2 data of bempedoic acid denotes a non-significant small increase in Lp(a) (115).

Mipomersen. Mipomersen is an antisense-oligonucleotide (ASO) targeting apo-B with a significant LDL-C lowering effect. Weekly injections of mipomersen has been shown to decrease Lp(a) levels by 21% in a study of 14 healthy individuals and by 26% in patients with or without FH in a meta-analysis of 4 trials (116, 117).

CETP inhibitors. Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl esters from HDL to apo-B containing particles, thereby raising the levels of HDL-C. These agents also lower Lp(a) levels and potent CETP inhibitors (except dalcetrapib) decrease apo-B and LDL-C (98). Torcetrapib and dalcetrapib lead to almost a 10% reduction in Lp(a) levels, while anacetrapib and evacetrapib induce a 25-31% decrease (98). The reduction in Lp(a) with anacetrapib, was documented to be due to reduced apo (a) production (118). Despite substantial lipoprotein changes, only anacetrapib demonstrated a modestly favorable clinical effect. Compared to placebo, anacetrapib significantly induced a 17% decrease in LDL-C levels, 25% in Lp(a) levels, and 9% reduction in the risk of MACE (98, 119). But CETP inhibitors are not approved for therapeutic use.

Targeted LPA therapeutics

Novel ASOs and small interfering RNA (siRNA) molecules, targeting apo(a) production in hepatocytes is underway as Lp(a) lowering therapeutics. These agents are currently investigated for efficacy, safety, and ASCVD outcomes in clinical trials.

Pelecarsen, is an ASO agonist apo(a)-mRNA that is conjugated to N-acetylgalactosamine (GalNAc), which enables specific targeting to hepatocytes. This technology provides enhanced potency, less toxicity, and infrequent dosing (3, 98). Pelecarsen therapy is associated with sustained dose-dependent Lp(a) reductions of 35-80% in patients with Lp(a) $\geq 60 \text{ mg/dL}$ ($\geq 150 \text{ nmol/L}$) and ASCVD (120). In early trials, mean decreases of 80% and 72% were reported with pelacarsen injected 20 mg weekly and 60 mg monthly, respectively, with 98% and 81% of participants attaining Lp(a) levels <125 nmo-1/L at the end of the study with mild adverse events related to the mild injection-site reactions (98, 120). The ongoing Horizon study (NCT04023552) will address the effect of lowering Lp(a) with pelacarsen on ASCVD outcomes in patients with established cardiovascular disease and elevated Lp(a) (≥70 mg/dL). Another trial on patients with GFR<30 ml/min/1.73m² is underway (121). So far, no difference has been reported regarding the efficacy of pelacarsen on Lp(a) isoforms (122).

Oplesiran is a GalNAc-conjugated siRNA with a Lp(a) lowering

effect ranging between 71% and 97% in patients with baseline Lp(a) ≥70 nmol/L to ≤199 nmol/L after the administration of a single-dose (123). Studies denote a favorable safety profile (97). The phase 3 trial investigating cardiovascular outcomes (OCEANS Study) is underway. Another GalNAc-conjugated siRNA (SLN360, Silence Therapeutics) is in early development (124).

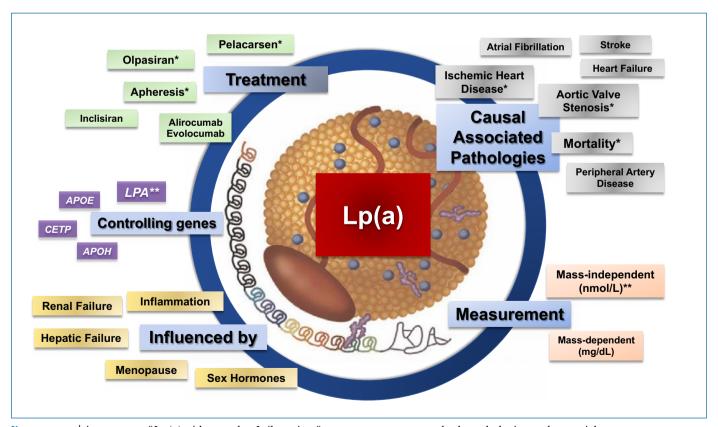
Lp(a) apheresis. Lipoprotein apheresis selectively eliminates apo-B containing particles including Lp(a). An apheresis session may lead to 50-75% acute Lp(a) reduction depending on the method used (125). Due to the lack of effective Lp(a)-lowering agents, in clinical practice Lp(a) apheresis is accepted as the most effective means of Lp(a) lowering therapy. With the special immune-adsorption polyclonal antibody columns being available since 1993, a large amount of experience is now present in some specialized centers. However, the awareness of Lp(a) as an AS-CVD risk factor is extremely low, consequently Lp(a) apheresis therapy is still not of widely available. Moreover, clinical benefits of Lp(a) specific apheresis requires more evidence in terms of effectivity in patients with isolated high Lp(a) (125), although it's known" known that Lp(a) specific apheresis can result in coronary atherosclerosis and carotid intima-media thickness regression if practiced consecutively for 18 months, provided that the patients reach their LDL-C goals (126). Lp(a) apheresis also attenuates refractory angina and provides improvement in atheromas, exercise capacity, and myocardial perfusion in patients with extremely high Lp(a) levels (>500 mg/dL) after 3 months of weekly apheresis (127, 128). Given the invasive nature of the procedure, large randomized controlled trials are lacking, but cumulative, consistent observational and cohort data denote an important role of Lp(a) apheresis in the secondary prevention of those with high Lp(a) (125-128).

Drugs other than lipid-modifying agents

Aspirin. Aspirin was shown to reduce the expression of apo(a) in cultured liver cells (129). An observational study of prospective use of aspirin (81 mg/day) in 70 subjects with a history of ASCVD showed a 15% decrease in Lp(a) levels from baseline in those with Lp(a) levels ≥ 30 mg/dL (130). However, in a placebo-controlled study of 56 patients with chronic ASCVD, aspirin showed no effect on Lp(a) levels over 3 months of therapy, irrespective of the baseline concentrations (98,130). Interestingly, Women's Health Study, also showed that aspirin reduced the risk of MACE in minor allele carriers of rs3798220 in the *LPA* gene, but not in non-carriers, with a significant interaction of carrier status and treatment (131). Of note, rs3798220 in the *LPA* gene is associated with high circulating Lp(a) levels. These scarce data may denote a possible benefit of aspirin on the prevention of MACE associated with Lp(a) levels. But prospective testing of this hypothesis is warranted.

Hormones. Anabolic steroids and estrogen treatment may decrease Lp(a), but the clinical benefit of this effect is uncertain. Though unblinded or uncontrolled studies denote an association between testosterone treatment and Lp(a) reduction, RCTs did not confirm such an association (3, 98, 132).

Estrogen and its analogues reduce the transcription of the LPA gene. Women already on hormone replacement therapy (HRT) have modestly lower Lp(a) levels compared to those not receiving HRT (9.4 mg/dL vs 11.6 mg/dL, respectively) in the baseline evaluation of Women's Health Study (133). Meta-analysis also revealed similar



results that HRT in post-menopausal women is associated with a 25% decrease in Lp(a) levels (134). In the Heart and Estrogen/progestin Replacement Study (HERS), though Lp(a) levels were reduced there was no overall benefit of HRT with regard to MACE in post-menopausal women with IHD (135).

Thyroid hormone analogues such as eprotirome may result in a significant dose-dependent reduction of Lp(a) up to 55% if combined with statins, however 6-month treatment with levothyroxine may also lower Lp(a) to some extent, in patients with primary hypothyroidism (3, 18, 98). Liver selective thyromimetics are being focused for treatment of nonalcoholic steatohepatitis. These agents also have beneficial lipid effects covering Lp(a), without adverse extrahepatic effects.

Conclusion

We are increasingly recognizing the importance of Lp(a) and cardiovascular pathologies, however we neither have a standardized measurement method nor an appropriate agent to intervene with this old threat that we have recognized 60 years ago (136). It is imperative to extend our knowledge about Lp(a), standardize its measurement, and make sure that it finds its well-deserved place in the daily clinical practice to prevent further ASCVD events.

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Conflict of Interest Statement

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References

- McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature. 1987; 330 (6144):132-7.
- Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Eur Heart J. 2020 Jan; 41 (1):111-88.
- Kronenberg F, Mora S, Stroes ESG, Ference BA, Arsenault BJ, Berglund L, et al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. Eur Heart J. 2022 Oct; 43 (39):3925-46.
- 4. Boffa MB. Beyond fibrinolysis: The confounding role of Lp(a) in thrombosis. Atherosclerosis. 2022 May; 349:72-81.
- Koschinsky ML, Marcovina SM. Structure-function relationships in apolipoprotein(a): Insights into lipoprotein(a) assembly and pathogenicity. Vol. 15, Current Opinion in Lipidology. Curr Opin Lipidol. 2004; 167-74.
- Hancock MA, Boffa MB, Marcovina SM, Nesheim ME, Koschinsky ML. Inhibition of plasminogen activation by lipoprotein(a). Critical domains in apolipoprotein(a) and mechanism of inhi-

- bition on fibrin and degraded fibrin surfaces. J Biol Chem. 2003 Jul; 278 (26):23260-9.
- Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: Insights from epidemiology, genetics, and biology. J Lipid Res. 2016 Nov; 57 (11):1953-75.
- 8. Di Maio S, Lamina C, Coassin S, Forer L, Würzner R, Schönherr S, et al. Lipoprotein (a) and SARS-CoV-2 infections: Susceptibility to infections, ischemic heart disease and thromboembolic events. J Intern Med. 2022 Jan; 291 (1):101-7.
- 9. Dzobo KE, Kraaijenhof JM, Stroes ESG, Nurmohamed NS, Kroon J. Lipoprotein(a): An underestimated inflammatory mastermind. Atherosclerosis. 2022 May; 349:101-9.
- Schnitzler JG, Hoogeveen RM, Ali L, Prange KHM, Waissi F, Van Weeghel M, et al. Atherogenic Lipoprotein(a) Increases Vascular Glycolysis, Thereby Facilitating Inflammation and Leukocyte Extravasation. Circ Res. 2020 May; 126 (10):1346-59.
- Liu Z, Li Y, Wang Y, Liu Z, Su Y, Ma Q, et al. Lipoprotein (a), an Opsonin, Enhances the Phagocytosis of Nontypeable Haemophilus influenzae by Macrophages. Ortega E, editor. J Immunol Res. 2021 Nov; 2021:1-14.
- 12. Coassin S, Kronenberg F. Lipoprotein (a) beyond the kringle IV repeat polymorphism: The complexity of genetic variation in the LPA gene. Atherosclerosis. 2022 May; 349:17-35.
- 13. Rader DJ, Cain W, Zech LA, Usher D, Brewer HB. Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein(a) isoform is determined by the rate of lipoprotein(a) production. J Clin Invest. 1993 Feb; 91 (2):443-7.
- Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Császár A, et al. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet. 1991; 86 (6):607-14.
- 15. de Boer LM, Hof MH, Wiegman A, Stroobants AK, Kastelein JJP, Hutten BA. Lipoprotein(a) levels from childhood to adulthood: Data in nearly 3,000 children who visited a pediatric lipid clinic. Atherosclerosis. 2022 May; 349:227-32.
- 16. Tsimikas S, Clopton P, Brilakis ES, Marcovina SM, Khera A, Miller ER, et al. Relationship of oxidized phospholipids on apolipoprotein B-100 particles to race/ethnicity, apolipoprotein(a) isoform size, and cardiovascular risk factors: results from the Dallas Heart Study. Circulation. 2009 Apr; 119 (13):1711-9.
- 17. Virani SS, Brautbar A, Davis BC, Nambi V, Hoogeveen RC, Sharrett AR, et al. Associations between lipoprotein(a) levels and cardiovascular outcomes in black and white subjects: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2012 Jan; 125 (2):241-9.
- 18. Enkhmaa B, Berglund L. Non-genetic influences on lipoprotein(a) concentrations. Atherosclerosis. 2022 May; 349:53-62.
- Varvel S, McConnell JP, Tsimikas S. Prevalence of Elevated Lp(a) Mass Levels and Patient Thresholds in 532 359 Patients in the United States. Arterioscler Thromb Vasc Biol. 2016 Nov; 36 (11):2239-45.
- Derby CA, Crawford SL, Pasternak RC, Sowers M, Sternfeld B, Matthews KA. Lipid changes during the menopause transition in relation to age and weight. Am J Epidemiol. 2009 Jun; 169 (11):1352-61.
- 21. Kronenberg F. Causes and consequences of lipoprotein(a) abnormalities in kidney disease. Clin Exp Nephrol. 2014 Apr; 18 (2):234-7.
- 22. Kronenberg F, König P, Neyer U, Auinger M, Pribasnig A, Lang U, et al. Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease

- treated by hemodialysis or continuous ambulatory peritoneal dialysis. J Am Soc Nephrol. 1995 Jul; 6 (1):110-20.
- 23. Kronenberg F, König P, Lhotta K, Öfner D, Sandholzer C, Margreiter R, et al. Apolipoprotein(a) phenotype-associated decrease in lipoprotein(a) plasma concentrations after renal transplantation. Arterioscler Thromb Vasc Biol. 1994 Sep; 14 (9):1399-404.
- Connolly CM, Li J, Goldman D, Fava A, Magder L, Petri M. Lipoprotein (a) in systemic lupus erythematosus is associated with history of proteinuria and reduced renal function. Lupus. 2022 Oct; 31 (11):1367-72.
- 25. Müller N, Schulte DM, Türk K, Freitag-Wolf S, Hampe J, Zeuner R, et al. IL-6 blockade by monoclonal antibodies inhibits apolipoprotein (a) expression and lipoprotein (a) synthesis in humans. J Lipid Res. 2015 May; 56 (5):1034-42.
- Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, Krone W, et al. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. PLoS One. 2010 Dec; 5 (12):e14328.
- 27. Enkhmaa B, Anuurad E, Zhang W, Li CS, Kaplan R, Lazar J, et al. Effect of antiretroviral therapy on allele-associated Lp(a) level in women with HIV in the Women's Interagency HIV Study. J Lipid Res. 2018 Oct; 59 (10):1967-76.
- Lippi G, Szergyuk I, de Oliveira MHS, Benoit SW, Benoit JL, Favaloro EJ, et al. The role of lipoprotein(a) in coronavirus disease 2019 (COVID-19) with relation to development of severe acute kidney injury. J Thromb Thrombolysis. 2021 Oct.
- Kaltoft M, Glavind KS, Nielsen SF, Langsted A, Iversen KK, Nordestgaard BG, et al. Lipoprotein(a) during COVID-19 hospitalization: Thrombosis, inflammation, and mortality. Atherosclerosis. 2022 Sep; 357:33-40.
- Melita H, Manolis AA, Manolis TA, Manolis AS. Lipoprotein(a) and Cardiovascular Disease: A Missing Link for Premature Atherosclerotic Heart Disease and/or Residual Risk. Vol. 79, Journal of cardiovascular pharmacology. J Cardiovasc Pharmacol. 2022; e18-35.
- Lin YK, Yeh CT, Kuo KT, Fong IH, Yadav VK, Kounis NG, et al. Apolipoprotein (a)/Lipoprotein (a)-Induced Oxidative-Inflammatory α 7-nAChR/p38 MAPK/IL-6/RhoA-GTP Signaling Axis and M1 Macrophage Polarization Modulate Inflammation-Associated Development of Coronary Artery Spasm. Oxid Med Cell Longev. 2022; 2022:9964689.
- 32. Kamstrup PR. Lipoprotein(a) and ischemic heart disease-A causal association? A review. Vol. 211, Atherosclerosis. 2010; 15-23.
- 33. Kamstrup PR, Benn M, Tybjærg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: The Copenhagen City Heart Study. Circulation. 2008 Jan; 117 (2):176-84.
- 34. Helgadottir A, Gretarsdottir S, Thorleifsson G, Holm H, Patel RS, Gudnason T, et al. Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. J Am Coll Cardiol. 2012 Aug; 60 (8):722-9.
- 35. Shi YP, Cao YX, Jin JL, Liu HH, Zhang HW, Guo YL, et al. Lipoprotein(a) as a predictor for the presence and severity of premature coronary artery disease: A cross-sectional analysis of 2433 patients. Coron Artery Dis. 2020; 32 (1):78-83.
- Huded CP, Shah NP, Puri R, Nicholls SJ, Wolski K, Nissen SE, et al. Association of serum lipoprotein (A) levels and coronary atheroma volume by intravascular ultrasound. J Am Heart Assoc. 2020 Dec; 9(23):e018023.
- 37. Chung YH, Lee BK, Kwon HM, Min PK, Choi EY, Yoon YW, et al.
 Coronary calcification is associated with elevated serum lipo-

