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Review Article

Mediterranean Diet and Its Protective Mechanisms against Cardiovascular Disease: An Insight into Platelet Activating Factor (PAF) and Diet Interplay

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Abstract

Several prospective studies and clinical trials have shown a protective effect of the Mediterranean Diet (MD) against cardiovascular disease, even after assessing for hard clinical end-points. However, the exact mechanisms through which MD exerts its actions have not been fully elucidated. The aim of the present review is to clarify the potential bio-protective mechanisms of MD against cardiovascular disease, i.e. the role of macro- and micro- nutrients, antioxidants, polyphenols, the effects of MD on postprandial metabolism and endothelial function and nutrient- gene interactions. A special emphasis is given on Platelet Activating Factor (PAF), which plays a key role in atherosclerosis. Its inhibitors are abundant in Mediterranean foods and have an anti-inflammatory and anti-thrombotic action.

Keywords: Mediterranean diet; Cardiovascular disease; Platelet activating factor; Postprandial state

Abbreviations

MD: Mediterranean Diet; PAF: Platelet Activating Factor; TNF-a: Tumor Necrosis Factor-alpha; Ox-LDL: Oxidized LDL; NF-kB: Nuclear Factor kappaB; Ox-PAPC: 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine; LTA: Lipoteichoic Acid, LPS: Lipopolysaccharides; TLR: Toll Like Receptor; Lyso-PAF-AT: acetyl-CoA:lyso-PAF acetyltransferase; DHA: Docosahexaenoic Acid; PAF-CPT: DTT-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase; PAF-AH: PAF-acetylhydrolase; Lp-PLA$_2$: Lipoprotein-associated phospholipase-A$_2$; PAMPS: Pathogen-Associated Molecular Patterns

Introduction

The Mediterranean basin is a cross roads between East and West mixing elements of the two cultures. Apart from its geographical position, it is distinguished by its unique climate and the warmth of residents. The available food, historical events, wars, customs, religious traditions, along with novel foods formed a particular diet of the inhabitants of the Mediterranean, the Mediterranean Diet (MD). The first systematic attempt to investigate the diet of Mediterranean countries was done after the Second World War by the Rockefeller Foundation [1]. The findings of the study were published in the institution's monograph in 1953 [2]. This document described the Cretan diet as a diet containing olives, olive oil, cereals, bread, legumes, wild greens, herbs and fruits along with small quantities of

goat meat, milk and fish [2]. The Cretans themselves, however, did not seem happy with their diet as only one in six said that it considers the standard diet as satisfactory [2] while members of one family felt "hungry most of the time of day" [2] probably due to the hard manual work.

Then, the Seven Countries study (Netherlands, USA, Japan, Finland, Italy, Former Yugoslavia, Greece), launched in 1960 and coordinated by Ancel Keys, Professor of the University of Minnesota (USA), demonstrated the protective effects of the traditional Cretan diet on mortality and cardiovascular disease [3,4]. The scientific community began to believe that the Cretans rather do or eat something right [5]. Ancel Keys also supported the motto "eat well and stay well" [6].

In general, the term MD describes the traditional Cretan diet which has the following characteristics [7]:

- High content in total fat (30-40% of energy)
- Low content in saturated fat (<9% of energy)
- High ratio of monounsaturated to saturated fatty acids.
- High content of fiber, micronutrients and natural antioxidants/phytochemicals.
- Moderate content of animal protein.
- Moderate content of alcohol, and especially wine.

However, it should be noted that there are marked differences in the diets of Mediterranean countries. Wine consumption is a central component of the diet of French and constitutes a possible interpretation of the "French paradox" [8] and the Spanish diet is rich in fish. Another notable difference is observed between Greece and Italy. In Greece whole grains are consumed, especially in Crete, while in Italy the most important source of carbohydrates is pasta [7]. In some cases, differences in food habits are observed within the same country. For example, in southern Italy, the consumption of cereals, fruits and vegetables is higher than in the north, where

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higher consumption of dairy products is observed [7]. Despite the differences in the various countries of the Mediterranean, the basic aspects of nutrition with the temperament of the people seem to make a "right recipe" confirming the words Umberto Echo that unity can found through diversity. Evaluating the benefits of MD in health as well as the importance of social development of the Mediterranean countries at the 5th Session of the Intergovernmental Committee for Intangible Cultural Heritage of UNESCO in November 2010, the UNESCO anointed the Mediterranean Diet as a cultural monument of Greece, Italy, Spain and Morocco (decision 5.COM 6.41) [9].

