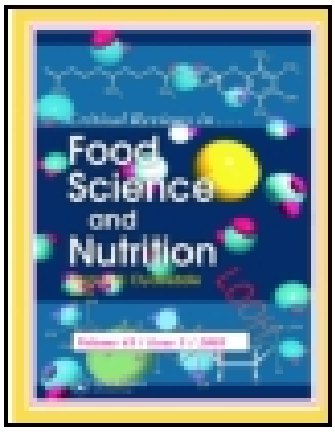

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

MEDITERRANEAN DIET AND CARDIOVASCULAR RISK: BEYOND TRADITIONAL RISK FACTORS

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Accepted author version posted online: 13 Aug 2014.

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SUMMARY:

A strict adherence to the Mediterranean Diet (MedDiet) has repeatedly been linked to a low risk of cardiovascular disease in several situations. Initially, the mechanisms considered as possible causes of this were based on the effects of this dietary pattern on the so-called traditional risk factors (especially lipids and blood pressure). However, the high relative reduction in the prevalence of cardiovascular morbidity and mortality were not proportional to the limited findings about regulation of those traditional risk factors. In addition to several studies confirming the above effects, current research on the MedDiet is being focused on defining its effects on non-traditional risk factors, such as endothelial function, inflammation, oxidative stress, or on controlling the conditions which predispose people to cardiovascular events, such as obesity, metabolic syndrome or type 2 diabetes mellitus. In the current article, after briefly reviewing the known effects of the MedDiet on the traditional risk factors, we will mainly focus on reviewing the current evidence about the effects that this dietary pattern exerts on alternative factors, including postprandial lipemia or coagulation, among others, as well as providing a short review on future directions.

Keywords: Mediterranean Diet. Cardiovascular prevention. Cardiovascular risk factors. Olive oil.

INTRODUCTION:

Cardiovascular diseases are the leading causes of mortality in developed countries. Furthermore, the age of onset of these conditions is earlier than in other chronic diseases, which means that, apart from total mortality, they are the primary cause of disability among young people in these countries, and cause a high impact in social costs and the use of health care services. The most effective strategies in public health to reduce the burden of cardiovascular disease are those which focus on lifestyle, especially those which can reduce cardiovascular risk factors. According to current knowledge, in theory, diet is the single lifestyle factor with the highest impact on cardiovascular risk factors. Furthermore, these interventions also influence the rates of incidence of obesity, as well as metabolic syndrome and type 2 diabetes mellitus, which are also linked to cardiovascular diseases.

Two main dietary patterns are associated with a lower rate of cardiovascular events: the low-fat diet and the MedDiet(Panagiotakos et al., 2008; Lopez-Miranda et al., 2010). This latter is, probably, the oldest dietary pattern in recent times, and, in addition to its effects on cardiovascular risk factors, has proved to be linked to other healthy effects, such as a decrease in age-associated cognitive age decline or the incidence of certain types of cancer(Lopez-Miranda et al., 2010).

The main foods or micronutrients involved in the above effects are still unknown. However, to date, fats are the single most important ingredients which have been shown to have a greater impact on health in general and cardiovascular diseases in particular, which leads us to infer that the distribution of the fat component in this diet (mainly monounsaturated fats from olive oil) has a significant importance. The abundance in this dietary pattern of additional foods associated

with healthy effects, such as vegetables and grains, or those rich in nutraceutical components, like virgin olive oil (rich in antioxidants) or fish (oily fish rich in n3 fatty acids), may be also implied.

Furthermore, the exact underlying mechanisms by which the MedDiet exerts its effects are not totally understood. Typically, it has been stated that it has favorable effects on lipids and blood pressure. As well as epidemiological studies observing the advantages in the classic risk factors, there is also other evidence from randomized clinical trials describing how the chronic consumption of the typical foods contained in the MedDiet (olive oil, vegetables, grains and fish) lowers triglycerides and LDL cholesterol, while maintaining or increasing HDL cholesterol and diminishing the atherogenic quotient total/HDL cholesterol and LDL concentration when compared to SFA rich diets(Fuentes et al., 2001; Jansen et al., 2000; Tripoli et al., 2005; Visioli and Galli, 1998; Bach-Faig et al., 2006; Lapointe et al., 2005; Pitsavos et al., 2005). In 2004, the FDA authorized a health claim for olive oil and coronary heart disease based on its effects on the lipid profile(CFSAN/Office of Nutritional Products, 2004). Further studies replicated these findings, and, in 2011 the European Food Safety Authority also authorized a health claim for the phenol compounds of olive oil in reducing the oxidation of LDL, a crucial step in the development of atherosclerosis(EFSA, 2011). Regarding blood pressure, many studies finding a direct relationship between adherence to the MedDiet and lower blood pressure in epidemiological and clinical trial settings have been published(Lopez-Miranda et al., 2010; Thomazella et al., 2011; Esposito et al., 2004; Shidfar et al., 2011; Bermudez et al., 2011; Estruch, 2010). The evidence linking the consumption of MUFA with lower blood pressure has been reviewed recently (Gillingham, Harris-Janz, and Jones, 2011), However, the advances in

our knowledge about the pathophysiology of atherosclerosis, and the study of new, important players in this process have allowed us to identify new effects of the MedDiet as a pattern, and of individual foods in particular. In this article, we will review the current evidence for these effects on non-traditional cardiovascular risk factors, such as endothelial function, inflammation, oxidative stress, postprandial lipemia or coagulation.

THE MEDITERRANEAN DIET AND THE ENDOTHELIUM.