- protein (a) levels in asymptomatic men over the age of 45 years: A cross-sectional study of the Korean national health checkup data. Medicine (Baltimore). 2021 Mar; 100 (9):e24962.
- 38. Cho JH, Lee DY, Lee ES, Kim J, Park SE, Park CY, et al. Increased risk of coronary artery calcification progression in subjects with high baseline Lp(a) levels: The Kangbuk Samsung Health Study. Int J Cardiol. 2016 Nov; 222:233-7.
- Cao YX, Zhang HW, Jin JL, Liu HH, Zhang Y, Zhang M, et al. Lipoprotein(a) and Cardiovascular Outcomes in Patients with Previous Myocardial Infarction: A Prospective Cohort Study. Thromb Haemost. 2020 Dec.
- **40.** Zhang Y, Jin JL, Cao YX, Liu HH, Zhang HW, Guo YL, et al. Prognostic utility of lipoprotein(a) combined with fibrinogen in patients with stable coronary artery disease: A prospective, large cohort study. J Transl Med. 2020 Oct; 18 (1).
- 41. Xu W, Guan H, Gao D, Wang Z, Ba Y, Yang H, et al. The association of syntax score with levels of lipoprotein(A) and inflammatory biomarkers in patients with stable coronary artery disease and different low-density lipoprotein cholesterol levels. Diabetes, Metab Syndr Obes Targets Ther. 2020; 13:4297-310.
- 42. Pokrovsky SN, Ezhov M V., Il'Ina LN, Afanasieva OI, Sinitsyn VY, Shiriaev AA, et al. Association of lipoprotein(a) excess with early vein graft occlusions in middle-aged men undergoing coronary artery bypass surgery. J Thorac Cardiovasc Surg. 2003 Oct; 126 (4):1071-5.
- 43. Huang Z, Shui X, Ling Y, Zhou L, Shi W, Luo Y, et al. Serum lipoprotein(a) and risk of periprocedural myocardial injury in patients undergoing percutaneous coronary intervention. Clin Cardiol. 2021 Feb; 44 (2):176-85.
- 44. Tao J, Dai W, Ye C, Yao Q, Zhou M, Li Y. Preprocedural Lp(a) level and ApoB/ApoA-I ratio and the risk for contrast-induced acute kidney injury in patients undergoing emergency PCI. Lipids Health Dis. 2021 Dec; 20 (1).
- 45. Li Q, Chen Y, Yu L, Zhu L, Wang Z, Jiao S, et al. The relationship between lipoprotein(a) and cardiovascular events in acute coronary syndrome patients with and without chronic kidney disease. Atherosclerosis. 2022 May; 349:204-10.
- **46.** Jin JL, Cao YX, Zhang HW, Sun D, Hua Q, Li YF, et al. Lipoprotein(a) and cardiovascular outcomes in patients with coronary artery disease and prediabetes or diabetes. Diabetes Care. 2019; 42 (7):1312-8.
- 47. Jang AY, Han SH, Sohn IS, Oh PC, Koh KK. Lipoprotein(a) and cardiovascular diseases revisited Vol. 84, Circulation Journal. Japanese Circulation Society; 2020; 867-74.
- 48. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Elevated lipoprotein(a) and risk of aortic valve stenosis in the general population. J Am Coll Cardiol. 2014 Feb; 63 (5):470-7.
- 49. Kaltoft M, Sigvardsen PE, Afzal S, Langsted A, Fuchs A, Kühl JT, et al. Elevated lipoprotein (a) in mitral and aortic valve calcification and disease: The Copenhagen General Population Study. Atherosclerosis. 2022 May; 349:166-74.
- Hovland A, Narverud I, Lie Øyri LK, Bogsrud MP, Aagnes I, Ueland T, et al. Subjects with familial hypercholesterolemia have lower aortic valve area and higher levels of inflammatory biomarkers. J Clin Lipidol. 2020 Jan; 15 (1).
- Pérez de Isla L, Watts GF, Alonso R, Díaz-Díaz JL, Muñiz-Grijalvo O, Zambón D, et al. Lipoprotein(a), LDL-cholesterol, and hypertension: predictors of the need for aortic valve replacement in familial hypercholesterolaemia. Eur Heart J. 2021 Jan.
- 52. Yang Y, Hong Y, Yang W, Zheng Z. Association of lipoprotein(a) with aortic dissection. Clin Cardiol. 2022 Sep; 45 (9):908.

- 53. Kamstrup PR, Nordestgaard BG. Elevated lipoprotein(a) levels, LPA risk genotypes, and increased risk of heart failure in the general population. JACC Hear Fail. 2016 Jan; 4 (1):78-87.
- 54. Wu B, Zhang Z, Long J, Zhao H, Zeng F. Association between lipoprotein (a) and heart failure with reduced ejection fraction development. J Clin Lab Anal. 2022 Jan; 36 (1):e24083.
- Li Z, Liu J, Shen J, Chen Y, He L, Li M, et al. Association of lipoprotein (a) and 1 year prognosis in patients with heart failure with reduced ejection fraction. ESC Hear Fail. 2022 Aug; 9 (4):2399-406.
- 56. Sakata K, Kumakura H, Funada R, Matsuo Y, Nakashima K, Iwasaki T, et al. Lipoprotein(a) is a Promising Residual Risk Factor for Long-Term Clinical Prognosis in Peripheral Arterial Disease. Ann Vasc Dis. 2022 Sep; 15 (3):186-92.
- Klarin D, Lynch J, Aragam K, Chaffin M, Assimes TL, Huang J, et al. Genome-wide association study of peripheral artery disease in the Million Veteran Program. Nat Med. 2019 Aug; 25 (8):1274-9.
- 58. Verwer MC, Waissi F, Mekke JM, Dekker M, Stroes ESG, de Borst GJ, et al. High lipoprotein(a) is associated with major adverse limb events after femoral artery endarterectomy. Atherosclerosis. 2022 May; 349:196-203.
- 59. Jun JE, Kang H, Hwang YC, Ahn KJ, Chung HY, Jeong IK. The association between lipoprotein (a) and carotid atherosclerosis in patients with type 2 diabetes without pre-existing cardiovascular disease: A cross-sectional study. Diabetes Res Clin Pract. 2021 Jan; 171.
- 60. Ishii D, Zanaty M, Roa JA, Li L, Lu Y, Sabotin R, et al. Concentration of Lp(a) (Lipoprotein[a]) in Aneurysm Sac Is Associated With Wall Enhancement of Unruptured Intracranial Aneurysm. Stroke. 2021 Feb; STROKEAHA120032304.
- 61. Arnold M, Schweizer J, Nakas CT, Schütz V, Westphal LP, Inauen C, et al. Lipoprotein(a) is associated with large artery atherosclerosis stroke aetiology and stroke recurrence among patients below the age of 60 years: results from the BIOSIGNAL study. Eur Heart J. 2021 Mar.
- 62. Waissi F, Dekker M, Timmerman N, Hoogeveen RM, Van Bennekom J, Dzobo KE, et al. Elevated Lp(a) (Lipoprotein[a]) Levels Increase Risk of 30-Day Major Adverse Cardiovascular Events in Patients following Carotid Endarterectomy. Stroke. 2020; 51 (10):2972-82.
- 63. Mohammadi-Shemirani P, Chong M, Narula S, Perrot N, Conen D, Roberts JD, et al. Elevated Lipoprotein(a) and Risk of Atrial Fibrillation: An Observational and Mendelian Randomization Study. J Am Coll Cardiol. 2022 Apr; 79 (16):1579-90.
- 64. Ding WY, Protty MB, Davies IG, Lip GYH. Relationship between lipoproteins, thrombosis, and atrial fibrillation. Cardiovasc Res. 2022 Feb; 118 (3):716-31.
- 65. Konrad E, Güralp O, Shaalan W, Elzarkaa AA, Moftah R, Alemam D, et al. Correlation of elevated levels of lipoprotein(a), high-density lipoprotein and low-density lipoprotein with severity of preeclampsia: a prospective longitudinal study. J Obstet Gynaecol (Lahore). 2020 Jan; 40 (1):53-8.
- 66. Jakimovski D, Zivadinov R, Pelizzari L, Browne RW, Weinstock-Guttman B, Ramanathan M. Lipoprotein(a) Levels Are Associated with the Size of Extracranial Arteries in Multiple Sclerosis. J Vasc Res. 2020 Jan; 57 (1):16-23.
- 67. Faruq RN, D'Silva P, Lau FD, Zhao C, Majumdar S. Early-Onset Vascular Dementia in a 43-Year-Old Man with Accelerated Atherosclerotic Disease, Elevated Lipoprotein (a), and a Missense DNA-JC5 Variant with Potential Association to Adult-Onset Ceroid Lipofuscinosis. Case Rep Neurol. 2021 Aug; 13 (2):565-71.

- 68. Choe C un, Petersen E, Lezius S, Cheng B, Schulz R, Buhmann C, et al. Association of lipid levels with motor and cognitive function and decline in advanced Parkinson's disease in the MARK-PD study. Parkinsonism Relat Disord. 2021 Apr; 85 (February 2020):5-10.
- 69. Moosaie F, Davatgari RM, Firouzabadi FD, Esteghamati S, Deravi N, Meysamie A, et al. Lipoprotein(a) and Apolipoproteins as Predictors for Diabetic Retinopathy and Its Severity in Adults With Type 2 Diabetes: A Case-Cohort Study. Can J Diabetes. 2020 Jul; 44 (5):414-21.
- Shao M, Li Y, Teng J, Li S, Cao W. Association Between Serum Lipid Levels and Patients With Primary Angle-Closure Glaucoma in China: A Cross Sectional, Case-Control Study. Front Med. 2021 Feb; 8:618970.
- 71. Chubykina U V, Ezhov M V, Afanasieva OI, Klesareva EA, Pokrovsky SN. Elevated Lipoprotein(a) Level Influences Familial Hypercholesterolemia Diagnosis. Dis (Basel, Switzerland). 2022 Jan; 10 (1).
- 72. Tsimikas S, Fazio S, Ferdinand KC, Ginsberg HN, Koschinsky ML, Marcovina SM, et al. NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. J Am Coll Cardiol. 2018; 71 (2):177-92.
- 73. Meroni M, Longo M, Lombardi R, Paolini E, Macchi C, Corsini A, et al. Low Lipoprotein(a) Levels Predict Hepatic Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Hepatol Commun. 2021.
- 74. Feng R, Guo X, Kou Y, Xu X, Hong C, Zhang W, et al. Association of lipid profile with decompensation, liver dysfunction, and mortality in patients with liver cirrhosis. Postgrad Med. 2021; 133 (6):626-38.
- 75. Ding L, Song A, Dai M, Xu M, Sun W, Xu B, et al. Serum lipoprotein (a) concentrations are inversely associated with T2D, prediabetes, and insulin resistance in a middle-aged and elderly Chinese population. J Lipid Res. 2015 Apr; 56 (4):920-6.
- A L, BG N, PR K. Low lipoprotein(a) levels and risk of disease in a large, contemporary, general population study. Eur Heart J. 2021
- 77. Xu J, Qiu X, Li Y, Sun N, Zhang Y, Shu J. Hyperlipoproteinemia (a) is associated with breast cancer in a Han Chinese population. Medicine (Baltimore). 2020 Sep; 99 (38):e22037.
- Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. Vol. 57, Journal of Lipid Research. American Society for Biochemistry and Molecular Biology Inc.; 2016; 526-37.
- 79. Gonen A, Yang X, Yeang C, Alekseeva E, Koschinsky M, Witztum JL, et al. Generation and characterization of LPA-KIV9, a murine monoclonal antibody binding a single site on apolipoprotein (a). J Lipid Res. 2020; 61(9):1263-70.
- 80. Marcovina SM, Albers JJ, Gabel B, Koschinsky ML, Gaur VP. Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). Clin Chem. 1995; 41 (2):246-55.
- 81. Kronenberg F. Lipoprotein(a) measurement issues: Are we making a mountain out of a molehill? Atherosclerosis. 2022; 349:123-35.
- 82. Cobbaert CM, Althaus H, Begcevic Brkovic I, Ceglarek U, Coassin S, Delatour V, et al. Towards an SI-Traceable Reference Measurement System for Seven Serum Apolipoproteins Using Bottom-Up Quantitative Proteomics: Conceptual Approach Enabled by Cross-Disciplinary/Cross-Sector Collaboration. Clin Chem. 2021 Mar; 67 (3):478-89.