MD and Cardiovascular disease

Several prospective studies and clinical trials have shown a protective effect of MD against cardiovascular events after assessing for hard clinical end-points. According to results of a meta-analysis an increased attachment to the MD by 2 units led to a reduction in cardiovascular mortality and the incidence of cardiovascular disease by 10% (RR = 0.90; 95% CI: 0.87- 0.93) [10]. Most recent prospective studies also point to a cardio-protective effect of this dietary pattern in Mediterranean and non-Mediterranean populations as well [11-19]. Moreover, the results of clinical trials for primary and secondary prevention of cardiovascular disease enable the confirmation of potential causal relationships. Recently, the PREDIMED Study (The Prevention con Diet Mediterranean) in 7447 high risk individuals from Spain revealed a protective effect of Mediterranean diet supplemented with extra virgin olive oil or nuts compared to a control diet (primary prevention) [20]. More particularly the Mediterranean diet groups had a ~30% reduction in the primary end point (composite end point of stroke, myocardial infarction, and cardiovascular deaths) [20]. A key study revealing the importance of adopting a Mediterranean-type diet in secondary prevention was the Lyon Heart Study [21-23], which involved 605 patients followed for 3.5 years. In this study patients with myocardial infarction were medically treated and were advised to follow the MD or the diet recommended by the American Heart Association. The group following the MD experienced had 70% fewer heart disease deaths. The studies of Singh et al [24,25] and the THIS study (The Heart Institute of Spokane Diet Intervention and Evaluation Trial) followed [26] with positive effects.

Atherosclerosis and cardio protective mechanisms of the MD

Despite the strong evidence supporting the beneficial role of the MD in cardiovascular disease the exact mechanisms through which it exerts its actions have not been fully elucidated and some biochemical aspects of the diet-disease interplay have been neglected. In the present review several protective properties of the MD will be presented pertaining to its macronutrient, micronutrient, polyphenol and antioxidant content, its beneficial effects on postprandiallipemia and glycemia and endothelial function, the presence of Platelet-Activating Factor (PAF) inhibitors with antithrombotic and anti-inflammatory activities and several gene-diet interactions.

Before analyzing the bioactive components of the MD one-by-one it is appropriate to briefly recall some key points of atherosclerosis and to enlighten the pathophysiological role of PAF and its analogs in this process. In the mid-1970s, the "lipid hypothesis" existed for atherosclerosis development was replaced by the "response to injury hypothesis" developed by Russel Ross, which supported that atherosclerotic lesions develop as a result of local injury to the arterial endothelium followed by platelet adhesion and accumulation [27]. Later, it was found that endothelium activation was sufficient for the activation of immune inflammatory responses related to atherogenic process. Moreover, cholesterol administration in rabbits was found to trigger monocyte adhesion onto the endothelium, followed by their migration through the morphologically intact endothelium in the sub-endothelial space [28]. This observation led Michael Gimbrone to propose that during atherosclerosis a modification of the normal endothelium takes place, resulting to a dysfunctional endothelium that loses its barrier function [29]. Today it is accepted that atherosclerosis is a chronic inflammatory disease, in the onset of which adhesion of monocytes/ lymphocytes to activated endothelium takes place [30].

A number of lipid bioactive mediators have been identified as primary initiators of atherogenesis. Among these, PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is the strongest lipid inflammatory mediator [31], while many structure analogs known as oxidized phospholipids, such as the oxidized form of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (Ox-PAPC), mimics its activity [32]. It is evidenced that PAF mediates the production of tumor necrosis factor-alpha (TNF-a) through monocyte activation with the same mechanism as oxidized LDLs (Ox-LDLs) [33] (Figure 1). The detection of PAF molecules on Ox-LDL particles [34] may suggest that Ox-LDL's lead to TNF-a production in part through PAF induced monocyte activation [35]. One of the most important atherogenetic actions of PAF is that it mediates the adhesion of monocytes to endothelium in synergy with P-select in [36]. Moreover, the adhesion of monocytes onto endothelium allows PAF to signal the transport of nuclear factor kappaB (NF-kB) in the nucleus of those monocytes, leading to the transcription of various genes and the biosynthesis of their related protein products such as