Endothelial dysfunction has been repeatedly reported as an important determinant of the initiation and the development of various clinical diseases and cardiovascular risk factors, including coronary heart disease, hypertension, diabetes or heart failure(Desjardins and Balligand, 2006; Halcox et al., 2002). Some factors have been identified which impair vascular endothelium function, both by their direct effects on the vascular vasomotor capacity, or by influencing cellular regulators, such as inflammatory mediators (ICAM, VCAM). These factors include smoking, high LDL-cholesterol concentration, hypertension, diabetes mellitus or advanced age, and their effects are mainly driven by an imbalance of the redox system, increasing oxidative stress, and decreasing nitric oxide availability at the vascular site(Ambrose and Barua, 2004; Brunner et al., 2005). Furthermore, there is an additional temporary impairment of endothelial function during the postprandial state, especially after meals rich in saturated fatty acids(Cuevas and Germain, 2004; Anderson et al., 2001; Bae et al., 2003).

Diet is another important regulator of endothelial function, and may exert its effects during both the fasting and postprandial states(Goode, Garcia, and Heagerty, 1997; Fuentes et al., 2008; Berry et al., 2008). The MedDiet has shown significant favorable effects on the endothelium,

especially when compared to high saturated fatty acid-rich diets: A four-week period following the MedDiet was associated to an improvement in endothelial function, as assessed by the flow-associated vasodilatation of the brachial artery versus a saturated fat diet (13.5% vs. 9.9%), in hypercholesterolemic patients(Fuentes et al., 2001). Curiously, in the same work, a low-fat diet failed to show similar findings, despite the fact that the two diets showed similar improvements in other endothelial regulators. The same improvement was eventually observed in obese participants who strictly adhered to a MedDiet versus regular counseling (Rallidis et al., 2009). In fact, starting a low-fat hypocaloric diet in obese persons deteriorated endothelial flow mediated dilatation, which does not happen with the MedDiet(Buscemi et al., 2009). Esposito et al. found in 180 Metabolic Syndrome patients that, after two years of diet, the MedDiet induced a better endothelial function as assessed by a combined score of reduction in blood pressure and platelet aggregation decreases in response to nitric oxide precursors (Esposito et al., 2004). Regarding postprandial endothelial function, many studies report the beneficial results of the MedDiet. Fuentes et al. reported that the MedDiet improves postprandial microvascular vasomotor function when compared to saturated fatty acid-rich and low-fat diets (enriched in alpha-linolenic) in healthy young men, in addition to a lower postprandial concentration of pro-inflammatory citokines and higher bioavailability of nitric oxide (NOx) (Fuentes et al., 2008), replicating a previous report(Fuentes et al., 2001). These results have recently been replicated by Perez-Martinez et al. in Metabolic Syndrome patients, when comparing MedDiet with an SFA-rich diet and two low-fat models (one of which was enriched with 1.24 g/day of long chain n-3 PUFA), and by the PREvencion con DIeta MEDiterranea (PREDIMED) trial, where following

the MedDiet induced a favorable chemokine profile for the endothelium(Estruch, 2010; Salas-Salvado et al., 2008).

As stated previously, although multiple factors have been linked to the physiology of the endothelial function, oxidative stress has been identified as one of its most important regulators. Looking for potential underlying mechanisms of the enhanced endothelial function in the MedDiet, an improvement of the redox balance, with increased nitric oxide bioavailability and decreased pro-inflammatory and pro-oxidant molecules in the plasma have been repeatedly reported (Esposito et al., 2004; Esposito, Ciotola, and Giugliano, 2006; Perez-Martinez et al., 2010; Perez-Martinez et al., 2010). In fact, some of the key components of the MedDiet may have a proportional effect on endothelial function through their antioxidant properties. We previously reported how the concentration of phenols (natural antioxidants present in virgin olive oil) influence endothelial function in a proportional way(Ruano et al., 2005). Furthermore, different antioxidants from the MedDiet may have synergic effects. Zampelas' team reported how the combination of high-phenol virgin olive oil combined with red wine (rich in antioxidants) induced the most favorable effect on postprandial flow-mediated dilatation in healthy young men(Karatzi et al., 2008)

Another key factor to comment on when assessing the influence of diets on the endothelium is not simply the functionality of the endothelium, but its capacity to react to different injuries. Tissue damage at the vascular wall, infections, or the aging process itself leads to stress in the endothelium, which has to respond with sufficient cell replacement. The level of endothelial damage may be estimated by the presence of microparticles released into the bloodstream, and conversely, the level of capacity of regeneration may be estimated by the proportion of

endothelial progenitor cells. We recently published how a chronic intervention with the MedDiet led to lower microparticle concentration and higher endothelial progenitor cells compared with an SFA-rich diet or a low-fat high-carbohydrate diet in the elderly, which demonstrates the protective effect of the MedDiet in the regenerative capacity of the endothelium(Marin et al., 2011). Furthermore, in these tests, the MedDiet period was followed by improved microvascular vasodilator endothelial function, and lower nitric oxide degradation products in urine(Marin et al., 2011). This endothelial cell protection may be driven by a lower level of oxidation and higher DNA stability in these cells: in another recent paper, we described how the reactive oxygen species in human umbilical endothelial cells incubated with serum from persons who had followed a MedDiet were lower than those in persons who had followed a high-SFA, low-fat, high-carbohydrate diet(Marin et al., 2011). Complementing these results, the cells incubated with MedDiet serum were less prone to suffer apoptosis, and presented less DNA-damage (assessed by telomere shortening)(Marin et al., 2011).

Although the exact molecular routes underlying the lower oxidative stress caused by the MedDiet are still being studied, research has been published showing that hydroxytyrosol (a key phenol antioxidant present in virgin olive oil) and other micronutrients typical of the MedDiet, such as resveratrol, may exert their vasculoprotective effects in response to oxidative injury by the activation of a nuclear factor-E(2)-related factor-2 (Nrf2) pathway, which, in turn, promotes the expression of other antioxidants, like NAD(P)H:quinone oxidoreductase 1(Zrelli et al., 2011; Ungvari et al., 2010). For more information, a recent work reviews the antioxidant effects of polyphenols, which induce nitric oxide, and endothelium-derived hyperpolarizing factor (EDHF)mediated relaxation(Schini-Kerth et al., 2010).