- 83. Kronenberg F, Trenkwalder E, Dieplinger H, Utermann G. Lipoprotein(a) in stored plasma samples and the ravages of time: Why epidemiological studies might fail. Arterioscler Thromb Vasc Biol. 1996; 16 (12):1568-72.
- 84. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J. 2010 Dec 1; 31 (23):2844-53.
- 85. Nurmohamed NS, Kaiser Y, Schuitema PCE, Ibrahim S, Nierman M, Fischer JC, et al. Finding very high lipoprotein(a): the need for routine assessment. Eur J Prev Cardiol. 2022 May; 29 (5):769-76.
- 86. Wilson DP, Jacobson TA, Jones PH, Koschinsky ML, McNeal CJ, Nordestgaard BG, et al. Use of Lipoprotein(a) in clinical practice: A biomarker whose time has come. A scientific statement from the National Lipid Association. J Clin Lipidol. 2022.
- Ho JH, Adam S, Liu Y, Azmi S, Dhage S, Syed AA, et al. Effect of bariatric surgery on plasma levels of oxidised phospholipids, biomarkers of oxidised LDL and lipoprotein(a). J Clin Lipidol. 2021.
- 88. Tsimikas S. Lipoprotein(a): Novel target and emergence of novel therapies to lower cardiovascular disease risk. Vol. 23, Current Opinion in Endocrinology, Diabetes and Obesity. Lippincott Williams and Wilkins; 2016; 157-64.
- 89. Cao YX, Jin JL, Guo YL, Sun D, Liu HH, Wu NQ, et al. Baseline and on-statin treatment lipoprotein(a) levels for predicting cardiovascular events in patients with familial hypercholesterolemia. Atherosclerosis. 2019 Dec; 291:27-33.
- 90. Lamina C, Kronenberg F. Estimation of the Required Lipoprotein (a)-Lowering Therapeutic Effect Size for Reduction in Coronary Heart Disease Outcomes: A Mendelian Randomization Analysis. JAMA Cardiol. 2019; 4(6):575-9.
- 91. Willeit P, Yeang C, Patrick ;, Moriarty M, Tschiderer L, Varvel SA, et al. Low-Density Lipoprotein Cholesterol Corrected for Lipoprotein(a) Cholesterol, Risk Thresholds, and Cardiovascular Events. J Am Hear Assoc J Am Hear Assoc. 2020; 9:16318.
- 92. Li C, Chen Q, Zhang M, Liu Y, Chu Y, Meng F, et al. The correlation between lipoprotein(a) and coronary atherosclerotic lesion is stronger than LDL-C, when LDL-C is less than 104 mg/dL. BMC Cardiovasc Disord. 2021 Dec; 21 (1):41.
- 93. Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein (a). Clin Chem. 2000 Dec; 46 (12):1956-67.
- 94. Kostner GM, Ibovnik A, Holzer H, Grillhofer H. Preparation of a stable fresh frozen primary lipoprotein[a] (Lp[a]) standard. J Lipid Res. 1999 Dec; 40 (12):2255-63.
- Yeang C, Witztum JL, Tsimikas S. Novel method for quantification of lipoprotein (a)-cholesterol: Implications for improving accuracy of LDL-C measurements. J Lipid Res. 2021; 62:100053.
- Kronenberg F, Mora S, Stroes ESG. Consensus and guidelines on lipoprotein(a) - seeing the forest through the trees. Curr Opin Lipidol. 2022 Dec; 33 (6):342-52.
- 97. Kronenberg F. Prediction of cardiovascular risk by Lp(a) concentrations or genetic variants within the LPA gene region. Clin Res Cardiol Suppl. 2019 Apr; 14 (Suppl. 1):5-12.
- **98.** Schwartz GG, Ballantyne CM. Existing and emerging strategies to lower Lipoprotein(a). Atherosclerosis. 2022 May; 349:110-22.
- Tsimikas S, Gordts PLSM, Nora C, Yeang C, Witztum JL. Statin therapy increases lipoprotein(a) levels. Eur Heart J. 2020 Jun; 41 (24):2275-84.
- 100. de Boer LM, Oorthuys AOJ, Wiegman A, Langendam MW, Kroon J, Spijker R, et al. Statin therapy and lipoprotein(a) levels: a

- systematic review and meta-analysis. Eur J Prev Cardiol. 2022 May; 29 (5):779-92.
- 101. Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014 Jul; 371 (3):203-12.
- 102. Sahebkar A, Reiner Ž, Simental-Mendía LE, Ferretti G, Cicero AFG. Effect of extended-release niacin on plasma lipoprotein(a) levels: A systematic review and meta-analysis of randomized placebo-controlled trials. Metabolism. 2016 Nov; 65 (11):1664-78.
- 103. Artemeva N V., Safarova MS, Ezhov M V., Afanasieva OI, Dmitrieva OA, Pokrovsky SN. Lowering of lipoprotein(a) level under niacin treatment is dependent on apolipoprotein(a) phenotype. Atheroscler Suppl. 2015 May; 18:53-8.
- 104. Albers JJ, Slee A, O'Brien KD, Robinson JG, Kashyap ML, Kwiterovich PO, et al. Relationship of apolipoproteins A-1 and b, and lipoprotein(a) to cardiovascular outcomes: The aim-high trial (atherothrombosis intervention in metabolic syndrome with low HDL/high triglyceride and impact on global health outcomes). J Am Coll Cardiol. 2013 Oct; 62 (17):1575-9.
- 105. Sahebkar A, Simental-Mendía LE, Pirro M, Banach M, Watts GF, Sirtori C, et al. Impact of ezetimibe on plasma lipoprotein(a) concentrations as monotherapy or in combination with statins: a systematic review and meta-analysis of randomized controlled trials. Sci Rep. 2018 Dec; 8 (1):17887.
- 106. Awad K, Mikhailidis DP, Katsiki N, Muntner P, Banach M. Effect of Ezetimibe Monotherapy on Plasma Lipoprotein(a) Concentrations in Patients with Primary Hypercholesterolemia: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Drugs. 2018 Mar; 78 (4):453-62.
- 107. Sahebkar A, Simental-Mendía LE, Watts GF, Serban MC, Banach M. Comparison of the effects of fibrates versus statins on plasma lipoprotein(a) concentrations: A systematic review and meta-analysis of head-to-head randomized controlled trials. BMC Med. 2017 Feb; 15 (1):22.
- 108. O'Donoghue ML, Fazio S, Giugliano RP, Stroes ESG, Kanevsky E, Gouni-Berthold I, et al. Lipoprotein(a), PCSK9 Inhibition, and Cardiovascular Risk. Circulation. 2019 Mar; 139 (12):1483-92.
- 109. Bittner VA, Szarek M, Aylward PE, Bhatt DL, Diaz R, Edelberg JM, et al. Effect of Alirocumab on Lipoprotein(a) and Cardiovascular Risk After Acute Coronary Syndrome. J Am Coll Cardiol. 2020 Jan; 75 (2):133-44.
- 110. Daviglus ML, Ferdinand KC, López JAG, Wu Y, Monsalvo ML, Rodriguez CJ. Effects of Evolocumab on Low-Density Lipoprotein Cholesterol, Non-High Density Lipoprotein Cholesterol, Apolipoprotein B, and Lipoprotein(a) by Race and Ethnicity: A Meta-Analysis of Individual Participant Data From Double-Blind and Open-Label Extension S. J Am Heart Assoc. 2021 Jan; 10 (1).
- 111. Ruscica M, Greco MF, Ferri N, Corsini A. Lipoprotein(a) and PCSK9 inhibition: clinical evidence. Eur Hear J Suppl. 2020 Nov; 22 (Suppl._L):L53-6.
- 112. Schwartz GG, Steg PG, Szarek M, Bittner VA, Diaz R, Goodman SG, et al. Peripheral Artery Disease and Venous Thromboembolic Events After Acute Coronary Syndrome. Circulation. 2020 May; 141 (20):1608-17.
- 113. Marston NA, Gurmu Y, Melloni GEM, Bonaca M, Gencer B, Sever PS, et al. The Effect of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) Inhibition on the Risk of Venous Thromboembolism. Circulation. 2020 May; 141 (20):1600-7.
- 114. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol. N Engl J Med. 2020 Mar; 382 (16):1507-19.

- 115. Rubino J, MacDougall DE, Sterling LR, Kelly SE, McKenney JM, Lalwani ND. Lipid lowering with bempedoic acid added to a proprotein convertase subtilisin/kexin type 9 inhibitor therapy: A randomized, controlled trial. J Clin Lipidol. 2021; 15 (4):593-601.
- 116. Santos RD, Raal FJ, Catapano AL, Witztum JL, Steinhagen-Thiessen E, Tsimikas S. Mipomersen, an antisense oligonucleotide to apolipoprotein B-100, reduces lipoprotein(a) in various populations with hypercholesterolemia: results of 4 phase III trials. Arterioscler Thromb Vasc Biol. 2015 Mar; 35 (3):689-99.
- 117. Nandakumar R, Matveyenko A, Thomas T, Pavlyha M, Ngai C, Holleran S, et al. Effects of mipomersen, an apolipoprotein B100 antisense, on lipoprotein (a) metabolism in healthy subjects. J Lipid Res. 2018 Dec; 59 (12):2397-402.
- 118. Thomas T, Zhou H, Karmally W, Ramakrishnan R, Holleran S, Liu Y, et al. CETP (Cholesteryl Ester Transfer Protein) Inhibition With Anacetrapib Decreases Production of Lipoprotein (a) in Mildly Hypercholesterolemic Subjects. Arterioscler Thromb Vasc Biol. 2017 Sep; 37 (9):1770-5.
- Bowman L, Hopewell JC, Chen F, Wallendszus K, Stevens W, Collins R, et al. Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. N Engl J Med. 2017 Sep; 377 (13):1217-27.
- 120. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. Lancet. 2016 Nov; 388 (10057):2239-53.
- 121. Fernandez-Prado R, Perez-Gomez MV, Ortiz A. Pelacarsen for lowering lipoprotein(a): implications for patients with chronic kidney disease. Vol. 13, Clinical kidney journal. 2020; 753-7.
- 122. Karwatowska-Prokopczuk E, Clouet-Foraison N, Xia S, Viney NJ, Witztum JL, Marcovina SM, et al. Prevalence and influence of LPA gene variants and isoform size on the Lp(a)-lowering effect of pelacarsen. Atherosclerosis. 2021 May; 324:102-8.
- 123. Koren MJ, Moriarty PM, Baum SJ, Neutel J, Hernandez-Illas M, Weintraub HS, et al. Preclinical development and phase 1 trial of a novel siRNA targeting lipoprotein(a). Nat Med. 2022 Jan; 28 (1):96-103.
- 124. Nissen SE, Wolski K, Balog C, Swerdlow DI, Scrimgeour AC, Rambaran C, et al. Single Ascending Dose Study of a Short Interfering RNA Targeting Lipoprotein(a) Production in Individuals With Elevated Plasma Lipoprotein(a) Levels. JAMA. 2022 May; 327 (17):1679-87.
- 125. Kayikcioglu M. LDL Apheresis and Lp (a) Apheresis: A Clinician's Perspective. Curr Atheroscler Rep. 2021; 23 (4).

- 126. Safarova MS, Ezhov M V., Afanasieva OI, Matchin YG, Atanesyan R V., Adamova IY, et al. Effect of specific lipoprotein(a) apheresis on coronary atherosclerosis regression assessed by quantitative coronary angiography. Atheroscler Suppl. 2013 Jan; 14 (1):93-9.
- 127. Khan TZ, Hsu LY, Arai AE, Rhodes S, Pottle A, Wage R, et al. Apheresis as novel treatment for refractory angina with raised lipoprotein(a): A randomized controlled cross-over trial. Eur Heart J. 2017 May; 38 (20):1561-9.
- 128. Jaeger BR, Richter Y, Nagel D, Heigl F, Vogt A, Roeseler E, et al. Longitudinal cohort study on the effectiveness of lipid apheresis treatment to reduce high lipoprotein(a) levels and prevent major adverse coronary events. Nat Clin Pract Cardiovasc Med. 2009; 6 (3):229-39.
- 129. Kagawa A, Azuma H, Akaike M, Kanagawa Y, Matsumoto T. Aspirin reduces apolipoprotein(a) (apo(a)) production in human hepatocytes by suppression of apo(a) gene transcription. J Biol Chem. 1999 Nov; 274 (48):34111-5.
- 130. Akaiek M, Azuma H, Kagawa A, Matsumoto K, Hayashi I, Tamura K, et al. Effect of aspirin treatment on serum concentrations of lipoprotein(a) in patients with atherosclerotic diseases. Clin Chem. 2002 Sep; 48 (9):1454-9.
- 131. Chasman DI, Shiffman D, Zee RYL, Louie JZ, Luke MM, Rowland CM, et al. Polymorphism in the apolipoprotein(a) gene, plasma lipoprotein(a), cardiovascular disease, and low-dose aspirin therapy. Atherosclerosis. 2009 Apr; 203 (2):371-6.
- 132. Marcovina SM, Lippi G, Bagatell CJ, Bremner WJ. Testoster-one-induced suppression of lipoprotein(a) in normal men; relation to basal lipoprotein(a) level. Atherosclerosis. 1996 Apr; 122 (1):89-95.
- 133. Suk Danik J, Rifai N, Buring JE, Ridker PM. Lipoprotein(a), Hormone Replacement Therapy, and Risk of Future Cardiovascular Events. J Am Coll Cardiol. 2008 Jul; 52 (2):124-31.
- 134. Salpeter SR, Walsh JME, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab. 2006 Sep; 8 (5):538-54
- 135. Shlipak MG, Simon JA, Vittinghoff E, Lin F, Barrett-Connor E, Knopp RH, et al. Estrogen and progestin, lipoprotein(a) and the risk of recurrent coronary heart disease events after menopause. J Am Med Assoc. 2000 Apr; 283 (14):1845-52.
- 136. Berg K. A new serum type system in man The Lp System. Acta Pathol Microbiol Scand. 1963 Nov; 59 (3):369-82.



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Nutraceutical alternatives to red yeast rice extract/monacolin K for moderate hypercholesterolaemia: Current evidence and knowledge gaps

Laura Comi^{1*}, Claudia Giglione^{1*}, Fationa Tolaj^{1*}, Cinzia Parolini¹, Chiara Olivieri¹, Marco Ruzza¹, Valentina Tollemeto², Maria Zurlo², Federico Pialorsi², Antonio Seneci², D Paolo Magni^{1,3}

¹Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", Università degli Studi di Milan, Milan, Italy

ABSTRACT

KeywordsNutraceutical:

hypercholesterolaemia; red yeast rice; monacolin K; LDL-cholesterol; phytosterol; berberine; probiotics



© 2022 The Authors Published by SITeCS The nutraceutical approach to moderate hypercholesterolaemia is an interesting option in the context of appropriate conditions associated with low cardiovascular risk, and red yeast rice (RYR) extract is one of the most utilized products in this field. Monacolin K, the RYR main active component, reduces serum LDL-C levels via inhibition of β -Hydroxy β -methylglutaryl-CoA reductase, similarly to statins. In 2011, EFSA approved the claim regarding monacolin K from RYR extract and maintenance of normal cholesterol levels. However, in 2018, EFSA issued a warning about potential adverse effects of this nutraceutical and, in 2022, the European Commission published a Regulation with several limitations of its use. Therefore, current research and development efforts are aiming at assessing efficacy and safety of other known and novel nutraceutical products which may benefit patients with moderate hypercholesterolaemia. These active agents range from phytosterols, probiotics and berberine to bergamot, cabbage, artichoke extracts and soy protein. Moreover, plant extracts from traditional medicine, for example from African countries, are also a subject of study in this field. The full clinical exploitation of many of them, however, still requires robust clinical evidence, which should be the objective of future research.

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Introduction

Cardiovascular disease (CVD) is the first cause of death world-wide and is specifically linked to increased premature mortality and raised health care costs (1). CVD prevalence has gradually increased over the last decades, along with the number of associated deaths, largely due to modifiable risk factors (2). According to the comparative risk assessment analysis provided by the Global Burden of Diseases, Injuries, and Risk Factors Study (1), high systolic blood pressure has been identified as the leading CVD risk factor, followed by unhealthy diet related risks (3), high low-density lipoprotein cholesterol (LDL-C) and fasting glucose plasma levels, high body-mass index, smoking (4), alcohol abuse (5), and physical inactivity (6). Moreover, sleep quality (7), and psychological stress (8) contribute to increase

the CVD risk (9). Therefore, the adjustment of these modifiable factors could help in limiting the development and in preventing of CVD (10, 11). Atherosclerosis is the dominant cause of CVD and results, when combined with thrombosis, in severe atherosclerotic CVD (ASCVD). Atherosclerosis is the result of several different pathophysiological events, including the deposition of cholesterol crystals at the level of the intima layer in the arteries, subsequently combined with a fibrous layer comprising smooth muscle cells, leukocytes, and connective tissue (12). As a direct consequence, lipid-lowering based therapy is indeed considered the first choice in patients with hypercholesterolaemia, combined or not with hyperlipidaemia (13-15). Lipid-lowering drugs include 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), Proprotein Convertase Subtilisin/Kexin type 9" (PCSK9) inhibitors, bempedoic acid,

²Truffini & Reggè, Milan, Italy

³IRCCS MultiMedica, Sesto S. Giovanni, Milan, Italy

^{*} These authors equally contributed to this work

fibrates, bile acid sequestrants (anion exchange resins), niacin (nicotinic acid), and selective cholesterol absorption inhibitors (e.g., ezetimibe) (15-17). Moreover, there is evidence of reduction of CVD risk by lifestyle modifications (18, 19), mainly focused in the control of LDL-C levels (20), and that lifestyle modification is recommended as the first line of management for subjects with LDL-C level up to 116 mg/dL, if at low CVD risk (SCORE <1%), or with lower LDL-C level, if at greater CVD risk (13). It is well known that the long-term prescription of lipid-lowering drugs could be associated with side effects (21), such as hepatotoxicity (22) and myopathy (23, 24), in addition to the increased risk of type 2 diabetes mellitus (T2DM) (25, 26). To overcome this issue, it has been recently identified a "treatment gap" between lifestyle changes and the standard drug therapy. This gap could be filled up by nutraceuticals (27), which are defined as "a food or part of a food that provides benefits to health in addition to its nutritional content", and thereby could be used as an alternative or in addition to pharmacological therapy (28). More specifically, nutraceuticals have been thought to help in preventing illness onset of a variety of pathological conditions such as hypertension (29), T2DM (30), hypertriglyceridemia (31), or hypercholesterolaemia (32, 33). In this review we discuss the current nutraceutical approaches for mild or moderate hypercholesterolaemia, focusing our overview on red yeast rice (RYR) extract/monacolin K properties and critical issues and on the nutraceutical alternatives to this popular product for hypercholesterolemia.