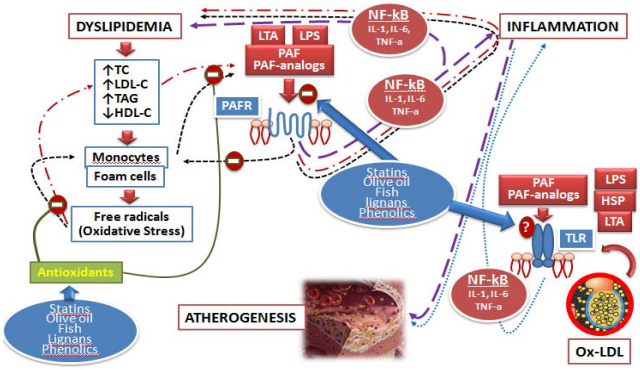

Figure 1: Simplified schematic representation for the role of PAF in the crosstalk of dyslipidemia, inflammation and atherogenesis. Chronic inflammation that may cause atherosclerosis may be the result of prolonged states of dyslipidemia, oxidative stress and acute inflammation mediated by various proinflammatory factors. Atherosclerosis prevention may be accomplished through down regulation of oxidative stress by antioxidants, regulation of physiological blood lipid profile, inhibition of inflammatory signal transduction systems at the level either of proinflammatory mediator biosynthesis or of their receptor signaling. These protective effects may be may exerted either by medication or by appropriate diet using appropriate combination of foods.
TC: Total Cholesterol; LDL-C: LDL-Cholesterol; HDL-C: HDL-Cholesterol; PAF-R: PAF-Receptor; TAG: Triacylglycerols; LPS: Lipopolysaccharides; LTA: Lipoteichoic Acid; HSP: Heat Shock Proteins

MCP-1, IL-8, TNF-a, associated with chronic inflammation [37]. Modified lipoproteins are able to cause oxidative stress resulting in the production of both free radicals and PAF or its analogs (Figure 1) [34,38]. Once produced, PAF and its analogs bind to PAF-receptors or Toll like receptors (TLRs) (see below) signaling in this way the inflammatory mechanisms in an uncontrolled and prolonged way that play a central role in triggering atherosclerosis [38-40]. Moreover, PAF orchestrates and reinforces these initial reactions since it causes the production of new free radicals and the biosynthesis and production of new PAF or its analogs [40,41]. In vivo studies in our laboratory on high-cholesterol fed rabbits show increased blood levels of PAF, as reflected by PAF attached on lipoprotein particles or blood albumin (free PAF) and PAF on blood cells (bound PAF) [42,43]. Similar studies on the activity of PAF metabolic enzymes in blood show an increase of circulating PAF in rabbits with diet-induced hyperlipidemia and point to leucocytes as the cells that provide the main newly biosynthesized PAF in blood in this case [44].

Interestingly, PAF receptor appears to have a particular role in controlling inflammation since it may mediate signaling in the pathogenesis of inflammatory diseases through other molecules outside of PAF and its analogs. Such molecules constitute Lipoteichoic Acid (LTA), and various lipopolysaccharides (LPS) as components of contaminant microorganisms. These molecules when recognized by PAF receptor cause the initiation of the inflammatory process, which if uncontrolled may result to chronic inflammation and initiation of atherosclerosis (Figure 1).