Finally, we must also cite the way in which genes may interact with diet to regulate endothelial function (the importance of the called nutrigenetics). The consumption of a MedDiet induces the downregulation of proinflammatory genes (which are pro-oxidant, and therefore deleterious to the endothelial cells)(Camargo et al., 2011), and, thus, the bloodstream may become less oxidized, and conserve the endothelial function better. As an example, it has been reported that individuals with variations in the nitric oxide synthase gene exhibit a worse endothelial function during the postprandial state(Delgado-Lista et al., 2011). However, the diet may somehow limit this circumstance. We recently showed that these carriers aleviate postprandial endothelial dysfunction when the meal ingested is rich in polyphenols, probably by increasing the nitric oxide bioavailability in the endothelial cells, therefore helping the partly inefficient nitric oxide synthase to work(Jimenez-Morales et al., 2011).

In conclusion, there is firm evidence to show that the MedDiet improves the endothelial function, in various places (**Table 1**). This improvement has been reported for large, medium and small size vessels, which indicates that these effects may be mediated by various mechanisms, taking into account the different physiology of these vessels. A combination of higher nitric oxide bioavailability, decreased pro-oxidant cytokine production and even stimulation of other antioxidant pathways like Nrf2 may also be involved. Some clues pointing towards an additional gene-environment interaction mechanism have been also suggested to date.

THE MEDITERRANEAN DIET AND COAGULATION.

Repair of the damaged vessels and the intravascular response to infectious agents by limiting their damage to the endothelial cells is a crucial process in human haemostasis, involving platelets and coagulation factors. However, chronic conditions, such as cardiovascular disease, often alter the ideal balance between pro and anti-cooagulant factors, favouring the existence of a prothrombotic environment, which, in turn, has being proven to be proatherogenic(Borissoff, Spronk, and ten Cate, 2011). A simple way to understand the importance of coagulation in cardiovascular disease is the fact that, these days, the main drug treatment for coronary heart disease is, in fact, aspirin, an antiplatelet therapy.

Diet may influence coagulation. The observation of a low incidence of coronary heart disease in Greenland Eskimos and their increased risk of hemorrhages led to research in the last century into the possible environment factors, and the discovery of marine omega-3 fatty acids as the main contributing factor. Since then, the anticoagulant properties of these fatty acids, and the inverse relationships of their intake and the prevalence of coronary heart disease have been established beyond doubt(Lichtenstein et al., 2006; Kris-Etherton, Harris, and Appel, 2002; Delgado-Lista et al., 2012). Evidence of the favorable effects of these fatty acids on coronary heart disease has led to the most prestigious scientific committees recommending two servings of blue fish a week for the general population (to attain a mean of 500 mg/d), and 1 g/d of marine omega-3 (EPA and DHA) in patients with coronary disease(Lichtenstein et al., 2006; Kris-Etherton, Harris, and Appel, 2002). Although the latter figures are difficult to achieve only by dietary intake, and most of coronary patients would need supplements of these fatty acids in the form of pills, the evidence showing the favorable effects of omega-3 for the heart come from studies using a very wide range of omega-3 doses, and some of the most significant evidence

comes from studies with modest quantities of omega-3, which can be obtained in some types of diet(Filion et al., 2010; Riediger et al., 2009; Mente et al., 2009; Lavie et al., 2009). Contrary to the western-type diet (which obtains most of its protein intake through meat), MedDiet gives preference to fish and poultry, including 2-3 servings of fish a week, a frequency which may allow for the 500mg/d recommended dose of omega-3. Most current research into omega-3 fatty acids is centered on assessing the underlying mechanisms behind these proven effects. In addition to other non-coagulant features observed, such as a better lipid profile(Harris et al., 2008), anti-inflammatory properties (Serhan, Chiang, and Van Dyke, 2008), blood pressure(Morris, Sacks, and Rosner, 1993), heart failure (Gissi et al., 2008; Yamagishi, Nettleton, and Folsom, 2008), or anti- arrhythmic properties(Mozaffarian et al., 2004; Leaf et al., 2005; Gillet et al., 2011), our current knowledge points to the way they reduce the adhesion rate of platelet metabolism as one of the main mechanisms(Renaud and Lanzmann-Petithory, 2002; Seo, Blaner, and Deckelbaum, 2005). However, other mechanisms related to atherothrombosis have been identified: Thies et al. administered sunflower oil or fish oil (omega-3) capsules to 188 patients awaiting carotid endarterectomy,, including a control group, for an average of 42 days. The group taking omega-3 exhibited fewer plaques with thin fibrous caps and signs of inflammation and more plaques with thick fibrous caps and no signs of inflammation, compared to the control group and sunflower oil groups. In addition, the number of macrophages in the plaques was also lower in the fish oil group compared with the other two groups(Thies et al., 2003).

When assessing the overall effects of oily fish on cardiovascular health, a recent topic of controversy is how safe it is to consume. Some studies (mostly observational) have reported

mixed or inconclusive results for the cardiovascular effects of the methylmercury and selenium in oily fish (especially large fish, such as tuna or shark). A recent review on the topic concluded that the "net health benefits of overall fish consumption in adults are clear"(Park and Mozaffarian, 2010). Future research into this field is of special interest to establish the cardiovascular effects of methylmercury and selenium. Until that time, the recommendation to eat oily fish must be taken with caution in specific groups, such as children or pregnant women.