Red yeast rice extract

Red yeast rice (RYR) extract is one of the most frequently used nutraceuticals for the management of mild/moderate hypercholesterolaemia. It is the product of *Monascus purpureus* yeast's fermentation of red rice (*Oryza sativa*). This fermentation process produces monacolins, about 2% in the commonly used RYR extract, and one of their subtypes is monacolin K. This is structurally identical to lovastatin, which was first isolated (under the name of mevinolin) at the end of the 1970s from the fungus *Aspergillus terreus* (34). The main mechanism of action of monacolin K is the inhibition of the β -Hydroxy β -methylglutaryl-CoA (HMG-CoA) reductase enzyme, a key player of the endogenous cholesterol synthesis pathway (**Figure 1**), making RYR a highly effective cholesterol-lowering nutraceutical on the market.

However, the pharmacokinetics and bioavailability of different RYR extract preparations may differ according to relative abundance of the several active components, which may affect the pharmacokinetic profile of monacolin K. On September 1987, the American Food and Drug Administration (FDA) approved lovastatin as a drug for clinical use under the name Mevacor. In 2011, the European

Food Safety Authority (EFSA) and the European Commission (EC) declared the existence of a causal relationship between the daily intake of monacolin K from RYR and the maintenance of normal concentrations of LDL-C in the blood (https://www.efsa.europa.eu/it/ efsajournal/pub/2304) (Figure 2). Both EFSA and the EC raised the warning that the RYR formulations could contain impurities, such as citrinin, which is a mycotoxin metabolite derived from Monascus purpureus fermentation (35). Several animal studies demonstrated that the chronic use of citrinin is nephrotoxic and gradually leads to hyperplasia of the renal tubular epithelium, renal adenomas, and sometimes to malignant renal tumors. Furthermore, citrinin may cause reproductive toxicity, malformations, or even embryo toxicity (36). In consideration of the presence of these impurities, in 2014 the EC established the maximum acceptable level of citrinin in food supplements based on rice fermented with Monascus purpureus as 2,000 pg of citrinin/1 kg of food supplement (EFSA Panel on Contaminants in the Food Chain (CONTAM)). Scientific opinion on the risk for public and animal health related to the presence of citrinin in food and feed. https://www.efsa.europa.eu/it/efsajournal/ pub/2605). In 2018, EFSA approved the opinion that monacolin K in the form of lactone is identical to lovastatin, a drug used in the treatment of hypercholesterolaemia. EFSA stated that taking monacolin K from RYR in the form of dietary supplements can lead to monacolin K exposure equal to therapeutic doses of lovastatin and has therefore emphasized the possibility of adverse reactions similar to those occurring while using lovastatin. Furthermore, the information available to date on adverse reactions are sufficient to state that RYR monacolins used in food supplements at doses of 20 mg/ day could cause serious health issues (37). Eventually, on October 2022, the EC reported that the label of nutraceuticals containing RYR extract must include the following warnings: "this food should not be consumed in a daily dose equal to or greater than 3 mg of monacolins; it must not be consumed by pregnant or in breastfeeding, by children below 18 years old and by adults of over 70 years old, by patients already taking statins and by people consuming other products containing RYR" (Commission Regulation (EU) 2022/860 - Publications Office. https://eur-lex.europa.eu > TXT > PDF > uri=-CEL...P). Nowadays, according to the above presented reasons the suggested dose of monacolins is below 3 mg. In consideration of these restrictions in the use of the RYR extracts, its future remains uncertain, although it seems that patients willing to pay for a natural alternative to statins are more compliant to therapy than those on conventional treatment (38).

In conclusion, the administration of RYR extract with low dose of monacolins (3 mg) could be considered a "relatively" safe nutraceutical with the aim of improving the CVD risk profile in those patients who have a mild or moderate hypercholesterolaemia. RYR should

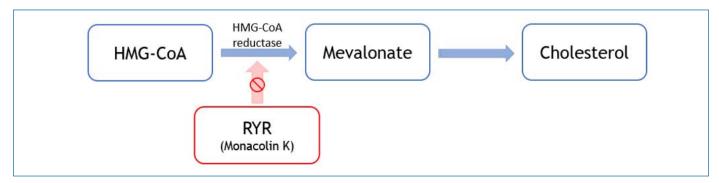


Figure 1 | Mechanism of action of monacolin k from red yeast rice (RYR) extract. HMG-CoA, β -Hydroxy β -methylglutaryl-CoA.

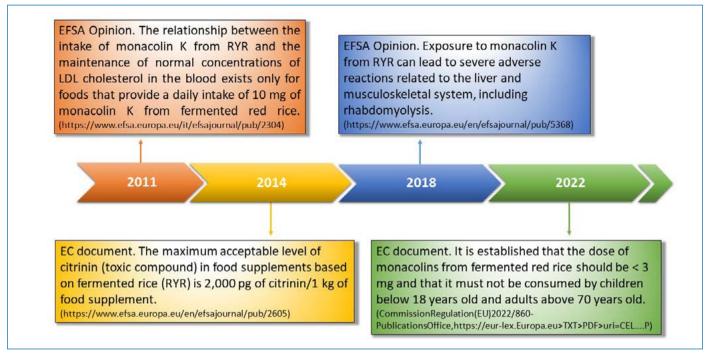


Figure 2 | Timeline of regulatory activities related to red yeast rice extract use. EFSA, European Food Safety Authority; EC, European Commission.

not be used, alone or in association with others hypocholesterolemic agents, when statins are not well-tolerated or when an evident nocebo effect is diagnosed (39). However, the available evidence supports that a large use of RYR should be still limited and RYR supplements should never replace statins or the other pharmacological approaches as first therapeutic option to effectively lower CVD risk (40).

Red yeast rice extract in combination with other active components

Similarly to canonical drug strategies, also in the case of RYR extract it has been demonstrated that treatment with a combination of active nutraceutical components with different mechanisms of action leads to an additive effect (32). A list of the main nutraceutical products for the treatment of moderate hypercolesterolaemia is shown in **Table 1**. For example, RYR extract has been used in combination with berberine, policosanol, astaxanthin, coenzyme Q10, and folic acid (41). Moreover, RYR extract has also been combined with the probiotic *Bifidobacterium Longum* BB536 (42, 43). Policosanols are a mixture of waxy alcohols derived from a variety of plants. They inhibit cholesterol synthesis by activation of AMP-kinase and modulating HMG-CoA reductase activity in hepatoma cells and

cultured fibroblasts, respectively, showing different mechanisms of action (44, 45). Astaxanthin is a carotenoid providing a pink color characteristic of few species and it is one of the most powerful biological antioxidant compounds. This molecule is able to prevent the infiltration of peroxidized LDL-C into the arterial intima and the formation of atherosclerotic plaques, thanks to its free radical scavenging activity. Due to these characteristics, astaxanthin protects cell membranes, LDL-C, endothelium of blood vessels and tissues against lipid peroxidation and oxidative damage (46). RYR, astaxanthin and policosanols were tested alone and in combination, using a model of experimental atherosclerosis elicited in rabbits with a cholesterol-enriched feed. It was shown that this combination of compounds could lead to additive or synergistic health effects, thanks to the different mechanism of action of each compound (47). Berberine is an isoquinoline alkaloid plant extract that has historically been used in traditional Chinese medicine. Berberine has been associated with hypolipidemic, anti-inflammatory and hypotensive properties, resulting in antiatherosclerotic effects. Berberine is also associated with serum glucose level reduction, increased expression of LDL receptors and reduction of serum cholesterol concentration by inhibiting lipid synthesis (48, 49).

Treatments with nutraceutical combinations containing chitosan,

Table 1 | Summary of the features of the main nutraceutical product for the treatment of mild/moderate hypercholesterolaemia, features based on evidence.

Nutraceutical	LDL-C reduction (%)	Mechanism of action	Daily dose	Evidence level	Recommendation class	Reference
RYR extract	-15 to 25	LDL synthesis inhibition (HMG-CoA reductase inhibition)	3 mg (monacolin k)	А	lla	51
Plant sterol/stanols	-12	Absorption inhibition	1500-3000 mg	В	IIb	54
Berberine	-15	LDL excretion Improvement (AMPK activation, other)	500-1500 mg	Α	I	62
Soy protein	-5	LDL excretion Improvement (various mechanisms)	25-100 g	Α	IIb	71
Artichoke	-10	HMG-CoA reductase inhibition, anti-inflammatory/anti-oxidant, othe	1-3 g	В	lla	84
Probiotics	-5	Absorption inhibition (various mechanisms)	dependent on strain	В	IIb	91
Bergamot	-15	HMG-CoA reductase inhibition, anti-inflammatory/anti-oxidant, othe	500-1000 mg	В	lla	84

RYR and berberine significantly reduced non-HDL-C and LDL-C in individuals with hypercholesterolaemia compared to placebo, without changes of PCSK9 plasma levels (50).

Moving beyond RYR extract: alternative nutraceutical products for the treatment of mild/moderate hypercholesterolaemia

In consideration of the above-mentioned critical issues and limitations in RYR extract use, a wide research effort has been devoted to the validation and development of well-established novel nutraceutical products with a good efficacy and safety profile. Some of them have already been mentioned above, in combination with RYR extract, while others are novel and have been proposed rightaway as alternative options (51).

Phytosterols. Phytosterols are triterpenes usually classified as sterols or stanols, according to the presence or absence of a double bond in position 5. A relevant structural difference is present between cholesterol and sitosterol, since the latter shows an additional ethyl group at position C-24, probably responsible for its relatively poor intestinal absorption (52, 53). The main mechanism responsible for phytosterol-induced reduction in circulating cholesterol levels resides in the competition with cholesterol for incorporation into mixed micelles in the intestinal tract (54). Phytosterols are more easily hydrolysable than cholesterol, and this leads to a lower solubilization of cholesterol into micelles, which decreases their absorption and increases fecal excretion of cholesterol and its metabolites. The cholesterol-lowering effect of phytosterols may be observed within a few weeks of treatment and remains stable upon supplementation (54). After interruption of phytosterol intake, circulating cholesterol concentrations return back to basal conditions (55). The reduction of cholesterol absorption in the intestine and reaching the liver through the chylomicron pathway promotes a greater endogenous synthesis of cholesterol as well as a greater uptake of plasma LDL-C by hepatocytes, in order to maintain cholesterol homeostasis. Such enhanced clearance of circulating LDL-C leads to a reduction of its plasma concentration, which is around 2-3% for a low (300-400 mg/ day) dietary intake of phytosterols (56) and reaches an average of 9% for an intake of 1500-2000 mg per day (57). Greater dosages of phytosterols up to 3 g/day have been shown to promote a 12–12.5% reduction (53, 54). Recent meta-analyses of published trials (58) indicate that the effect of the intake of phytosterols on plasma LDL-C levels in humans is within the range indicated by EFSA in its 2008 Opinion (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j. efsa.2008.781).

Berberine. Berberine is well known to be able to reduce intestinal cholesterol absorption by increasing the elimination of cholesterol through the faecal material and to stimulate the formation of bile acids. It also stimulates adenosine monophosphate activated protein kinase (AMPK), which can limit the synthesis of fatty acids (59). Berberine also increases glucose transporter-4 (GLUT-4) and glucagon like peptide-1 (GLP-1) levels (60). Several studies reported that three months of treatment with berberine (500 mg/day) results in reduction of plasma concentrations of total cholesterol, triglyceride and LDL-C and in increased concentration of HDL-C (61). Moreover, data obtained from a recent metanalysis of 19 clinical studies showed a significant reduction in total cholesterol and LDL-C. As regards HDL-C, a slight non-significant increase was observed (62). Moreover, in vitro studies on HepG2 human hepatocarcinoma cell line demonstrated that berberine inhibits the synthesis of cholesterol and triglycerides (63) and reduces the gene and protein expression of PCSK9 (64). Berberine has been shown to be safe in the majority of clinical trials. In a small percentage of patients, berberine has been reported to cause nausea, vomiting, constipation, hypertension, respiratory failure and paresthesia; however, clinical evidence of such adverse effects is not prominent in the literature. Rare adverse effects including headache, skin irritation, facial flushing, bradycardia have also been reported with the use of berberine (60).

Soy proteins. A large number of published studies has evaluated the hypocholesterolaemic potential of a regular consumption of soy protein. A health claim has been approved by the Food and Drug Administration stating that diets low in saturated fat and cholesterol that substitute 25-30 g/day protein from animal sources with an equal amount of soy protein, may reduce the risk of coronary heart disease (65). Some evidence show that the cholesterol-lowering effect of soy derivatives is due almost entirely to soy protein, while isoflavones have only a limited effect on blood lipids, but could play a key role in inflammation (66). In fact, the isoflavones are present as β-glucosides, after ingestion the glycosidic bond is hydrolyzed by the microbiota to release free aglycones, which can be absorbed or further metabolized. Isoflavones or their metabolized forms could enter in the blood circulation and act on endothelial function, in particular daidzein, dihydrodaidzein (DHD), equol, O-desmethylangolensin (O-DMA), genistein (67). Soy proteins are largely composed of storage proteins, and the two main components are known as β-conglycinin (7S globulin) and glycinin (11S globulin) (68, 66). The proposed cholesterol-lowering mechanisms of soy proteins are still unclear and may include down regulation of the expression of sterol regulatory element-binding protein (SREBP-1), modulation of the PI3K/Akt/GSK3b pathways, decrease of cholesterol synthesis, increase of ApoB receptor activity, modulation of the activity of bile acids (69). Most randomized controlled trials that evaluated the lipidemic profile after purified protein diet in adults with normal or moderate hypercholesterolaemia, resulted in a significant reduction in total cholesterol (at least - 4%) and LDL-C (about - 6%) (70). It should be noted that patients do not take soy protein in a purified form; therefore, a novel approach was used in a recent study in which patients were given whole soy foods (30 g of soy protein) possibly commercially available, on a low lipid diet for 12 weeks. Compared to a standard low-lipid diet with the same amount of animal protein, there was a reduction of total cholesterol (-4.8%), LDL-C (-5.2%), non-HDL-C (-7.1%) and apoB (-14.8%) levels in the circulation (71). Products containing soy could have beneficial effects because of their content of polyunsaturated fatty acids, fiber, vitamins and minerals and their low SFA content (72). The role of soy in the reduction of coronary artery disease remains controversial. As of today, EFSA has concluded that the hypocholesterolemic effect of isolated soy protein has not been established (73). In addition, the 2011 version of the ESC/EAS Guidelines suggested that soy can be used as a substitute for animal protein foods, despite the fact that LDL cholesterol lowering is modest and greater than subjects with hypercholesterolaemia (74). In contrast, the most recent 2019 version (75) downsized the role of this nutraceutical: "LDL-C-lowering effect was not confirmed when changes in other dietary components were taken into account" (76). In conclusion, the evidence currently available in the literature and the statements made by health agencies do not appear to reveal a clinical importance of soy protein in the management of hypercholesterolaemia.