Apart from PAF-receptors, PAF and its analogs also bind to Toll like receptors (TLRs), who's signaling is associated with signaling of new PAF production and atherogenesis promotion [45]. TLRs are type I transmembrane glycoproteins with extracellular transmembrane and intracellular part and belong to the pattern recognition receptors of the innate immune system. They are expressed in cells of the immune system and other tissues, such as those of the cardiovascular system. The activation of TLRs activates the NF-kB pathway leading to production of cytokines that in turn evoke the expression and production of adhesion molecules, chemokines, interferons and NO (I and II) that all may have beneficial effect in acute inflammation but harmful effects in the uncontrolled mode of chronic inflammation [46] (Figure 1). In particular, Toll like receptors TLR-2and TLR-4 play an important role in atherosclerosis as activators of the innate immune system and a major part of the mechanism underlying the state of chronic inflammation. Administration of exogenous binding molecules or pathogens themselves, accelerate the atherosclerotic process, and exogenous binding molecules such as PAF analogues lead to increased atherosclerosis. Binding of the above molecules onto TLR-2 and TLR-4 causes their activation followed by recruitment of several proteins known as the adaptor proteins. After that, MAP kinases (JNK, ERK, p38a) and pro-inflammatory transcription factors (NF-kB, AP1, Elk1) are activated and followed by the expression of various inflammatory molecules such as IL-6, IL-1b and TNF-a [46]. It is also noted that the PAF analog Ox-PAPC inhibits the binding of LPS to their binding proteins (LPS-binding protein), a step that is required in order for LPS to be exposed for recognition from TLR-4. This suggests a dual role for the activity of PAF and its analogs since it seems that in acute inflammation conditions through bacterial agents act as anti-inflammatory agents inhibiting the pathway of NF-kB [47] (Figure 1), while in chronic inflammation conditions their activity appears to be responsible for development of the pathological condition, this time through NF-kB pathway enhancement [32].

Macronutrients and micronutrients

The MD is high in monounsaturated fatty acids, which have beneficial effects on HDL- and LDL- cholesterol and improve endothelial function, increase insulin sensitivity, reduce platelet aggregation and increase fibrinolysis [48]. The main source of monounsaturated fatty acids in the MD is olive oil [7]. Despite research data indicating that oleic acid may exert a protective effect against cardiovascular diseases [49], it is worth mentioning that oleic acid is a key component not only of the MD but also of a Western-style diet, as meat contains a considerable amount of it [50]. This observation supports the view that possibly other olive oil components contribute to its protective effects (see below). Furthermore, the MD is low in cholesterol, saturated and Tran's fatty acids and high in omega-3 fatty acids due to the presence of fish, vegetables, fruit and snails [51].

Indeed, omega-3 fatty acids have beneficial effects in reducing triacylglycerols, heart rate, blood pressure, platelet aggregation while they improve inflammation and atherosclerosis [52]. Moreover, they may prevent sudden death of cardiovascular cause and have an antiarrhythmic effect [53]. The actions of fish oils are extended to the reduction of heart rate [54,55], and the improvement of ventricular function in humans [54,56], possibly by increasing the elastic properties of myocardial cells [57] and increasing the release of NO [58]. Omega-3 fatty acids exert their antithrombotic effect possibly by reducing thromboxane synthesis [59], the expression of platelet derived growth factor –A and –B [60] and PAF production [61]. More specifically linoleic (18:2) inhibits the binding of monocytes to endothelium by inhibiting PAF biosynthesis [62] and docosahexaenoic acid (DHA, 22: 6) inhibits the *in vivo* actions of PAF [63,64]. Preliminary results of our group show that omega-3 inhibit the *in vitro* PAF induced platelet aggregation. It is likely that the protective effect of omega-3 in atherogenesis, depend on the relative ratio of omega-3/ omega-6, since - as demonstrated by Jian-Bo Wan et al.- omega-3 inhibit atherogenesis in modified mice at a ratio of omega-6/omega-3 = 1 [65]. It is noted that the ratio of omega-6 / omega-3 fatty acids was 1 to 2/1 in the traditional Cretan diet while in Europe and the United States, the ratio is about 10 to 20/1 [51]. Moreover, the intake of omega-3 fatty acids should be viewed within the context of the MD, since their supplementary intake in secondary prevention have not shown any beneficial effects according to recent studies: Diet and Omega-3 Intervention Trial [66], ORIGIN [67] and The Risk and Prevention Study [68].