Complementing the effects of the omega-3 fatty acids, the other main food present in the MedDiet which has been studied in relation to the haemostatic system is olive oil (especially virgin olive oil). Our group has summarized the evidence on the anti-coagulant and anti-thrombotic features associated with the intake of a MedDiet rich in olive oil in previous works(Delgado-Lista et al., 2011; Lopez-Miranda et al., 2007; Perez-Jimenez et al., 2006). Olive oil exerts its function on the haemostatic system in a double axis, downregulating both platelet metabolism and the concentration of some coagulation factors.

As regards platelet metabolism, olive oil acts by decreasing thromboxane generation, the responsiveness of platelets to some of their inductors, like ADP, and the release of platelet-activating factor(Perez-Jimenez et al., 2006; Karantonis, Antonopoulou, and Demopoulos, 2002; Karantonis et al., 2006; Sirtori et al., 1986; Smith et al., 2003; Singh et al., 2008; Antonopoulou et al., 2006). In some *in vitro* and animal studies, it has even been reported that the antiaggregant effects of virgin olive oil may be almost as efficient as those of acetylsalicylic acid (ASA), and that virgin olive oil may improve the inhibitory effects of ASA on platelet activation and aggregation(De La Cruz et al., 2010; Gonzalez-Correa et al., 2008). However, the latter results

must be taken with caution, as they come from experimental, non-clinical settings and should be confirmed in human *in vivo* studies.

Regarding coagulation factors, the anticoagulant effects of virgin olive oil have been clearly established and recently re-confirmed(Delgado-Lista et al., 2011). These effects include those which influence the baseline fasting state and those which influence the postprandial state, where there is an increase in vascular pro-thrombotic molecules, and where, therefore, the anticoagulant properties may be of greater interest(Delgado-Lista et al., 2011). To date, meals rich in virgin olive oil have been shown to reduce FVII (Smith et al., 2003; Delgado-Lista et al., 2008; Temme, Mensink, and Hornstra, 1999; Turpeinen and Mutanen, 1999; Junker et al., 2001; Junker et al., 2001; Mezzano et al., 2003; Williams, 2001; Mezzano and Leighton, 2003), tissue factor (Bravo-Herrera et al., 2004), fibrinogen (Mezzano and Leighton, 2003), PAI-1 factor (Perez-Jimenez, 2005; Perez-Jimenez et al., 1999; Perez-Jimenez, Lopez-Miranda, and Mata, 2002; Avellone et al., 1998) or von Willebrand Factor (Perez-Jimenez et al., 1999; Rasmussen et al., 1994), when compared to high SFA meals.

Although it is not a vital component of the MedDiet, a moderate consumption of red wine is a usual feature in some Mediterranean countries. Unlike the above elements, we are less sure of the overall influence of red wine on haemostasis, since although it has been linked to a decrease in some procoagulant species, such as fibrinogen, and to an increase in the natural anticoagulant TPA, it has been also connected to increases in some proinflammatory markers (ICAM-1, E-Selectin, interleukin-6), and even to increased platelet aggregation (Mezzano and Leighton, 2003; Tozzi Ciancarelli et al., 2011).

Finally, the MedDiet is rich in vegetables, grains and nuts. Although these foods have not studied as extensively as the above elements, the overall influence of this combination is clearly anti-thrombotic(Phang et al., 2011; Torres-Urrutia et al., 2011). A recent study reported the *in vitro* capacities of a variety of fruit and vegetables, and found anti-thrombotic properties in green beans and tomatoes, by inhibiting platelet aggregation, and anti-coagulant effects in grapes and raspberries(Torres-Urrutia et al., 2011).

Proteins originating from plants are less prothrombotic than those in meat (Sawashita et al., 2006). One of the possible mechanisms by which this works is the fact that animal proteins are an important source of methionine, which is converted into homocysteine when it loses a methyl group. Normally, the homocysteine must be then restored again to methionine in the methylation cycle, but an excessive dietary intake of methionine, or a decrease in some nutrients and vitamins (such as folic acid from vegetables or VitB12) may lead to an accumulation of homocysteine, which has been linked to an increased risk of thrombosis in venous and arterial blood vessels, although this relationship has failed to show a causal effect, and homocysteine-lowering trials have been unsuccessful(Zhu et al., 2012; Homocysteine Studies Collaboration, 2002; Kang, 2011; Clarke et al., 2011; Finch and Joseph, 2010; Di Minno et al., 2010; Williams and Schalinske, 2010; Den Heijer, Lewington, and Clarke, 2005). A recent work has shown that, in the presence of a high homocysteine concentration, endothelial cells promote clot formation(Zhu et al., 2012).

To sum up, many of the components of the MedDiet have shown anti-thrombotic and/or anti-coagulant properties (**Table 2**). These include olive oil, vegetables, grains and nuts. Furthermore,

when assessing the global influence of the MedDiet as a whole, it is important to note that a large epidemiological cohort has recently linked adherence to the MedDiet with lower levels of some pro-thrombotic molecules, such as fibrinogen(Carter et al., 2010).

THE MEDITERRANEAN DIET AND INFLAMMATION.

What we currently know about the etiopathogeny of atherosclerosis is that it is a chronic inflammatory disease, defined by low-grade chronic inflammation, which is mainly caused by monocytes, but also by other key players of inflammation like neutrophils, natural killers, or immune cytokines.

Many observational studies and clinical trials have reported reduced inflammatory biomarkers when the MedDiet is consumed, compared with SFA-rich diets(Lopez-Miranda et al., 2010; Carter et al., 2010; Pellegrini et al., 2010; Perez-Jimenez et al., 2005; Giugliano and Esposito, 2008; Fung et al., 2005). As with the rest of the factors included in this review, two different research strategies have been followed when assessing the effects of the MedDiet in the factor evaluated (in this case, inflammation). Some authors look for the effect of the dietary pattern as a whole, while others try to identify the actions of some of the individual foods which are most characteristic of the MedDiet.