Lupin proteins. Yellow lupin (*Lupinus luteus*) contains fiber (30%), protein (30-35%), carbohydrates, unsatured fat and also phosphorus, calcium, and magnesium. Lupin is different from other legumes, such as soy, because of the absence of phytoestrogens and low sodium content (77, 69). The observed activities of lupin

could be explained by the peptides deriving from the hydrolysis of lupin proteins. The mechanism of action of absorbed lupin peptides has been investigated in some molecular studies. They seem to interfere with HMG-CoA reductase activity, up-regulating LDL receptor and SREBP-2 (78), as already observed in the case of soy protein. Some works provided evidence that lupin peptides may also inhibit PCSK9 (79). The lipid-lowering effects of lupin may be also linked to the formation of short-chain fatty acids, specifically propionate and acetate (80). Clinical studies confirmed these experimental investigations (81), however others do not agree about the effects of lupin consumption. For example a study has shown that a 12-week treatment of individuals in low-lipid diet with a moderate dyslipidaemia with a lupin protein concentrate (30 g/day) leads to a non-significant reduction of total cholesterol, LDL-C, non-HDL-C, in comparison with those consumed a lactose-free skimmed milk powder (82). In conclusion, lupin protein intake represents a relatively weak adjuvant therapy in the treatment of hypercholesterolaemia, also according to most guidelines (76).

Artichoke. Artichoke (Cynara scolymus L.) is a plant native from the Mediterranean area (North Africa and Southern Europe). Receptacle of C. scolymus L., also known as "artichoke heart", is commonly consumed as a food. In addition, a traditional use of artichoke leaf extracts is reported, especially for its antioxidant, liver-protective, anti-microbial, choleretic, hepatoprotective, bile-enhancing and lipid-lowering effects. It is related to beneficial effects in atherosclerosis, biliary duct, digestive tract, and the treatment of scurvy and anemia (83). A recent review summarized beneficial effects of artichoke extracts on blood lipid concentrations after consumption of artichoke leaf extracts compared with placebo or reference pharmacological treatment (84). The active components of artichoke have been found to inhibit cholesterol synthesis and show relevant anti-inflammatory and antioxidant properties (85). Clinical trials showed a significant reduction of total cholesterol concentrations compared to placebo after 6 or 12 weeks of supplementation. Trials reports indicate mild, transient and infrequent adverse events. In addition, a recent meta-analysis analyzed data from 9 trials including 702 participants with mostly mild to moderate hypercholesterolemia. Consumption of artichoke extract, for 6 to 12 weeks, significantly decreased plasma concentrations of total cholesterol and LDL-C with no significant alteration in plasma HDL-C concentrations (86).

Probiotics. The gut microbiome has been shown to affect human health and disease, including hypercholesterolemia. A disbalance of the microbiota (i.e. dysbiosis), caused by different factors, can increase the intestinal permeability and eventually lead to higher levels of bacterial metabolites. This event together with other mechanisms promotes low-grade inflammation, which has been shown to be one of the main main contributor of atherosclerosis development (87, 88). Based on these observations, probiotics, prebiotics and their combinations have gained interest as a new promising approach for dyslipidemia treatment. The World Health Organization defines probiotics as: "live microorganisms that, when administered in adequate quantities, confer health benefits to the host" (89). Probiotics insinuate additional microorganisms in the host, prebiotics stimulate their growth in the intestinal tract, while synbiotics combine these applications to improve the viability. The hypocholesterolaemic effect of probiotics may result from several mechanisms, including:

- bile salt hydrolase activity regulation, thus reducing the bile salts enterohepatic circulation;
- 2) cholesterol incorporation into cellular membranes of bacteria;
- cholesterol conversion into prostanol, hence reducing its absorption and facilitating the excretion via the feces;

- 4) cholesterol transport modulation by NPC1L1, ABCA1, CD36, and SR-B1 down-regulating gene expression;
- 5) cholesterol synthesis modulation through short-chain fatty acids production;
- 6) exopolysaccharides production, which then bind to free bile acids and increase their elimination through the feces (90, 91).

Previous clinical studies described controversial results regarding probiotics effect on dyslipidemia. Several meta-analyses of human trials suggest that, in most cases, probiotic treatment significantly reduced total cholesterol and LDL-C, with little effect on triglycerides and HDL-C (92). Differently from others, a significant TG level reduction has also been reported (93). There are difficulties since the effects on lipid profile depended on intervention times and probiotic strains, which are different in each study. Hence, many of these mechanisms still need to be clarified in humans.

Bergamot. Bergamot (*C. bergamia*) belongs to the Citrus fruits and differs from others Citrus fruits for its high-content of flavonoids and statin-like compounds (94). Bergamot-derived molecules have been shown to ameliorate plasma lipid profile and visceral fat in clinical trials (84, 95). Specifically, among bergamot constituents, naringin, neoeriocitrin, and rutin were found to inhibit the oxidation of LDL particles (96). Moreover, the scientific literature of the last decade has provided evidence about the potential anti-inflammatory and antioxidant effects of bergamot extracts (97). Interestingly, clinical studies have also shown lipid-lowering effects of bergamot-derived compounds such as the reduction of LDL-C, triglycerides, non-HDL-C, and malonyl dialdehyde (98).

Cabbage. Brassicaceae (crucifers) is one of the most extensive angiosperm families, composed of 360 genera and approximately 3709 species distributed worldwide. One important species is cabbage, that is an essential part of the human diet thanks to its high nutritional value, mainly due to its content in fiber, minerals and vitamins (99). Cabbage exhibits anti-aging, anticancer and antioxidant properties, and presents multiple health benefits (100). Cabbage's dietary fiber (DF) can be classified into insoluble DF (IDF) and soluble DF (SDF). Pectin and oligosaccharides, the main components of SDF, can decrease blood glucose and cholesterol, prevent cardiovascular diseases, and serve as important probiotic sources. Soluble DF also generates short-chain fatty acids, which can regulate immunity and promote the growth of probiotics in the digestive system to prevent inflammatory bowel diseases (101). Moreover, several studies suggest that cabbage has a functional potential to regulate glucose homeostasis and improves health in people with T2DM (102). Plant food groups such as berries, green leaves, and crucifers (including cabbage) would play a significant role in reducing the risk of T2DM (103). A prospective study in Japan analyzed the relationship between the intake of different plant food groups and the incidence of T2DM. The results showed a significant higher risk reduction among the male population and consumption of vegetables, including cabbage (104). Moreover, the effects of cabbage on serum lipid levels in hypercholesterolaemic patients were investigated. Their serum total cholesterol levels significantly decreased from 6.7 ± 0.8 to 6.1± 0.6 mmol/L, and, more strikingly, the level of LDL-C significantly decreased from 4.4 \pm 0.8 to 3.8 \pm 0.7 mmol/L. At 9 weeks after the cessation of administration, these levels returned to the preadministration levels (105).

Goji (Lycium barbarum L.). Lycium barbarum is a well-known traditional Chinese herbal medicine. Supplemental L. barbarum is beneficial to nourish the liver and kidney and brighten the eyes. In support of these traditional properties, scientific evidence has shown that L. barbarum fruit possesses a variety of biological activities, such as anti-aging, neuroprotection, anti-fatigue/endurance, increased me-

tabolism, glucose control in diabetics, glaucoma, antioxidant properties, immunomodulation, anti-tumor activity and cytoprotection (106). L. barbarum includes polysaccharides, carotenoids, flavonoids, betaine, cerebroside, beta-sitosterol, p-coumaric acid, vitamins and other phytochemicals. Among them, L. barbarum polysaccharides (LBP) have various physiological effects, such as antioxidant effects (107), anti-diabetes (108) and cardiovascular benefits (109). The effect of L. barbarum on the cardiometabolic risk factors was investigated in a meta-analysis (106), combining seven randomized controlled trials with a total of 548 participants. Authors concluded that L. barbarum significantly reduced total cholesterol and triglycerides concentrations in participants aged ≥60 years or intervention period duration of ≥3 months (106). In another clinical trial, 50 participants with a metabolic syndrome were randomly divided into two groups, control and supplemented with 14 g/day of goji berry in the diet. Both LDL-C and VLDL-C were significantly reduced in the supplemented group (110).

Olea europaea. The olive tree leaves (Olea europaea L.), native from the Mediterranean area, have been used in traditional herbal medicine with the aim of preventing or treating several conditions like hypertension, diabetes and inflammation. Potential effects could be related to the rich content of polyphenols oleuropein and hydroxytyrosol that benefits human health (111). Current studies show great potential in these effects: in a recent meta-analysis, olive leaf extract shows improvement of lipid profile in normal-weight subjects (trigliceride (- 9.21 mg/dL), total cholesterol (- 6.69 mg/dL), systolic blood pressure (-7.05 mmHg), as well as in patients with hypertension (triglyceride (-14.42 mg/dl), total cholesterol (-9.14 mg/ dL), LDL-C (- 4.6 mg/dL) (112). In a randomized, double-blind, controlled crossover trial, olive leaf extract (providing 136.2 mg oleuropein and 6.4 mg hydroxytyrosol per day) significantly reduced plasma LDL-C, total cholesterol and triglyceride concentrations in 60 men with slightly elevated blood pressure (113). Furthermore, in another randomized placebo-controlled clinical trial, there was a significant improvement in the triglyceride-to-HDL-cholesterol ratio and a significant decrease in triglyceride levels (114). Olmez et al. (115) described a lipid-lowering effect in rats supplemented with olive leaf extract (50 or 100 mg/kg/day) for 8 weeks. LDL-C and total cholesterol were significantly lower than in rats fed with a high cholesterol diet only. Overall results from literature seem to be promising, although there is a need for further studies to fully understand the related mechanisms.

Ethnic medicine and cardiometabolic disease: experimental evidence

In many countries, herbs and spices have been traditionally used as remedies for a wide range of pathological conditions, including cardiovascular and metabolic diseases, and some of them, like berberine, have been discussed above. Recently, experimental studies have been focused on additional extracts, especially from plants of African origin, which appear to have been less exploited by appropriate research. Indeed, before human use as potential nutraceuticals, it is crucial to better understand the active components and the potentially toxic compounds of such botanical preparations, highlighting their activity in modulating specific molecular pathways linked to cardiometabolic diseases, including hypercholesterolaemia. In this field, extracts from Adansonia digitata L. (also known as baobab) and from a series of Cameroonian spices have been studied for their potential usefulness in cardiometabolic disease using cell-based and in vivo models. In particular, the fruit pulp and leaf extracts of Adansonia digitata L. have been shown a good inhibitory activity against relevant enzymes such as HMG-CoA reductase, in addition to alpha-amylase, alpha-glucosidase, angiotensin-converting enzyme, and pancreatic lipase (116). Moreover, Adansonia digitata leaf extracts were found to reduce hyperglycaemia and hyperlipidaemia (including LDL-C reduction) of diabetic rats (117). A series of spice extracts from Cameroon has also been extensively studied in different experimental settings, showing that some of them may positively modulate enzymes relevant to arbohydrate/lipid metabolism and cardiometabolic disorders (118), oxidative stress, inflammation and metabolic pathways related to lipid biosynthesis in hepatic and adipose human cell models (119, 120) and in animal models of diet-induced obesity (121). Taken together, well-characterized extracts from plants used in African traditional medicine have been shown to positively modulate molecular pathways associated with hypercholesterolaemia and other events promoting atherosclerosis, suggesting their use in cardiometabolic conditions of moderate severity. These promising data should prompt further research, especially in the context of clinical trials.

Concluding remarks

The use of nutraceutical products represents a feasible option for the management of mild to moderate hypercholesterolaemia in subjects with low cardiovascular risk, and RYR extract is one of the most effective and used products in this field. The current warnings for potential adverse effects and the limitations of use of RYR extract/monacolin K highlight the need to identify and validate additional active compounds for the management of this condition. The use of these nutraceuticals as add-on therapy on top of current drug treatments for hypercholesterolaemia is also an area deserving to be studied. Moreover, important socio-economical aspects, like their global sustainability and the potential exploitation of agro-food waste in terms of human health benefit, should also be taken into consideration in the context of such nutraceutical development.

Conflict of interest

All authors have no conflict of interest to disclose.

Authors' contribution

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References

- Roth GA, Mensah GA, Johnson CO, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol 2020; 76:2982-3021.
- Wang T, Zhao Z, Yu X, et al. Age-specific modifiable risk factor profiles for cardiovascular disease and all-cause mortality: a nationwide, population-based, prospective cohort study. Lancet Reg Health West Pac 2021; 17:100277.
- Chareonrungrueangchai K, Wongkawinwoot K, Anothaisintawee T, Reutrakul S. Dietary Factors and Risks of Cardiovascular Diseases: An Umbrella Review. Nutrients 2020; 12.
- Gallucci G, Tartarone A, Lerose R, Lalinga AV, Capobianco AM. Cardiovascular risk of smoking and benefits of smoking cessation. J Thorac Dis 2020; 12:3866-76.
- Hoek AG, van Oort S, Mukamal KJ, Beulens JWJ. Alcohol Consumption and Cardiovascular Disease Risk: Placing New Data in Context. Curr Atheroscler Rep 2022; 24:51-9.
- Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary Behavior, Exercise, and Cardiovascular Health. Circ Res 2019; 124:799-815.
- Lao XQ, Liu X, Deng HB, et al. Sleep Quality, Sleep Duration, and the Risk of Coronary Heart Disease: A Prospective Cohort Study With 60,586 Adults. J Clin Sleep Med 2018; 14:109-17.
- Osborne MT, Shin LM, Mehta NN, et al. Disentangling the Links Between Psychosocial Stress and Cardiovascular Disease. Circ Cardiovasc Imaging 2020; 13:e010931.
- Perk J, De Backer G, Gohlke H, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 2012; 33:1635-701.
- Rippe JM. Lifestyle Strategies for Risk Factor Reduction, Prevention, and Treatment of Cardiovascular Disease. Am J Lifestyle Med 2019; 13:204-12.
- Dimovski K, Orho-Melander M, Drake I. A favorable lifestyle lowers the risk of coronary artery disease consistently across strata of non-modifiable risk factors in a population-based cohort. BMC Public Health 2019;19:1575.
- Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med 2014;5:927-946.
- 13. Mach F, Baigent C, Catapano A, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. European heart journal 2020;41.
- Visseren FLJ, Mach F, Smulders YM, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 2021;42:3227-3337.
- 15. Averna M, Banach M, Bruckert E, et al. Practical guidance for combination lipid-modifying therapy in high- and very-high-risk patients: A statement from a European Atherosclerosis Society Task Force. Atherosclerosis 2021; 325:99-109.
- Parham JS, Goldberg AC. Review of recent clinical trials and their impact on the treatment of hypercholesterolemia. Prog Cardiovasc Dis 2022; 75:90-6.
- Kim KA, Park HJ. New Therapeutic Approaches to the Treatment of Dyslipidemia 2: LDL-C and Lp(a). J Lipid Atheroscler 2023; 12:37-46.
- 18. Claas SA, Arnett DK. The Role of Healthy Lifestyle in the Pri-