It is worth mentioning that a small number of studies on the interaction of food ingredients with TLRs have shown that saturated fatty acids stimulate the inflammatory process by activating NF-kB through TLR-4. On the other hand monounsaturated fatty acids and fish origin omega-3 fatty acids inhibit the binding of saturated fatty acids onto TLRs, inhibiting this way the activation of TLR and finally the activation of NF-kB [69]. Food that may seem with naked eye unspoiled are believed to contain LPS that, as a heat-stable molecule under cooking conditions, results to cooked food containing active molecules that may stimulate TLRs. In a recent study these active molecules were detected in meat and processed food products, typical

in western diet, while they were in minute quantities or undetectable in fruits and vegetables, typical in Mediterranean type diets [70]. Although PAF inhibitors of food origin have not been studied for their effect on TLR's activation, the fact that Ox-LDL (containing PAF), PAF and PAF analogs bind to TLR-4, strongly indicates that food origin PAF inhibitors, such as those found in Mediterranean type diet foodstuffs, may inhibit TLR's activation. In a recent study, statins, medicines that exert pleiotropic effects and are well known for their hypolipidemic effect, have been shown to inhibit TLR's stimulation, exerting an anti-inflammatory activity [71]. Interestingly we have previously shown that statins are also PAF inhibitors [72].

The MD also includes foods rich in fiber, such as whole grains, legumes, fruits and vegetables. The high dietary fiber content of the MD goes hand in hand with its low glycemic load and glycemic index [73]. Furthermore, foods rich in fiber can also act as prebiotics inducing the production of short chain fatty acids by colonic bacteria, which in turn inhibit cholesterol synthesis, glucose digestion and thus influence systemic lipid and carbohydrate metabolism [74].

The traditional Mediterranean diet meets the requirements for micronutrients as it was demonstrated though menu analysis [75]. Several foods i.e. wine, fruit, vegetables, herbs and olive oil, which are key features of the MD, contain a plethora of micronutrients such as vitamin C, E, folate and carotenoids and phytochemicals [51]. It is also worth mentioning the low sodium and high potassium content of the MD which is a key element for proper endothelial function and blood pressure regulation [76].

Phytochemicals and antioxidants

Polyphenols exert pleiotropic cardio protective actions as it has been shown by in vivo and in vitro studies: they reduce the oxidation of LDL, increase the release of NO, reduce lipid concentration, have an anti-inflammatory activity, protect against atherothrombotic events, and reduce platelet aggregation [77] and PAF production [78-80]. MD foods such as wine, fruit, vegetables, herbs and olive oil are rich in polyphenols and other phytochemicals [51]. Olive oil, in particular, contains terpenes, phytosterols, phenolics, beta carotene [48] and polar lipids, which act as PAF antagonists [43,81]. Its phenolics have antioxidant and anti-inflammatory properties, inhibit lipoproteins oxidation and improve endothelial function [48]. Moreover, frying with olive oil form sonly few oxidized fatty acids, unlike other vegetable oils, as a result of monounsaturated fat and antioxidants [82]. Interestingly, the long term adoption of the MD results in beneficial effects in oxidative balance, as it was recently shown in the PREDIMED study [83,84] and previously shown in the ATTICA study [85] and other works [86].

Recently, our research team showed that circulating PAF was inversely correlated to the dietary antioxidant capacity and antioxidant-rich foods intake (herbal drinks and coffee) in healthy volunteers after adjustment for multiple covariates [87]. Moreover, the biosynthetic enzyme of PAF, Lyso-PAF-AT, was also negatively associated with the dietary antioxidant capacity and a healthy eating pattern (rich in fruits, nuts and herbal drinks, and a pattern rich in olive oil and whole-wheat products) [87] (Figure 2).

Postprandial metabolism

Deregulation of postprandial metabolism coupled with high glucose and lipids spikes is considered as a risk factor for diabetes, atherosclerosis and cardiovascular disease [88,89] since it triggers inflammation, oxidative stress, endothelial dysfunction and hypercoagulability [73]. Mediterranean diet may favorably affect postprandial metabolism as recently reviewed by our group [90]. From the few studies examining the long-term effects of MD on the postprandial state it was shown that postprandial glucose and insulin levels [91,92], postprandial TAG [93,94] and B-48 [93] were reduced.