The components of the MedDiet have been proven to elicit lower activation of the main inflammatory trigger, the NFkB, both after an isolated meal, in the postprandial state, or after a chronic dietary intervention(Perez-Martinez et al., 2007; Bellido et al., 2004). Results of the PREDIMED study (a chronic dietary intervention with MedDiet or low-fat diets in the primary

prevention of subjects with high cardiovascular risk), reported that chronic consumption of the MedDiet led to an anti-inflammatory effect (assessed by a reduction in C-reactive protein, interleukin 6 and proinflammatory chemokines). Interestingly, these markers rose after the low-fat diet intervention(Estruch, 2010; Mena et al., 2009). Panogiatakos et al. reported that, after a three year follow-up of the AIRGENE project (performed on myocardial infarction survivors from six different geographical areas in Europe), adherence to the traditional MedDiet was associated with a reduction in the concentrations of inflammatory markers (C-reactive protein and interleukin 6)(Panagiotakos et al., 2009); in the ZINCAGE study in elderly persons, adherence to the MedDiet was inversely correlated to interleukin 18(Dedoussis et al., 2008); and, in the ATTICA study, performed with over 3,000 participants, those who were in the highest tertile of adherence to the MedDiet score exhibited 14-20% lower CRP, interleukin 6, and white blood cell counts(Chrysohoou et al., 2004). Another report by Esposito et al. showed that, after two years of MedDiet counseling versus a prudent, low-fat diet, 180 Metabolic Syndrome patients exhibited reduced serum concentrations of C-reactive protein and several proinflammatory interleukins (IL-6, IL-7 and IL-18), in addition to enhanced endothelial function and insulin sensitivity(Esposito et al., 2004). Moreover, it has been observed that the expression of VCAM-1 and E-selectin in human umbilical vascular endothelial cells (HUVECs), following the addition of minimally-oxidised LDL, was lower with LDL obtained from subjects who had followed a diet rich in olive oil than from those whose diet was rich in SFA(Bellido et al., 2006).

To discriminate the possibility of shared environmental and genetic factors, Dai et al. published that, in 345 middle-aged male twins, adherence to the MedDiet was associated with lower levels

of interleukin 6 but not of C-reactive protein. The fact that adherence to a dietary model is linked to phenotypical differences in plasma concentration in persons with the same DNA adds strength to the idea that it is the diet itself, and not the different environments in which the person lives, that determines the changes observed in these inflammatory markers(Dai et al., 2008)

We recently published that after a dietary period, Med Diet consumption induces favorable anti-inflammatory gene expression changes in mononuclear cells. More specifically, in the fasting state, we observed lower gene expression of the activator subunit of NFkB (p65) compared with an SFA-rich diet, and, in the postprandial state, lower gene expression of this subunit together with that of the monocyte chemoattractant protein 1 and the metalloproteinase 9, two proinflammatory chemokines, compared with the SFA-rich diet, and a lower gene postprandial expression of p65 and TNF-alpha compared with a low-fat, high-carbohydrate diet enriched in vegetable omega-3. Findings along the same lines were reported by Llorente-Cortes et al, who found a downregulation of proinflammatory, prothrombotic genes after three months of allocation to a MedDiet rich in virgin olive oil, compared to a MedDiet rich in nuts or a control diet, in persons with high cardiovascular risk(Llorente-Cortes et al., 2010). Additional studies showing similar findings in the postprandial state have been also published(Konstantinidou et al., 2009; Jimenez-Gomez et al., 2009; Pacheco et al., 2007), and these anti-inflammatory effects also seem to be linked to an enhancement of the postprandial endothelial vasomotor function, as stated above(Fuentes et al., 2008).

In the search for individual macro- or micronutrients responsible for these effects, many studies have singled out olive oil as a food, and its minor anti-inflammatory compounds (such as phenols) (Salas-Salvado et al., 2008; Lucas, Russell, and Keast, 2011; Camargo et al., 2010;

Brunelleschi et al., 2007; Carluccio et al., 1999). The expression of inflammatory genes is lower when the olive oil used in the meals is rich in phenols, compared with meals that included olive oil with a low phenol concentration(Camargo et al., 2010). The cooking method may also influence these findings. It has been reported that the intake of a meal containing sunflower oil which has been subjected to twenty heating cycles leads to a proinflammatory reaction in plasma and mononuclear cells (with increases in NFkB, other proinflammatory activators -MIF and JNK- and proinflammatory interleukins) which does not occur with a high phenolic virgin olive oil or a mixed-seed oil (sunflower/canola oil) artificially enriched with phenols from olive mill wastewater(Perez-Herrera et al., 2012).

Other foods present in the MedDiet also may play a role in the lower level of inflammation. Serrano-Martinez showed that adherence to the MedDiet was inversely correlated with TNF-alpha and the chemokine VCAM-1 in coronary sinus blood, and that the link was maintained both when assessing only the olive oil consumption, and when using a modified MedDiet score excluding olive oil(Serrano-Martinez et al., 2005). As an example, as stated above, fish is a major source of animal protein in the MedDiet. Marine omega-3 from fish may exert a favorable effect by a negative regulation of inflammation(Serhan, Chiang, and Van Dyke, 2008). More specifically, it has been identified as a precursor of some lipoxins, resolvins and protectins. All these molecules are the main components of the inflammatory “resolution”. Resolution is a molecular term used to describe those mechanisms which, as well as limiting neutrophil infiltration, promote the “clearance” of pro-inflammatory stimuli, like the retirement of apoptotic cells or substances of microbial origin. Stated simply, the function of resolution is to bring back the inflammatory homeostasis from the activated to the normal status(Serhan, Chiang, and Van

Dyke, 2008). Salas-Salvado reported that those subjects who regularly consumed fruit and cereals had lower concentrations of interleukin 6, in a cross-sectional study of the PREDIMED study(Salas-Salvado et al., 2008)

To sum up, there is a large body of evidence to indicate that MedDiet consumption is linked to a lower degree of inflammation both in the fasting and the postprandial state (**Table 3**). This fact includes a downregulation of the expression of proinflammatory genes in mononuclear cells, but also a lower total plasma concentration of proinflammatory markers, such as C-reactive protein, proinflammatory interleukins and adhesion molecules. While this feature has been found in studies assessing the effects of the MedDiet as a pattern, the individual contributions of some of its components, like fish, vegetables and olive oil, has also been shown.