- mordial Prevention of Cardiovascular Disease. Curr Cardiol Rep 2016: 18:56.
- Doughty KN, Del Pilar NX, Audette A, Katz DL. Lifestyle Medicine and the Management of Cardiovascular Disease. Curr Cardiol Rep 2017; 19:116.
- Janse Van Rensburg WJ. Lifestyle Change Alone Sufficient to Lower Cholesterol in Male Patient With Moderately Elevated Cholesterol: A Case Report. Am J Lifestyle Med 2018; 13:148-55.
- 21. Wilkinson MJ, Laffin LJ, Davidson MH. Overcoming toxicity and side-effects of lipid-lowering therapies. Best Pract Res Clin Endocrinol Metab 2014; 28:439-52.
- 22. Björnsson E. Hepatotoxicity of statins and other lipid-lowering agents. Liver international: official journal of the International Association for the Study of the Liver 2017; 37.
- 23. Magni P, Macchi C, Morlotti B, Sirtori CR, Ruscica M. Risk identification and possible countermeasures for muscle adverse effects during statin therapy. European Journal of Internal Medicine 2015; 26.
- 24. Mancini GB, Baker S, Bergeron J, et al. Diagnosis, prevention, and management of statin adverse effects and intolerance: proceedings of a Canadian Working Group Consensus Conference. Can J Cardiol 2011; 27:635-62.
- Carter AA, Gomes T, Camacho X, et al. Risk of incident diabetes among patients treated with statins: population based study. BMJ 2013; 346:f2610.
- 26. Ruscica M, Macchi C, Morlotti B, Sirtori CR, Magni P. Statin therapy and related risk of new-onset type 2 diabetes mellitus. Eur J Intern Med 2014; 25:401-6.
- 27. Santini A, Tenore GC, Novellino E. Nutraceuticals: A paradigm of proactive medicine. Eur J Pharm Sci 2017; 96:53-61.
- Puri V, Nagpal M, Singh I, et al. A Comprehensive Review on Nutraceuticals: Therapy Support and Formulation Challenges. Nutrients 2022; 14.
- Borghi C, Cicero AF. Nutraceuticals with a clinically detectable blood pressure-lowering effect: a review of available randomized clinical trials and their meta-analyses. Br J Clin Pharmacol 2017; 83:163-71.
- Fernandes I, Oliveira J, Pinho A, Carvalho E. The Role of Nutraceutical Containing Polyphenols in Diabetes Prevention. Metabolites 2022; 12.
- **31.** Penson PE, Banach M. Nutraceuticals for the Control of Dyslipidaemias in Clinical Practice. Nutrients 2021; 13.
- Protic O, Bonfigli AR, Antonicelli R. Nutraceutical Combinations in Hypercholesterolemia: Evidence from Randomized, Placebo-Controlled Clinical Trials. Nutrients 2021; 13.
- 33. Osadnik T, Goławski M, Lewandowski P, et al. A network meta-analysis on the comparative effect of nutraceuticals on lipid profile in adults. Pharmacol Res 2022; 183:106402.
- 34. Alberts A, Chen J, Kuron G, et al. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proceedings of the National Academy of Sciences of the United States of America 1980; 77.
- **35.** Rasheva T, Nedeva T, Hallet J, Kujumdzieva A. Characterization of a non-pigment producing Monascus purpureus mutant strain. Antonie van Leeuwenhoek 2003; 83.
- Singh ND SA, Dwivedi P, Patil RD, Kumar M. Experimentally induced citrinin and endosulfan toxicity in pregnant Wistar rats: histopathological alterations in liver and kidneys of fetuses. Journal of applied toxicology: JAT 2008; 28.
- Scientific opinion on the safety of monacolins in red yeast rice
 2018 EFSA Journal Wiley Online Library.

- Fernández-Friera L, Fuster V, López-Melgar B, et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. J Am Coll Cardiol 2017; 70:2979-91.
- 39. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. European heart journal 2016; 37.
- Cicero A, Fogacci F, Zambon A. Red Yeast Rice for Hypercholesterolemia: JACC Focus Seminar. Journal of the American College of Cardiology 2021; 77.
- 41. Ruscica M, Gomaraschi M, Mombelli G, et al. Nutraceutical approach to moderate cardiometabolic risk: results of a randomized, double-blind and crossover study with Armolipid Plus. J Clin Lipidol 2014; 8:61-8.
- 42. Ruscica M, Pavanello C, Gandini S, et al. Nutraceutical approach for the management of cardiovascular risk a combination containing the probiotic Bifidobacterium longum BB536 and red yeast rice extract: results from a randomized, double-blind, placebo-controlled study. Nutr J 2019; 18:13.
- Cicolari S, Pavanello C, Olmastroni E, et al. Interactions of Oxysterols with Atherosclerosis Biomarkers in Subjects with Moderate Hypercholesterolemia and Effects of a Nutraceutical Combination Nutrients 2021; 13.
- Singh DK, Li L, Porter TD. Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase. J Pharmacol Exp Ther 2006; 318:1020-6.
- **45.** Menéndez R AA, Rodeiro I, González RM, González PC, Alfonso JL, Más R. Policosanol modulates HMG-CoA reductase activity in cultured fibroblasts. Archives of medical research 2001; 32.
- Pereira CPM, Souza ACR, Vasconcelos AR, Prado PS, Name JJ. Antioxidant and anti-inflammatory mechanisms of action of astaxanthin in cardiovascular diseases (Review). Int J Mol Med 2021; 47:37-48.
- **47.** Setnikar I, Senin P, Rovati LC. Antiatherosclerotic efficacy of policosanol, red yeast rice extract and astaxanthin in the rabbit. Arzneimittelforschung 2005; 55:312-7.
- Wu M WJ, Liu LT. Advance of studies on anti-atherosclerosis mechanism of berberine. Chinese journal of integrative medicine 2010; 16.
- 49. Xie X MX, Zeng S, Tang W, Xiao L, Zhu C, Yu R. Mechanisms of Berberine for the Treatment of Atherosclerosis Based on Network Pharmacology. Evidence-based complementary and alternative medicine: eCAM 2020; 2020.
- 50. Spigoni V, Aldigeri R, Antonini M, et al. Effects of a New Nutraceutical Formulation (Berberine, Red Yeast Rice and Chitosan) on Non-HDL Cholesterol Levels in Individuals with Dyslipidemia: Results from a Randomized, Double Blind, Placebo-Controlled Study. International journal of molecular sciences 2017; 18.
- Cicero AFG, Fogacci F, Stoian AP, et al. Nutraceuticals in the Management of Dyslipidemia: Which, When, and for Whom? Could Nutraceuticals Help Low-Risk Individuals with Non-optimal Lipid Levels? Curr Atheroscler Rep 2021; 23:57.
- Marangoni F, Poli A. Phytosterols and cardiovascular health. Pharmacological research 2010; 61.
- 53. Poli A, Marangoni F, Corsini A, et al. Phytosterols, Cholesterol Control, and Cardiovascular Disease. Nutrients 2021; 13.
- 54. Ras RT, Geleijnse JM, Trautwein EA. LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomised controlled studies. Br J Nutr 2014; 112:214-9.

- 55. Visioli F, Poli A. Prevention and Treatment of Atherosclerosis: The Use of Nutraceuticals and Functional Foods. 2022.
- Klingberg S, Ellegård L, Johansson I, et al. Inverse relation between dietary intake of naturally occurring plant sterols and serum cholesterol in northern Sweden. Am J Clin Nutr 2008; 87:993-1001.
- 57. Katan MB, Grundy SM, Jones P, et al. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003; 78:965-78.
- 58. Ying J, Zhang Y, Yu K. Phytosterol compositions of enriched products influence their cholesterol-lowering efficacy: a meta-analysis of randomized controlled trials. Eur J Clin Nutr 2019; 73:1579-93.
- Li X, Zhao Z, Huang M, et al. Effect of Berberine on promoting the excretion of cholesterol in high-fat diet-induced hyperlipidemic hamsters. Journal of translational medicine 2015; 13.
- 60. Derosa G, Maffioli P, Cicero A. Berberine on metabolic and cardiovascular risk factors: an analysis from preclinical evidences to clinical trials. Expert opinion on biological therapy 9019: 19
- 61. Lan J, Zhao Y, Dong F, et al. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol 2015; 161:69-81.
- 62. Bertuccioli A, Moricoli S, Amatori S, et al. Berberine and Dyslipidemia: Different Applications and Biopharmaceutical Formulations Without Statin-Like Molecules-A Meta-Analysis. J Med Food 2020; 23:101-3.
- 63. Liu X, Li W, Zhang H, et al. Biodistribution and pharmacokinetic profile of berberine and its metabolites in hepatocytes. Phytomedicine: international journal of phytotherapy and phytopharmacology 2022; 104.
- **64.** Adorni MP, Zimetti F, Lupo MG, Ruscica M, Ferri N. Naturally Occurring PCSK9 Inhibitors. Nutrients 2020; 12.
- 65. Stein K. FDA approves health claim labeling for foods containing soy protein. Journal of the American Dietetic Association 2000; 100.
- 66. Sirtori C, Lovati M. Soy proteins and cardiovascular disease. Current atherosclerosis reports 2001; 3.
- 67. Dewell A, Piper L, Hollenbeck C. A critical evaluation of the role of soy protein and isoflavone supplementation in the control of plasma cholesterol concentrations. The Journal of Clinical Endocrinology & Metabolism 2006; 91:772-80.
- 68. KeShun L. Soybeans: Chemistry, Technology, and Utilization. Springer New York, NY; 2012.
- **69.** Cicero AFG, Colletti A, Bajraktari G, et al. Lipid-lowering nutraceuticals in clinical practice: position paper from an International Lipid Expert Panel. Nutr Rev 2017; 75:731-67.
- Harland J, Haffner T. Systematic review, meta-analysis and regression of randomised controlled trials reporting an association between an intake of circa 25 g soya protein per day and blood cholesterol. 2008.
- 71. Ruscica M, Pavanello C, Gandini S, et al. Effect of soy on metabolic syndrome and cardiovascular risk factors: a randomized controlled trial. European journal of nutrition 2018; 57.
- 72. Mannarino M, Ministrini S, Pirro M. Nutraceuticals for the treatment of hypercholesterolemia. European journal of internal medicine 2014: 25.
- 73. Scientific Opinion on the substantiation of a health claim related to isolated soy protein and reduction of blood LDL-cholesterol concentrations pursuant to Article 14 of Regulation (EC) No 1924/2006 2012 EFSA Journal Wiley Online Library.

- 74. Reiner Z, Catapano A, De Backer G, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). European heart journal 2011; 32.
- 75. Cosentino F, Grant P, Aboyans V, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. European heart journal 2020; 41.
- Casula M, Catapano AL, Magni P. Nutraceuticals for Dyslipidaemia and Glucometabolic Diseases: What the Guidelines Tell Us (and Do Not Tell, Yet). Nutrients 2022; 14.
- 77. Parolini C, Rigamonti E, Marchesi M, et al. Cholesterol-lowering effect of dietary Lupinus angustifolius proteins in adult rats through regulation of genes involved in cholesterol homeostasis. Food chemistry 2012; 132.
- 78. Lammi C, Zanoni C, Scigliuolo G, D'Amato A, Arnoldi A. Lupin peptides lower low-density lipoprotein (LDL) cholesterol through an up-regulation of the LDL receptor/sterol regulatory element binding protein 2 (SREBP2) pathway at HepG2 cell line. Journal of agricultural and food chemistry 2014; 62.
- Lammi C, Zanoni C, Aiello G, Arnoldi A, Grazioso G. Lupin Peptides Modulate the Protein-Protein Interaction of PCSK9 with the Low Density Lipoprotein Receptor in HepG2 Cells. Scientific reports 2016; 6.
- 80. Fechner A, Kiehntopf M, Jahreis G. The formation of short-chain fatty acids is positively associated with the blood lipid-lowering effect of lupin kernel fiber in moderately hyper-cholesterolemic adults. The Journal of nutrition 2014; 144.
- 81. Bähr M, Fechner A, Kiehntopf M, Jahreis G. Consuming a mixed diet enriched with lupin protein beneficially affects plasma lipids in hypercholesterolemic subjects: a randomized controlled trial. Clinical nutrition (Edinburgh, Scotland) 2015: 34.
- 82. Pavanello C, Lammi C, Ruscica M, et al. Effects of a lupin protein concentrate on lipids, blood pressure and insulin resistance in moderately dyslipidaemic patients: A randomised controlled trial | Elsevier Enhanced Reader. 2017; 37:8-15.
- 83. Ben Salem M, Affes H, Ksouda K, et al. Pharmacological Studies of Artichoke Leaf Extract and Their Health Benefits. Plant foods for human nutrition (Dordrecht, Netherlands) 2015; 70.
- 84. Arnaboldi L, Corsini A, Bellosta S. Artichoke and bergamot extracts: a new opportunity for the management of dyslipidemia and related risk factors. Minerva Med 2022; 113:141-57.
- 85. Santos HO, Bueno AA, Mota JF. The effect of artichoke on lipid profile: A review of possible mechanisms of action. Pharmacol Res 2018; 137:170-8.
- Sahebkar A, Serban MC, Gluba-Brzózka A, et al. Lipid-modifying effects of nutraceuticals: An evidence-based approach. Nutrition 2016; 32:1179-92.
- Illiano P, Brambilla R, Parolini C. The mutual interplay of gut microbiota, diet and human disease. The FEBS journal 2020; 287
- 88. Gomaa E. Human gut microbiota/microbiome in health and diseases: a review. Antonie van Leeuwenhoek 2020; 113.
- 89. Hill C, Guarner F, Reid G, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology 2014; 11:506-14.
- Vourakis M, Mayer G, Rousseau G. The Role of Gut Microbiota on Cholesterol Metabolism in Atherosclerosis. Int J Mol Sci 2021; 22.