The beneficial effects of the MD are explained if its macronutrient content is considered. Briefly, the high fat content of the MD delays gastric emptying, resulting in a lower glycemic response. Moreover, the type of fat included in the MD seems to play a beneficial role in postprandial lipemia. Monounsaturated fatty acids may attenuate postprandial lipemia [95], increase chylomicron clearance [94], increase HDL-cholesterol and GLP-1 [89,96] and decrease FVIIc [97]. Moreover, omega-3 fatty acids reduce postprandial triacylglycerols up to 16-40% via a reduction in VLDL secretion and an efficient chylomicrons clearance [73,98,99]. Dietary fiber is related to the decreased glycemic index and load of the Mediterranean diet [73]. Indeed, diets containing dietary fiber, vegetables, fruits, whole grains, olive oil, fish, legumes, fruit and vegetable drinks, such as the MD can improve the postprandial glycemic and lipid response [100,101]. For example, the consumption of nuts together with bread or mashed potatoes reduces postprandial glucose levels by 30-50% and also reduces postprandial oxidative stress [73]. Additionally, the mixture of olive oil and vinegar added abundantly in Mediterranean salads reduces postprandial glucose levels [100]. This is probably due to slowed gastric emptying due to acetic acid (vinegar) [100]. The cinnamon is used in many Mediterranean sweets and when added to a high glycemic index meal also reduces postprandial glucose levels by partly slowing gastric emptying [73]. The intake of protein of high biological value without parallel intake of saturated fat (e.g. egg white, fish, and whey proteins) improves postprandial hyperglycemia and inflammation [73].

Wine with food has been shown to reduce LDL oxidation and improve endothelial function compared to an isocaloric aqueous alcoholic solution, probably because of contained antioxidants [102]. Dark chocolate, tea and pomegranate also have beneficial effects on postprandial oxidative stress [73]. Finally, data from our research group as part of a European study showed that the incorporation of wild greens of Crete in a meal with bread and olive oil resulted in lower postprandial glucose levels in healthy subjects [103] and reduced platelet aggregation in individuals with the metabolic syndrome [104].

Endothelial function

Endothelial dysfunction resulting from reduced NO production and/or increased oxidative stress is implicated in the development of hypertension and other diseases of the cardiovascular system [105]. The MD and its components may have a protective effect in this process by increasing NO bioavailability and decreasing cytokine production and oxidative stress [106]. Indeed, MD improves endothelial function in patients with abdominal obesity [107] and hypercholesterolemia [108] and acts favorably on the regenerative activity of the endothelium [109], although not all studies have shown an effect [110]. As far as postprandial endothelial function is

concerned, MD as well as several of its components has been reported to exert beneficial effects [106]. Moreover, the combination of foods within the MD may also play a role in endothelium function. For example, the combination of olive oil and red wine has a beneficial effect on postprandial endothelial function [111]. It is noteworthy that NO perturbations are connected to PAF synthesis modulation, since NO inhibits PAF action as well as PAF synthesis [112], which may have further beneficial results regarding cardiovascular disease.

PAF inhibitors and PAF levels

An additional mechanism by which the MD exerts its beneficial effects is through PAF inhibitors, which reduce platelet aggregation and possibly inflammation [113-115]. A particular class of PAF inhibitors includes compounds found in food extracts. Our research group has examined many foods of the MD for the presence of PAF inhibitors. In particular, extracts of olive oil [116], wine [115,117], fish [118,119], honey [120], egg yolk [121], milk and yoghurt [122] have a PAF inhibitory activity. PAF inhibitors have been also found in extracts of Mediterranean meals [113,123]. In all these cases PAF inhibitors were isolated, tested and identified. Search for PAF inhibitors in various stages of olive oil production and olive pomace processing showed that the bioactive ingredients against PAF are mainly the byproducts of the procedure [114] and are removed during refining and treatment. Studies by other researchers have shown the existence of PAF inhibitors in garlic [124] and onion [125], which are also used in many recipes of the MD. It is also noted that vitamin E, which is one of the main micronutrients of the MD displays inhibitory activity against PAF-induced platelet aggregation [126] and has been found to inhibit atherogenesis *in vitro* [127]. Vitamin D, which is a PAF inhibitor, has been also found to inhibit the formation of atherosclerotic plaques in animals [128].