THE MEDITERRANEAN DIET AND POSTPRANDIAL LIPEMIA

Postprandial lipemia is defined as the rise in circulating TG and TG-rich lipoproteins which occurs after a fatty meal. Although not strictly delimited, it is believed that meals containing 30-50g of fat cause such a response, while those below this figure do not(Lopez-Miranda and Marin, 2010; Jackson, Poppitt, and Minihane, 2012; Lopez-Miranda, Williams, and Lairon, 2007). Although the importance of lipids in cardiovascular disease has traditionally been confined to the fasting concentration of cholesterol (mainly LDL cholesterol), which is still the main factor influencing most treatment guidelines, increasing importance is now being given to postprandial lipemia, not only for the multiple pro-oxidative, pro-thrombotic, pro-inflammatory biological changes that accompany it but also for the evidence suggesting a direct link to cardiovascular events(Mihas et al., 2011; Kolovou et al., 2011).

The influence of different dietary models on postprandial lipids is, however, not fully understood. The primordial importance of fats in this state has led research in this field to be centered on the effects of the different types of fat, discarding the other usual differences between whole meals of the different models. Therefore, most of the research has been performed using the same (or very similar) meal base, enriched with different sources of fat. In such a setting, the use of omega-3 as the main source of fat has been shown to reduce the rise in postprandial triglyceride, while MUFA-rich fat (such as olive oil) leads to a higher and earlier triglyceride peak. It may seem surprising at first sight that some of the studies have shown a smaller increase in triglycerides after the consumption of SFA fats from dairy products. However, this is easy to understand if we remember that these fats are rich in small, medium-chain fatty acids that enter the portal route, rather than the chylomicron route, through general circulation(Lopez-Miranda, Williams, and Lairon, 2007). In general, it is now widely recognized that MUFA rich meals provoke a sharper, earlier rise in triglycerides, followed by a faster lipid clearance than with non-dairy SFA-rich meals(Sanders et al., 2000; Roche et al., 1998). Although the area under the curve for triglycerides after the different types of meals do not seem to differ much, a more extended postprandial lipemia has been proven to be proatherogenic(Lopez-Miranda, Williams, and Lairon, 2007; Patsch et al., 1992). One of the explanations suggested for the rapid triglyceride clearance after the MUFA-rich diets is the larger chylomicron size found after the chronic consumption of this type of diet. Larger chylomicrons are able to manage a higher quantity of triglycerides, and reduce the time it takes to process them(Silva et al., 2003). On the other hand, it has been also proposed that MUFA are easier to exchange with triglycerides than SFA due to their distribution in positions 1 and 3 of

the triglyceride molecule. However, other studies have found higher total Large- and Small-TRL particle production after isolated MUFA-rich meals(Jackson, Poppitt, and Minihane, 2012). Recently, we have demonstrated that consumption of an olive oil-rich meal leads to the formation of a smaller number of TRL particles compared with butter and walnut-based meals. Moreover, TRL particle size was greater after the intake of an olive oil meal compared with a walnut meal(Perez-Martinez et al., 2011). In general, current evidence points to the fact that the differences in postprandial lipemia between SFA and MUFA diets lie mainly in the kinetics of the response, with a sharper, earlier response with MUFA-rich diets, and a longer response after SFA diets. Omega-3 rich meals, on the other hand, lead to lower postprandial hypertriglyceridemia, but the amount of omega-3 necessary to cause such effects is difficult to attain in a regular meal. Some recent articles have reviewed in depth the relationships between diet and postprandial lipemia(Jackson, Poppitt, and Minihane, 2012; Lopez-Miranda, Williams, and Lairon, 2007; Lopez-Miranda and Marin, 2010).

Genes also may interact with diet to regulate postprandial response. In this context, over the past few years, we have produced different evidence linking a number of candidate genes to the modulation of the postprandial lipid metabolism(Perez-Martinez et al., 2011; Perez-Martinez et al., 2010; Perez-Martinez et al., 2008). Furthermore, some of these interactions may link lipids to inflammation during the postprandial state, and we recently described how alterations in the genes of inflammatory particles may condition the postprandial response to fatty meals(Delgado-Lista et al., 2011).

Table 4 summarizes the influence of the MedDiet on the postprandial lipemia

THE MEDITERRANEAN DIET AND CARDIOVASCULAR DISEASE: FUTURE DIRECTIONS.

Although we have reviewed in this article some of the current lines of research into the biological effects of the MedDiet on cardiovascular disease, many others are also open, which cannot be reviewed here, which include the effects that it may exert on the genetic expression of proteins, hormones or other molecules (Nutrigenetics-Nutrigenomics), the epigenetic regulation of the proteins and metabolites produced by the cell (epigenetics, microRNA studies), oxidative stress, proteomics and metabolomics. In addition, other non-cardiovascular processes are also being investigated, such as its effects on cell stability and cancer, neurotransmitters and their effects on cognitive function, as well as many others.