- Li HY, Zhou DD, Gan RY, et al. Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics on Metabolic Diseases Targeting Gut Microbiota: A Narrative Review. Nutrients 2021; 13.
- 92. Mo R, Zhang X, Yang Y. Effect of probiotics on lipid profiles in hypercholesterolaemic adults: A meta-analysis of randomized controlled trials. Med Clin (Barc) 2019; 152:473-81.
- 93. Wang C, Li S, Xue P, et al. The effect of probiotic supplementation on lipid profiles in adults with overweight or obesity: A meta-analysis of randomized controlled trials, 2021.
- 94. Di Donna L, De Luca G, Mazzotti F, et al. Statin-like principles of bergamot fruit (Citrus bergamia): isolation of 3-hydroxymethylglutaryl flavonoid glycosides. J Nat Prod 2009; 72:1352-4.
- 95. Rondanelli M, Peroni G, Riva A, et al. Bergamot phytosome improved visceral fat and plasma lipid profiles in overweight and obese class I subject with mild hypercholesterolemia: A randomized placebo controlled trial. Phytother Res 2021; 35:2045-56
- **96.** Nauman MC, Johnson JJ. Clinical application of bergamot (Integr Food Nutr Metab 2019; 6.
- 97. Perna S, Spadaccini D, Botteri L, et al. Efficacy of bergamot: From anti-inflammatory and anti-oxidative mechanisms to clinical applications as preventive agent for cardiovascular morbidity, skin diseases, and mood alterations. Food Sci Nutr 2019; 7:369-84.
- 98. Gliozzi M, Walker R, Muscoli S, et al. Bergamot polyphenolic fraction enhances rosuvastatin-induced effect on LDL-cholesterol, LOX-1 expression and protein kinase B phosphorylation in patients with hyperlipidemia. Int J Cardiol 2013; 170:140-5.
- 99. Uuh-Narvaez JJ S-CM. Cabbage (Brassica oleracea var. capitata): A food with functional properties aimed to type 2 diabetes prevention and management. Journal of food science 2021; 86.
- 100. Rungapamestry V DA, Fuller Z, Ratcliffe B. Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (Brassica oleracea Var. capitata) cooked for different durations. Journal of agricultural and food chemistry 2006; 54.
- Galvez J, Rodríguez-Cabezas M, Zarzuelo A. Effects of dietary fiber on inflammatory bowel disease. Molecular nutrition & food research 2005; 49.
- Nawaz H, Shad MA, Muzaffar S, et al. Phytochemical Composition and Antioxidant Potential of Brassica. 2018.
- 103. Wang P, Fang J, Gao Z, Zhang C, Xie S. Higher intake of fruits, vegetables or their fiber reduces the risk of type 2 diabetes: A meta-analysis. Journal of diabetes investigation 2016; 7.
- 104. Kurotani K, Nanri A, Goto A, et al. Vegetable and fruit intake and risk of type 2 diabetes: Japan Public Health Center-based Prospective Study. Br J Nutr 2013; 109:709-17.
- 105. Suido H, Tanaka T, Tabei T, et al. A mixed green vegetable and fruit beverage decreased the serum level of low-density lipoprotein cholesterol in hypercholesterolemic patients. J Agric Food Chem 2002; 50:3346-50.
- 106. Guo X-f, Li Z-h, Cai H, Li D. The effects of Lycium barbarum L. (L. barbarum) on cardiometabolic risk factors: a meta-analysis of randomized controlled trials. 2017.
- Luo Q CY, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life sciences 2004; 76.
- 108. Zhao R LQ, Li J, Zhang T. Protective effect of Lycium barbarum polysaccharide 4 on kidneys in streptozotocin-induced diabetic rats. Canadian journal of physiology and pharmacology 2009; 87.

- 109. Jing L, Cui G, Feng Q, Xiao Y. Evaluation of hypoglycemic activity of the polysaccharides extracted from Lycium barbarum. African journal of traditional, complementary, and alternative medicines: AJTCAM 2009; 6.
- 110. De Souza Zanchet M, Nardi G, de Oliveira Souza Bratti L, Filippin-Monteiro F, Locatelli C. Lycium barbarum Reduces Abdominal Fat and Improves Lipid Profile and Antioxidant Status in Patients with Metabolic Syndrome. Oxidative medicine and cellular longevity 2017; 2017.
- 111. Romani A, Ieri F, Urciuoli S, et al. Health Effects of Phenolic Compounds Found in Extra-Virgin Olive Oil, By-Products, and Leaf of. Nutrients 2019; 11.
- 112. Razmpoosh E, Abdollahi S, Mousavirad M, Clark CCT, Soltani S. The effects of olive leaf extract on cardiovascular risk factors in the general adult population: a systematic review and meta-analysis of randomized controlled trials. Diabetol Metab Syndr 2022; 14:151.
- 113. Lockyer S, Rowland I, Spencer JPE, Yaqoob P, Stonehouse W. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. Eur J Nutr 2017; 56:1421-32.
- 114. Stevens Y, Winkens B, Jonkers D, Masclee A. The effect of olive leaf extract on cardiovascular health markers: a randomized placebo-controlled clinical trial. European Journal of Nutrition 2020; 60:2111-20.
- 115. Olmez E, Vural K, Gok S, et al. Olive Leaf Extract Improves the

- Atherogenic Lipid Profile in Rats Fed a High Cholesterol Diet. Phytother Res 2015; 29:1652-7.
- 116. Cicolari S, Dacrema M, Tsetegho S, AJ, et al. Hydromethanolic Extracts from Adansonia digitata L. Edible Parts Positively Modulate Pathophysiological Mechanisms Related to the Metabolic Syndrome. Molecules (Basel, Switzerland) 2020; 25.
- 117. Ebaid H, Bashandy SAE, Alhazza IM, Hassan I, Al-Tamimi J. Efficacy of a Methanolic Extract of Adansonia digitata Leaf in Alleviating Hyperglycemia, Hyperlipidemia, and Oxidative Stress of Diabetic Rats. Biomed Res Int 2019; 2019:2835152.
- 118. Atchan Nwakiban AP, Sokeng AJ, Dell'Agli M, et al. Hydroethanolic plant extracts from Cameroon positively modulate enzymes relevant to carbohydrate/lipid digestion and cardio-metabolic diseases. Food Funct 2019; 10:6533-42.
- 119. Nwakiban APA, Cicolari S, Piazza S, et al. Oxidative Stress Modulation by Cameroonian Spice Extracts in HepG2 Cells: Involvement of Nrf2 and Improvement of Glucose Uptake. Metabolites 2020; 10.
- 120. Atchan Nwakiban AP, Passarelli A, Da Dalt L, et al. Cameroonian Spice Extracts Modulate Molecular Mechanisms Relevant to Cardiometabolic Diseases in SW 872 Human Liposarcoma Cells. Nutrients 2021; 13.
- 121. Nwakiban Atchan AP, Shivashankara ST, Piazza S, et al. Polyphenol-Rich Extracts of Xylopia and Aframomum Species Show Metabolic Benefits by Lowering Hepatic Lipid Accumulation in Diet-Induced Obese Mice. ACS Omega 2022; 7:11914-28.



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The XVI National Congress of the Società Italiana di Terapia Clinica e Sperimentale (SITeCS)

№ Manuela Casula^{1,2}, Alberto Aronica³, Maurizio Averna⁴, Stefano Carugo⁵, Andrea Poli⁶, Alberico L. Catapano^{1,2}

¹Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy e IRCSS Multimedica, Sesto San Giovanni (MI)

CONFERENCE REPORT



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The XVI National Congress of the Società Italiana di Terapia Clinica e Sperimentale (SITeCS) was held in Milan on September 22-24, 2022. As is now customary, the Congress was organised in collaboration with the Italian Society for the Study of Atherosclerosis (SISA) Lombardy Region. The Congress included the discussion of the most recent evidence or the most topical issues in clinical and pharmacological research as well as presentations of scientific work by young researchers.

A short report of the main issues debated during the 2022 Congress lectures is offered in this issue, for the benefit of the *European Atherosclerosis Journal* readers.

The first session focused on the role of genetics and environment in susceptibility in cardiovascular diseases (CVDs). It is well known that CVDs have a complex multifactorial aetiology. Professor Gianluigi Condorelli thoroughly discussed this issue, focusing specifically on the interaction between environmental factors and genes that contributes to the complexity of CVD. Among the environmental factors, Doctor Andrea Poli showed how the evaluation of diet-related factors has proven over time to be limited, often not so robust, and strongly confounded by environmental influences. It remains a huge chal-

lenge to define the authentic cardiovascular effects of diet, largely due to the difficulty of separating the effects of each food or food component from the overall effects of dietary habits taken as a whole. On the other hand, the role of genes, which can be considered relatively fixed, must be considered. Neither genetics nor environmental agents acting independently cause the disease. Full knowledge about an individual's genetic frame or exposures to environmental factors cannot predict with certainty the onset, progression, or severity of the disease. The genes, or rather the combination of genes determining genetic predisposition, and environmental factors causing a particular multifactorial trait may vary from person to person. The disease develops as a consequence of interactions between the baseline conditions, coded in the genotype, and exposures to environmental agents indexed by time and space that are integrated at levels above the genome. Professor Condorelli also emphasised how the technologies available for genetic investigation as well as the knowledge on the interaction between genetic and environmental factors have changed rapidly over time. This enormous bulk of data in turn requires innovative analysis techniques. Artificial intelligence methods will likely respond to this need.

Corresponding Author

Manuela Casula: manuela.casula@unimi.it

²Center for the Study of Dyslipidaemias IRCCS MultiMedica, Sesto S. Giovanni, Milan, Italy

³CoS (Consorzio Sanità) Study Center, Italy;

⁴Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G. D'Alessandro" (PROMISE), University of Palermo, Palermo, Italy

⁵Department of Clinical Sciences and Community Health, University of Milano, Italy

⁶Nutrition Foundation of Italy, Milan, Italy

In the session dedicated to the patient at high cardiovascular risk, three different case studies have been discussed. Professor Stefano Carugo described the challenges in managing patients with polyvascular disease, emphasising the importance of a comprehensive assessment of the patient. As polyvascular disease is indicative of systemic atherosclerosis, individuals with polyvascular disease are at heightened risk for cardiovascular events in all vascular districts. However, it should be considered that multiple vessel involvement is not an uncommon feature and that the use of simple measurements, such as the ankle-brachial index (ABI), may be very useful for estimating the overall cardiovascular risk. The second case, addressed by Doctor Gianluca Perseghin, concerns the diabetic patient. Since diabetes is not only a well-known cardiovascular risk factor, but is considered a CVD-equivalent, patients with diabetes are to be considered on a par with individuals in secondary prevention. Additional features that typically accompany diabetes, such as insulin resistance, vascular alterations, inflammation, and oxidative stress, together with lifestyle aspects. While the complexity of cardiovascular prevention in the diabetic patient is well recognised, it must also be acknowledged how the most recent classes of antidiabetic drugs on the market, first of all the sodium-glucose co-transporter 2 (SGLT-2) inhibitors, have revolutionised the therapeutic algorithms. Finally, Dr Paolo Fabbrini described the cardiovascular prevention approach in the chronic kidney disease (CKD) patient. He emphasized how patients with CKD have so far had few treatment options to slow down progression, and how the introduction of SGLT-2 inhibitors had a strongly positive effect in this patient population. The link between the kidney and heart is complex. Kidney disease is often secondary to diseases leading to increased cardiovascular risk. Although hypertension and diabetes explain the vast majority of renal failure incidence in the Western world, the sum of these two causes does not justify the cardiovascular risk observed in CKD patients. The development of renal disease aggravates an already complicated cardiovascular condition. From this point of view, mention must certainly be made of uremic toxins, molecules present in very small concentrations in healthy subjects but at much higher levels in the subject with impaired glomerular filtration. These toxins can also have adverse effects on the cardiovascular system. Overall, all three presentations emphasised the need for a joint effort among cardiologists, diabetologists, and nephrologists in intervening as early as possible to delay the progression of these pathological conditions and avoid organ impairment t which may further increase the risk of cardiovascular events. Despite the considerable progress in the field of therapies, much work still needs to be done in these high cardiovascular risk patients.

The Congress traditionally hosts a joint symposium of the Lombardy sections of AMD (Association of Diabetes Physicians), SID (Italian Society of Diabetology), and SISA. This year, the presentations have addressed the lipid-lowering therapy in diabetic patients and discussed the most recent evidence on the efficacy and positioning of the newest anti-diabetic drugs. In his lecture, Professor Alberto Corsini reiterated the role of diabetes as CVD-equivalent in defining CV risk. The management of cholesterol levels is of paramount importance in these patients. Lipid abnormalities beyond elevated low-density lipoprotein (LDL) cholesterol contribute to increase the risk of atherosclerotic CVD in type 2 diabetes (T2D). After almost 30 years of widespread clinical use in diabetes, statin treatment remains the cornerstone of drug therapy to prevent CVD. Ezetimibe appears to be particularly beneficial as add-on for high-risk statin-treated patients with diabetes. Similarly, currently available proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors reduce CVD risk in statin-treated diabetic patients. Bempedoic acid is an interesting new oral agent inhibiting the cholesterol biosynthetic pathway, at an earlier step than 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, namely ATP-citrate lyase. Although one might imagine that this would result in an effect equivalent in magnitude to statins, the clinical trial experience has shown that LDL reduction with bempedoic acid is about half of that observed with statin treatment. The efficacy and safety of this molecule were recently demonstrated in a randomized trial of patients at high CV risk, of whom about 30% also had diabetes. Finally, fibrates might reduce CVD risk in patients with diabetes with high triglyceride and low high-density lipoprotein cholesterol levels and may also slow the progression of diabetic retinopathy. The two subsequent presentations by Doctor Alberto Rocca and Laura Montefusco reported the most updated evidence about SGLT2 inhibitors and glucagon-like peptide 1 (GLP1) receptor agonists, respectively. SGLT2 inhibitors were originally developed as anti-diabetic agents, with clinical trials demonstrating improved CV outcomes in diabetic patients. Secondary analyses of CV outcome trials and results from kidney outcome trials consistently reported improved kidney-related outcomes independent of T2D status and across a range of kidney functions and albuminuria. SGLT2 inhibitors are generally safe and well tolerated, with clinical trials and real-world analyses demonstrating a decrease in the risk of acute kidney injury. GLP1 receptor agonists, long-acting analogues of incretin, have shown high glucose-lowering and weight-lowering efficacy when administered as once-weekly subcutaneous injection. Moreover, they have demonstrated robust and significant reductions in CV outcomes, such as hospitalizations for heart failure. This evidence has changed the landscape for the treatment of patients with T2D. Both diabetes and cardiology guidelines have responded to this paradigm shift by including strong recommendations to use SGLT2i and/or GLP-1 RA, with evidence-based benefits to reduce cardiovascular risk in high-risk individuals with T2D.

In the session dedicated to pathology registers, Doctor Manuela Casula described the virtuous example of LIPIGEN. The LIPIGEN (Lipid TransPort Disorder Italian Genetic Network) Network was created in 2009 by the Italian Atherosclerosis Society (Società Italiana per lo Studio dell'Aterosclerosi - SISA) through its Foundation (Fondazione SISA) to promote and facilitate the clinical and genetic diagnosis of familial dyslipidaemias. Until now, the network involves more than 50 Italian centres specialized in the management of patients affected by primary dyslipidemias throughout the national territory, including paediatric clinics and LDL apheresis centres. The LIPIGEN Network structure is based on a close interaction between clinical centres, general practitioners, and patient organizations. The main objectives are to create a structured nationwide network for the identification of patients with genetic dyslipidaemias, to facilitate molecular genetic testing, and to promote research in the field. This initiative also aims at raising awareness and culture of the medical community, patients, and regulatory authorities in our country in the area of genetic dyslipidaemias and encouraging the exchange of information and knowledge according to recommendations from scientific societies. The clinical activity of the centres is complemented by the work of specialized genetic laboratories. Doctor Aldo Maggioni described registries by the European Society of Cardiology (ESC) and the Italian Cardiology Society (SIC). The Europe Observational Research Programme (EORP) was launched in 2009 by ESC to assess how European cardiology centres were adhering to the ESC guidelines and highlight any gaps that could become the topics of training interventions. Another objective was to identify rare diseases, collecting data on their characteristics, management, and outcome, to gather evidence that could support the recommendations of the guidelines in a context where large clinical trials are lacking. The

different registries implemented in Italy by the National Association of Hospital Cardiologists (ANMCO) were then described. The main advantage of these experiences is the creation of a network of clinical cardiologists collecting information from the real world. This consents to improve the quality of care and clinical outcomes. An example is acute coronary syndromes: while the GISSI-1 study showed a 13% in-hospital mortality in 1987 registries show that it has now fallen to 4%.

Finally, several hot topics were discussed during the last day.