It is noted that PAF inhibitors have been found in many traditional plants of Mediterranean and non-Mediterranean origin, which are used as drugs and have also an atheroprotective effects. One of the best known PAF antagonists is Gingolide B or BN 52021, which derives from the Ginkgo biloba tree and it, inhibits the formation of atherosclerotic plaques [129]. Another traditional herbal medicine is flaxseed containing lignans, which are widespread in nature. A specific lignan, secoisolariciresinol diglucoside, is a PAF inhibitor and it also inhibits the development of atherosclerotic plaques in experimental animals [130]. Moreover, the black pepper extract inhibits atherogenesis in rabbits [131] and contains PAF inhibitors [132]. The results of our group and other investigators have shown that several phenolic compounds inhibit the activity of PAF *in vitro*, *in vivo* and its biosynthesis as well [87,133,134]. A PAF inhibitory activity has been reported foroleuropein, tyrosol and resveratrol, which indeed is modified and enhanced by acetylation [135,136].

It can be thus assumed that the MD contains foods with PAF inhibitors, which protect against atherosclerosis. This viewpoint has been also demonstrated from *in vivo* animal experiments. Specifically, rabbits fed an atherorogenic diet supplemented with PAF inhibitors (for example olive oil or its by-products) or statins do not develop atherosclerotic plaques [43,137]. This supports the theory of atherogenesis with PAF involvement, which recognizes that even with the presence of high levels of cholesterol inhibitors of PAF can inhibit atherogenesis [138]. In addition, we have shown that some fish oil polar lipids (other than omega-3 fatty acids) have anti-atherogenic actions through their ability to inhibit the biological actions of PAF *in vitro* [139] and *in vivo* by inhibiting the formation of atherosclerotic plaques [140].

From human studies it has been shown that the adoption of the

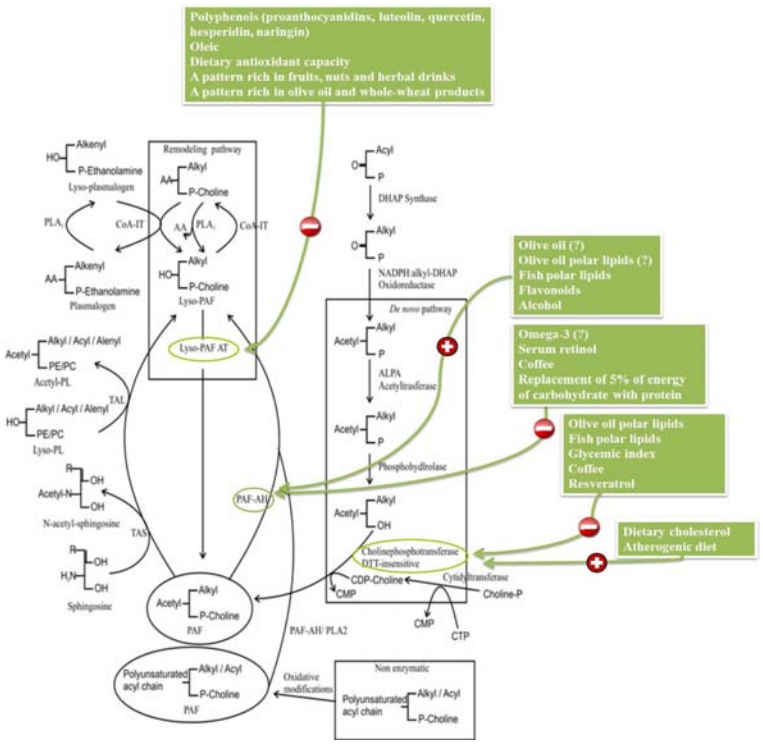

Figure 2: PAF metabolism and effect of dietary components on its key metabolic enzymes.

MD from patients with type 2diabetes for one month reduces PAF induced platelet aggregation [113,123]. A recent study even showed that wild greens of Crete, when consumed by patients with the metabolic syndrome have a postprandial PAF inhibitory effect [104].