As a complement to these extraordinarily important functional studies, the main milestone for MedDiet research in the present decade is to establish its capacity to reduce the incidence of cardiovascular events in long-term dietary intervention trials, and to prove in those settings that it is at least as effective as low-fat diets. The conceptual change that a high-fat diet may be at least as effective as low-fat diets in terms of the rate of incidence of cardiovascular events may help to generalize the use of this dietary pattern, which could also provide an opportunity to increase research into the beneficial effects of this diet in other health problems, such as age-associated cognitive decline, the control of metabolic diseases like metabolic syndrome or type 2 diabetes, or the incidence of certain types of cancer. Furthermore, the high palatability of this dietary pattern may help the population to follow a healthy diet on a long-term basis with good adherence rates. In this area, PREDIMED, a study on the long-term intervention effects of the

MedDiet in primary cardiovascular prevention which has recently concluded, and CORDIOPREV, an ongoing intervention trial into the effects of the MedDiet on secondary cardiovascular prevention, will both help to unveil the causal effect of this dietary pattern underlying the lower rates of cardiovascular disease associated with the consumption of the MedDiet.

ACKNOWLEDGMENTS:

Supported partly by public funding: research grants from the Spanish Ministry of Science and Innovation (AGL2009-12270 to J L-M, SAF07-62005 to F P-J and FIS PI10/01041 to P P-M, PI10/02412 to F P-J); Consejería de Economía, Innovación y Ciencia, Proyectos de Investigación de Excelencia, Junta de Andalucía (P06-CTS-01425 to J L-M, CTS5015 and AGR922 to F P-J); Consejería de Salud, Junta de Andalucía (07/43, and PI0193/09 to J L-M, 0118/08 to F F-J, PI-0252/09 to J D-L, and PI-0058/10 to P P-M); Fondo Europeo de Desarrollo Regional (FEDER). The CIBEROBN is an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

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Table 1. Effects of Mediterranean diet on the endothelium

Evidence	Effects
Fuentes et al., 2001	A four-weeks period of MedDiet was associated to an improvement in endothelial function, as assessed by the flow-associated vasodilatation of the brachial artery versus a saturated fat diet (13.5% vs. 9.9%), in hypercholesterolemic patients
Rallidis et al., 2009; Buscemi et al., 2009	The start of a low-fat hypocaloric diet in obese persons deteriorates endothelial flow mediated dilatation, something that does not happen with the MedDiet
Esposito et al., 2004	In 180 Metabolic Syndrome patients, after two years of diet, MedDiet induced a better endothelial function as assessed by a combined score of reduction in blood pressure and platelet aggregation decreases in response to nitric oxide precursors
Fuentes et al., 2008	MedDiet improves postprandial microvascular vasomotor function when compared to a saturated fatty acids rich and a low-fat diet (enriched in alpha-linolenic) in healthy young men, additionally to a lower postprandial concentration of pro-inflammatory citokines and higher bioavailability of nitric oxide
Estruch, 2010; Salas-Salvado et al., 2008	Allocation to MedDiet induced a favorable chemokine profile for the endothelium
Esposito, Ciotola, and Giugliano, 2006; Perez-Martinez et al., 2010	MedDiet increased nitric oxide bioavailability and decreased pro-inflammatory and pro-oxidant molecules in the plasma
Marin et al., 2011	The MedDiet led to lower microparticle concentration and higher endothelial progenitor cells compared with a SFA rich diet or a low-fat high-carbohydrate diet in the elderly, which denotes the protective effect of the MedDiet in the regenerative capacity of the endothelium. Complementing these results, the cells incubated with MedDiet serum were less prone to undergo apoptosis, and presented less DNA-damage (assessed by telomere shortening)
Camargo et al., 2011	The consumption of a MedDiet induces the downregulation of proinflammatory genes (pro-oxidant, and therefore deleterious for the endothelial cells)

Table 2. Effects of Mediterranean diet on the coagulation

Evidence	Effects
Lichtenstein et al., 2006; Kris-Etherton, Harris, and Appel, 2002; Delgado-Lista et al., 2012	The anticoagulant properties of omega-3 fatty acids have been clearly established, as well as the inverse relationships of their intake and the prevalence of coronary heart disease.
Perez-Jimenez et al., 2006; Karantonis, Antonopoulou, and Demopoulos, 2002; Karantonis et al., 2006; Sirtori et al., 1986; Smith et al., 2003	Regarding the platelet metabolism, olive oil acts decreasing the thromboxane generation, the platelet responsiveness to some of their inductors, like ADP, and the release of platelet-activating factor
Delgado-Lista et al., 2008; Bravo-Herrera et al., 2004; Mezzano and Leighton, 2003; Perez-Jimenez, 2005; Perez-Jimenez et al., 1999	With respect to coagulation factors, meals rich in virgin olive oil have shown effects reducing FVII, tissue factor, fibrinogen, PAI-1 factor or von Willebrand Factor, when compared to high SFA meals.
Delgado-Lista et al., 2011; Lopez-Miranda et al., 2007; Perez-Jimenez et al., 2006	Summarized evidence on the anti-coagulant, anti-thrombotic features associated with the intake of the MedDiet rich in olive oil