Professor Alberico Catapano and Doctor Marta Gazzotti reviewed the evidence on homozygous familial hypercholesterolemia (HoFH) and familial chylomicronemia syndrome (FCS). The different genetic causes and the presence of a heterozygous or homozygous condition influence the phenotypic presentation, but sometimes the less severe homozygous forms are at the interface with heterozygotes. Consequently, HoFH is difficult to be confirmed without genetic testing. T Within the LIPIGEN Network, the main aim of the Italian Study Group on Homozygous FH is to provide a complete molecular characterization of HoFH patients. These results are then merged in the global registry "HoFH International Clinical Collaborators (HICC)". Familial chylomicronemia syndrome (FCS) is a very rare autosomal recessive disorder of triglyceride-rich lipoproteins, characterized by severe hypertriglyceridemia, the presence of chylomicrons in fasting condition, fasting triglyceride levels higher than 885 mg/dL, and an increased risk to develop recurrent episodes of potentially lethal pancreatitis. The extension of the LIPIGEN register to FCS is expected to improve the detection of affected subjects in Italy, promoting the use of shared protocols and validating diagnostic suspicion with genetic testing. The analysis of the collected data will allow estimating the prevalence of rare forms of genetic dyslipidaemias and the identification of clusters and/or subpopulations at higher risk, as well as to evaluate response to treatments.

Another topic which is becoming of increasing interest is Lipoprotein (a) [Lp(a)]. Lp(a) consists of an LDL particle in which apolipoprotein B100 (apoB) is covalently bound to an apolipoprotein(a) unit. The first associations between Lp(a) and coronary heart disease were reported in the early 1970s. In the last decade, following large epidemiological, genome-wide association (GWAS), and Mendelian randomization studies, together with the development of more reliable immunoassays, Lp(a) has been recognized as an important CV risk factor. Given the frequency of high Lp(a) levels and the lack of effective Lp(a) lowering therapies, the potentially modifiable Lp(a) burden should be considered one of the most important risk factors to target in the coming decade. The rationale is not only to be found in genetic studies. Recently, it has been suggested that the failure to reduce Lp(a) in subjects treated with evolocumab, a PCSK9 inhibitor able of reducing LDL-cholesterol (LDL-C) levels by 60%, may explain persistent arterial wall inflammation. The same outcome trials on PCSK9 inhibitors showed a reduction in the incidence of CV events associated with the reduction of Lp(a), which was independent of the reduction in LDL-C levels. Several experimental therapies targeting Lp(a) are in development, including an antisense oligonucleotide (pelacarsen) and two small interfering ribonucleic acid (olpasiran and SLN-360).

Three lipid-lowering therapies were then discussed. Doctor Andrea Baragetti critically evaluated recent data about omega-3 fatty acids (FAs). Eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA) have recently undergone testing for their ability to reduce residual CV risk among statin-treated subjects. The outcome trials have yielded highly inconsistent results, perhaps attributable to variations in dosage and composition. Among high-risk patients in contemporary care, mixed n3-FA formulations showed no reduction in CV events. Notably, CV trials using icosapent ethyl (IPE), a highly purified ethyl ester of EPA, reduced CV events and progression of atherosclerosis compared with mixed EPA/DHA treatments. This inconsistency justifies the lack of consensus among experts regarding the use of omega-3 fatty acids in CVD. Professor Alberto Zambon reviewed the most recent evidence from an open-label extension trial on a PCSK9 inhibitor, specifically evolocumab. These long-term data (7 years of follow-up) confirmed and even reinforced the efficacy and safety of evolocumab. The comparison between subjects treated from the start of the trial and those who only started treatment at the beginning of the open-label phase (with comparable LDL-C levels) showed that long-term treatment with evolocumab is associated with a significant reduction in clinical outcomes, including cardiovascular mortality, compared with patients who delay starting PCSK9 inhibitor therapy. Professor Marcello Arca described the characteristics and the therapeutic role of bempedoic acid, a new cholesterol-lowering drug, recently approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA). Bempedoic acid is a pro-drug: it is converted to its active moiety by an enzyme which is present mostly in the liver and absent in skeletal muscles, leading to a tissue-specific effect and limiting the risk of myalgia and myopathy. Clinical trials have shown that bempedoic acid is generally well-tolerated alone, or in combination with statins, ezetimibe, or PCSK9 inhibitors. Based on the data collected so far, bempedoic acid represents an additional effective and safe option to reduce LDL-C levels; it is a cost-effective therapeutic choice, to be considered in combination therapy in patients not reaching the recommended LDL-C goal, or in statin-intolerant patients, alone or in combination with

Finally, Professor Maurizio Averna addressed the issue of the combination therapy in in the management of hypercholesterolemia. Prof. Averna leads the European Atherosclerosis Society Task Force in working on a statement about Practical guidance for combination lipid-modifying therapy in high- and very-high-risk patients. The starting point is the recognition of the role of LDL-C and triglyceride-rich lipoproteins as primary targets for therapeutic interventions. Although many patients achieve lipid goals with high-intensity statins, certain patients do not have an optimal response because of genetic issues, intolerance, nonadherence, or therapeutic inertia. Over the past decade, clinical trials have provided strong evidence demonstrating the effectiveness and safety of non-statin medications added to statin therapy in high-risk patients. Combining statins with ezetimibe, PCSK9 inhibitors, bempedoic acid, or inclisiran has additive effects on LDL-C reduction. Moreover, fixed-dose combination tablets lower LDL-C more effectively than two separate tablets. Current clinical guidelines are shifting towards the recommendation for combination therapy, rather than the use of statin monotherapy titrated to the highest tolerated dose to reach the goals. Combination therapies increase the efficacy and reduce the side effects associated with higher doses, increasing tolerability and leading to higher adherence. Higher efficacy and adherence will result in a higher number of patients achieving recommended goals.



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XVI SITeCS Congress 2022 - Selected Abstracts

Effect of the deletion of Prenylcysteine oxidase 1 (PCYOX1) on arterial thrombosis in an animal model

Patrizia Amadio¹, Cristina Banfi², Marta Zarà¹, Maura Brioschi², Stefania Ghilardi², Leonardo Sandrini¹, Silvia Stella Barbieri¹

¹Brain-Heart axis: cellular and molecular mechanisms, Centro Cardiologico Monzino IRCCS, Milan, Italy; ²Unit of Functional proteomics, metabolomics and network analysis, Centro Cardiologico Monzino IRCCS, Milan, Italy https://doi.org/10.56095/eaj.v1i3.19 Patrizia Amadio: pamadio@ccfm.it

Prenilcistein oxidase 1 (PCYOX1) enzyme, involved in the degradation of prenylated proteins, is expressed in different types of cells, among which vascular and blood cells. Previous studies demonstrated that the secretome of cells silenced for PCYOX1 reduced platelet adhesion on both fibrinogen and endothelial cells, suggesting its possible involvement in thrombotic mechanisms.

In this study we analyzed the role of PCYOX1 in arterial thrombosis by the use of an animal model. All the procedures have been carried on mice knock-out for PCYOX1 (Pcyox1KO) that were compared with wild-type (WT) mice. Arterial thrombosis was induced by Ferric chloride application on carotid artery, while pulmonary thromboembolism was induced by the injection of collagen-epinephrine. The phenotype and the functionality of platelets were analyzed by cytofluorimetry and functional tests. The expression of PCYOX1 on platelets was evaluated by mass spectrometry.

Thrombus formation induced by Ferric Chloride was reduced in Pcyox1KO mice, that were also protected from pulmonary thromboembolism. No differences were identified in blood cells count, vascular pro-coagulant activity and functional fibrinogen. Interestingly, Pcyox1KO mice displayed a marked reduction in the number of platelets-leukocytes aggregates, in the release of alpha granules, in the activation of receptor allb\beta3 and in platelets aggregation induced by ADP e TRAP (analyzed on whole blood or platelets rich plasma). Mass spectrometry showed that PCYOX1 was highly expressed in WT platelets. However, the deletion of PCYOX1 did not alter platelets phosphorylation pathways, and platelets adhesion and aggregation (analyzed on washed platelets), in respect of WT mice. Of note, when platelets aggregation was performed on washed platelets isolated from WT mice in the presence of plasma derived from Pcyox-1KO mice, we observed a strong impairment in comparison with the aggregation obtained on the same platelets resuspended in plasma derived from WT mice.

In conclusion, our results, showing an ipo-reactivity of platelets and a reduced arterial and pulmonary thrombosis in Pcyox1KO mice, suggest that this protein could represent a new potential target in antithrombotic therapy.

Impact of dietary choline on lipid metabolism and atherosclerosis development in apoEKO mice deficient or overexpressing apolipoprotein A-I

♠ Alice Colombo¹, Marco Busnelli¹, Stefano Manzini¹, Elsa Franchi¹, Mariel Garcia Rivera², Jennifer Kirwan², Giulia Chiesa¹

¹Università degli studi di Milano, Dipartimento di Scienze Farmacologiche e Biomolecolari, Milano, Italy ²Max Delbrück Center for Molecular Medicine, BIH Core Facility Metabolomics, Berlin, Germany https://doi.org/10.56095/eaj.v1i3.23 Alice Colombo: alicecolombo10@gmail.com

TMAO, a metabolite of dietary choline, is considered a pro-atherogenic molecule for its ability to interfere with the reverse cholesterol transport, in which apolipoprotein A-I and HDL play a key role. In the present work it was evaluated how TMAO impacts on the development of atherosclerosis in mice with different levels of apoA-I/HDL. Mice deficient in both murine apoA-I and apoE (DKO) and DKO mice overexpressing human apoA-I (DKO/hA-I), characterized by extremely low or high plasma HDL levels respectively, were fed for 16 weeks two standard rodent diets, differing only in their choline content (0.09% or 1.2%). At the end of the dietary treatment, atherosclerosis development was quantified at the aortic sinus, targeted plasma metabolomics was performed, and gene expression was evaluated in liver, duodenum, jejunum and ileum.

With both diets, DKO mice developed much larger plaques than DKO/hA-I mice. High-choline diet increased plasma TMAO levels in both genotypes. Interestingly, a worsening of plaque development by high choline diet occurred in DKO/hA-I mice only $(0.057\pm0.048~\rm mm^2~vs~0.0988\pm0.064~\rm mm^2$, p<0.01). Plasma metabolomics indicated that choline supplementation, only in the presence of HDL, significantly increased the concentration of some ceramide species in addition to several markers of impaired kidney function.

High-choline diet increased the hepatic gene expression of Fmol and Fmo2 in DKO/hA-I, whereas the expression of Scarb1 was lower in DKO/hA-I compared to DKO mice, regardless of the dietary treat-

ment. Intestinal expression of genes involved in inflammatory response and in lipid metabolism was comparable between genotypes and was not modified by choline supplementation.

In conclusion, high choline diet increased plasma TMAO concentration in both genotypes, but affected atherosclerosis development, plasma metabolome and hepatic gene expression only in high HDL mice. Intestinal gene expression was not affected neither by genotype nor by dietary choline content.

Role of histone deacetylase 3 (HDAC3) in adipose tissue metabolism and immunophenotype

Lara Coppi¹, Carolina Peri¹, Fabrizia Bonacina¹, Raffaella Longo¹, Dalma Cricrí¹, Silvia Pedretti¹, Rui Silva¹, Ilenia Severi², Antonio Giordano², Danilo Norata¹, Alberico Catapano¹, Nico Mitro¹, Emma De Fabiani¹, Maurizio Crestani¹

¹Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan, Milan, Italy ²Department of Experimental and Clinical Medicine, Marche Polytechnic University https://doi.org/10.56095/eaj.v1i3.22 Lara Coppi: lara.coppi@unimi.it

Introduction: Obesity is associated with comorbidities such as cardio-vascular disease and type 2 diabetes. HDAC3 regulates adipose tissue physiology (WAT), and its genetic inactivation causes metabolic reprogramming of white adipocytes toward browning. The aim of this work is to evaluate the effect of HDAC3 silencing at different stages of differentiation and investigate the influence of adipocyte metabolism on the immunophenotype of WAT.

Materials and Methods: Following HDAC3 silencing in mesenchymal stem cells and mature adipocytes, adipocyte function, RNA, DNA and protein levels, and proliferation at the end of differentiation were analyzed. Visceral WAT immunophenotype (vWAT) of *Hdac3* KO mice in WAT (Hdac3fatKO) and controls (FL) was performed by FACS.

Results: Silencing HDAC3 in precursors amplifies the expression of genes and proteins that regulate differentiation, oxidative metabolism, browning and mitochondrial activity. Following silencing, we found increased 1)phosphorylation of AKT (1.64 fold change, P<0.0001), indicative of increased insulin signaling, and 2)proliferation, characteristic of the early phase of differentiation. Mitochondrial content was unchanged, but increased mitochondrial activity was observed in terms of maximal respiration (1.42 fold change, P=0.0151) and uncoupling of the electron transport chain (+11.6%, P<0.0001). No difference was observed following HDAC3 silencing in mature adipocytes.

We hypothesized that the enhancement of oxidative metabolism may cause cellular damage or senescence and, consequently, the immunophenotype of vWAT might be affected by HDAC3 ablation. Analysis reveals an increase of macrophages (2.48 fold change, P=0.0311) in the vWAT of Hdac3fatKO mice polarizing toward the M2 population. Coculture of adipocytes with macrophages from bone marrow indicates that HDAC3 silencing in adipocytes stimulates macrophage activation

Conclusions: HDAC3 is a key factor in the WAT phenotype, and its inactivation triggers mechanisms that support browning. Early epi-

genetic events mediated by HDAC3 silencing are crucial in directing adipocyte precursors toward the oxidative phenotype. Finally, results obtained from *ex vivo* and *in vitro* studies suggest that specific factors produced by KO adipocytes may be involved in determining the observed immunophenotype.

Combining family history of coronary heart disease and individual genetic predisposition to predict the risk of major coronary events

Elena Olmastroni¹, Federica Galimberti², Alberico L. Catapano^{1,2}, Brian A. Ference³

¹Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy ²IRCCS MultiMedica, Sesto S. Giovanni (MI), Italy ³Centre for Naturally Randomized Trials, University of Cambridge, Cambridge, UK

https://doi.org/10.56095/eaj.v1i3.17 Elena Olmastroni: elena.olmastroni@unimi.it

Background: Inherited predisposition to atherosclerosis leads to higher risk for developing coronary heart disease (CHD). There are mainly two ways to conceptualize inherited risk of CHD: family history and polygenic predisposition. We aimed at assessing the impact of family history of CHD and genetic predisposition in predicting the individual lifetime risk of major coronary events (MCE).

Methods: Using adjusted Cox proportional hazard models, we estimated the lifetime risk of MCE associated with parental family history of CHD and individual genetic predisposition (estimated by a polygenic risk score including 350 variants).

Results: A total of 445,744 UK-Biobank participants were included in the study (mean age 57 years; 54.3% females). Having one parent with a history of CHD increased the lifetime risk of MCE by 75%(HR 1.75, 95%CI 1.70-1.82). Having both parents with a history of CHD further increased the risk (HR 2.78, 95%CI 2.64-2.92) Similarly, a dose-dependent step-wise increase in MCE risk was observed moving from the lowest to the highest decile of the polygenic score. Compared to subjects without family history of CHD and with average level of the polygenic score, having a parental history of CHD determined an increase in lifetime risk of MCE (HR 1.90, 95%CI 1.82-1.98) comparable to belonging to the highest decile of the polygenic score (HR 1.89, 95%CI 1.76-2.02). However, if subjects present both parents with family history of CHD and a very high polygenic predisposition, the risk was even higher (HR 3.54, 95%CI 3.34-3.75), suggesting an additive contribution to the characterization of the lifetime risk.

Conclusions: We described the addictive impact of family history of CHD and individual polygenic predisposition in predicting lifetime risk of MCE. In order to identify subjects at higher risk of having an early event, it is essential to retrieve information about both these hereditary components.



European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly journal covering all topics within atherosclerosis and cardiovascular disease areas.

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