Apart from the action of the MD in inhibiting the actions of PAF, it should be noted that several of its components may act on its metabolic enzymes, affecting the final levels of circulating PAF (Figure 2) [141]. Briefly, PAF is synthesized via the remodeling and the *de novo* pathway, key enzymes of which are acetyl-CoA: lyso-PAF acetyltransferase (lyso-PAF-AT) and DTT-insensitive CDP-choline: 1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (PAF-CPT), correspondingly. PAF-acetylhydrolase (PAF-AH) and its extracellular isoform lipoprotein-associated phospholipase-A_2 (Lp-PLA_2) catabolize and deactivate PAF. In this context, Proanthocyanidins [78], luteolin [80], quercetin [80,142], hesperidin [142] naringin and oleic acid [142] reduce the activity of lyso-PAF-AT. Moreover, lyso-PAF-AT, has been negatively associated with the dietary antioxidant capacity and a healthy diet (a pattern rich in fruits, nuts and herbal drinks, and a pattern rich in olive oil and whole-wheat products) [87]. From *in vitro* data of our research group in mesangial cells it has been shown that olive oil polar lipids, fish polar lipids as well as resveratrol are inhibitors of another PAF biosynthetic enzyme, PAF-CPT [143]. Recently, PAF-CPT was negatively associated with the glycemic index of the diet, coffee intake and positively associated with dietary cholesterol in healthy volunteers [87]. Furthermore, fish polar lipids were found to decrease PAF biosynthesis [44] and increase PAF catabolism [44,137], while an atherogenic diet was found to increase PAF-CPT in rabbits [44].

As far as the PAF catabolic enzyme is concerned PAF-AH or its extracellular isoform Lp-PLA_2, the bibliography is less clear regarding its relation to diet. Olive oil and/ or its polar lipids appear to increase the enzyme's activity in hypercholesterolemia rabbits [42,137] or to have no effect [43]. Flavonoids have also been shown to increase or not to affect the enzyme activity *in vitro* [142,144]. Two studies in humans have focused on the relationship of omega-3 fatty acids on Lp-PLA_2 and have shown reverse [145] or no association [146]. Additional data on the correlation between the activity of Lp-PLA_2 with dietary factors derive from the Bruneck study in which, the enzyme activity was inversely related to serum retinol levels while no significant relation with energy intake, fat intake, levels of carotene and tocopherol in plasma was observed [147]. Furthermore, it has been found that the replacement of 5% of energy of carbohydrate with protein was associated with decreased activity of Lp-PLA_2 [148]. Recently, PAF-AH was negatively associated to coffee and positively associated to alcohol consumption [87]. In summary, various components of the diet may reduce the actions of PAF and its production while the relation between diet and the PAF's catabolic enzyme Lp-PLA_2 is less clear.

Gene- diet interactions

Recent data suggest that the MD can affect the expression of genes associated with inflammation and oxidative stress, such as the gene for interferon-γ, interleukin receptor, the β2 adrenergic receptor, etc. [149]. The adoption of the MD in the elderly for 3 months led to lower postprandial expression of MCP-1and metalloproteinase MMP-9relative to a diet rich in saturated fat and a lower expression of TNF-αcompared to a diet rich in carbohydrates [150]. The adoption of a MD pattern enriched with olive oil also resulted in lower expression of proinflammatory molecules, cyclooxygenase-1 andMCP-1 [151]. Various components of olive oil can affect gene expression directly or indirectly (through reduction of oxidative stress). Such gene examples are those of vascular cell adhesion molecule-1, NF-kB, cyclooxygenase-2, and antioxidant enzymes (catalase and glutathione peroxidase) [152]. Several interactions of MD components and polymorphisms have been identified: tetrahydrofolic acid reductase [153], PPARγ [154], IL-6 [155], adiponectin etc.[156]. Last but not least, MD components may regulate gene expression via epigenetic mechanisms [157].

Conclusion

The MD is a pattern high in monounsaturated fat, low in saturated and trans fats, which provides a high amount of dietary fiber, vitamins, folic acid and natural antioxidants, moderate amounts of animal protein and a moderate amount of alcohol mainly in the form of wine. The Seven Countries firstly highlighted the beneficial effects of MD for cardiovascular disease prevention, while further studies have corroborated its results. Mediterranean diet interventions have shown a reduction of cardiovascular events in primary (PREDIMED study) and secondary prevention (Lyon Heart Study and other studies). The main mechanisms of action include its macronutrients and micronutrients profile, the content of antioxidants, its beneficial effects on postprandial lipemia and glycemia as well as endothelial function and gene-diet interactions. Finally, the proposed theory of atherosclerosis with PAF implication in conjunction with the presence of PAF inhibitors in the MD provides an additional explanation of its cardio-protective effects.

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