Table 3. Effects of Mediterranean diet on the inflammation

Evidence	Effects
Lopez-Miranda et al., 2010; Carter et al., 2010; Pellegrini et al., 2010; Perez-Jimenez et al., 2005	Observational studies and clinical trials have reported reduced inflammatory biomarkers when MedDiet is consumed, as compared to SFA rich diets
Perez-Martinez et al., 2007; Bellido et al., 2004	Components of MedDiet have proven to elicit a lower activation of the main inflammatory trigger, the NFkB, both after an isolated meal, in the postprandial state, or after a chronic dietary intervention
Estruch, 2010; Mena et al., 2009	Results of the PREDIMED study, reported that the chronic consumption of the MedDiet induced an anti-inflammatory effect (as assessed by a reduction of C-reactive protein, interleukin 6 and proinflammatory chemokines). Interestingly, these markers augmented after the low-fat diet intervention
Panagiotakos et al., 2009	After three years of follow up of the AIRGENE project (performed in myocardial infarction survivors from six different geographic areas in Europe), adherence to the traditional MedDiet was associated with a reduction of the concentrations of inflammatory markers (C-reactive protein and interleukin 6)
Bellido et al., 2006	The expression of VCAM-1 and E-selectin in human umbilical vascular endothelial cells, following the addition of minimally oxidized LDL, was less with LDL obtained from persons who had followed a diet rich in olive oil than from persons whose diet was rich in SFA
Llorente-Cortes et al., 2010; Camargo et al., 2010	The Med Diet consumption induces in mononuclear cells favorable anti-inflammatory gene expression changes
Serrano-Martinez et al., 2005	Adherence to MedDiet was inversely correlated with TNF-alpha and the chemokine VCAM-1 in coronary sinus blood, and that the association was conserved both when assessing only the olive oil consumption, and when using a modified MedDiet score excluding olive oil

Table 4. Effects of Mediterranean diet on the postprandial lipemia

Evidence	Effects
Sanders et al., 2000; Roche et al., 1998	MUFA rich meals provoke a sharper, sooner triglyceride rising, followed by a faster lipid clearance that non-dairy SFA rich meals
Perez-Martinez et al., 2011	Consumption of an olive oil-rich meal leads to the formation of a reduced number of TRL particles compared with butter and walnut-based meals. Moreover, TRL particle size was greater after the intake of the olive oil meal compared with the walnut meal
Perez-Martinez et al., 2011; Perez-Martinez et al., 2010; Perez-Martinez et al., 2008	Genes also may interact with diet to regulate postprandial response
Delgado-Lista et al., 2011	Alterations in genes of inflammatory particles may condition the postprandial response to fat meals
Jackson, Poppitt, and Minihane, 2012; Lopez-Miranda and Marin, 2010; Lopez-Miranda, Williams, and Lairon, 2007	Recent articles review in deep the relationships between diet and the postprandial lipemia

Circulating markers:

MedDiet reduces vasoconstrictor cytokines, reduces oxidative stress and increases NOx bioavailability, versus SFA and low-fat diets, in different settings (fasting and postprandial states)(Esposito et al., 2004; Estruch, 2010; Fuentes et al., 2008; Salas-Salvado et al., 2008; Esposito, Ciotola, and Giugliano, 2006; Perez-Martinez et al., 2010; Perez-Martinez et al., 2010), reduces the activation of proinflammatory pathways, like NFkb(Perez-Martinez et al., 2007; Bellido et al., 2004), and induces the activation of antioxidant pathways like Nrf2(Zrelli et al., 2011; Ungvari et al., 2010). Phenols present in virgin olive oil induce nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) mediated relaxation(Schini-Kerth et al., 2010)

Large Vessels:

Metabolic Syndrome patients improve their endothelial function (combined blood pressure and platelet aggregation) after long term intervention with MedDiet(Esposito et al., 2004). Other favorable effects on blood pressure have been also reported(Lopez-Miranda et al., 2010; Thomazella et al., 2011; Shidfar et al., 2011; Bermudez et al., 2011; Estruch, 2010)

Medium Vessels:

MedDiet increases FMD in brachial arteries compared to SFA-rich diet(Fuentes et al., 2001). Obese persons increase FMD when they adhere to MedDiet versus regular counseling(Rallidis et al., 2009). These effects may be partly mediated by the phenols in virgin olive oil(Ruano et al., 2005), and virgin olive oil and red wine seem to be synergic in this repect(Karatzi et al., 2008).

Small Vessels/Microcirculation

MedDiet improve microvascular endothelial reactivity versus SFA-rich diets and low-fat diets(Fuentes et al., 2001; Fuentes et al., 2008; Perez-Martinez et al., 2010; Ruano et al., 2005). Marine omega-3 (2g/d) improves microvascular reactivity, although evidence comes from capsule origin, not from food(Stirban et al., 2010).

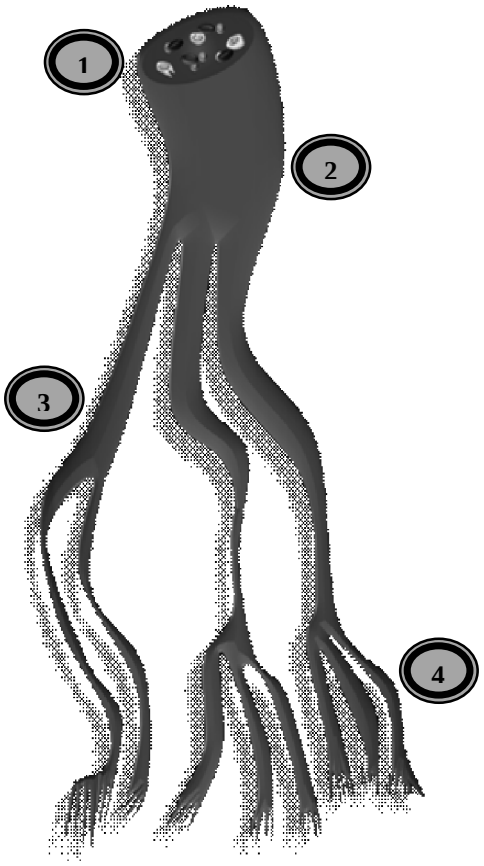

Figure 1: Studies evaluating the effects of MedDiet on endothelial function.