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Session 4: Dietary strategies to prevent and mitigate inflammatory diseases
Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study

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Several epidemiological and clinical studies have evaluated the effects of a Mediterranean diet (Med-Diet) on total cardiovascular mortality, and all concluded that adherence to the traditional Med-Diet is associated with reduced cardiovascular risk. However, the molecular mechanisms involved are not fully understood. Since atherosclerosis is nowadays considered a low-grade inflammatory disease, recent studies have explored the anti-inflammatory effects of a Med-Diet intervention on serum and cellular biomarkers related to atherosclerosis. In a pilot study of the PREvencion con DIeta MEDiterranea (PREDIMED) trial, we analysed the short-term effects of two Med-Diet interventions, one supplemented with virgin olive oil and another with nuts, on vascular risk factors in 772 subjects at high risk for CVD, and in a second study we evaluated the effects of these interventions on cellular and serum inflammatory biomarkers in 106 high-risk subjects. Compared to a low-fat diet, the Med-Diet produced favourable changes in all risk factors. Thus, participants in both Med-Diet groups reduced blood pressure, improved lipid profile and diminished insulin resistance compared to those allocated a low-fat diet. In addition, the Med-Diet supplemented with virgin olive oil or nuts showed an anti-inflammatory effect reducing serum C-reactive protein, IL-6 and endothelial and monocytary adhesion molecules and chemokines, whereas these parameters increased after the low-fat diet intervention. In conclusion, Med-Diets down-regulate cellular and circulating inflammatory biomarkers related to atherogenesis in subjects at high cardiovascular risk. These results support the recommendation of the Med-Diet as a useful tool against CVD.

Inflammation: Mediterranean diet: Atherosclerosis

CVD is the main cause of death worldwide at the turn of the 21st century. Western countries, including the USA, currently continue to exhibit unacceptably high absolute rates of cardiovascular morbidity and mortality, but incidence rates show marked geographical differences[1]. Surprisingly, a low incidence of CHD is found in some developed countries such as France, Spain, Greece, Italy and Portugal, leading to a higher life expectancy as compared with Northern European countries or the USA[1,2]. The Mediterranean food pattern (Mediterranean diet (Med-Diet)) has been the factor most frequently invoked to explain this health advantage.

The Med-Diet was identified as the traditional dietary pattern found in olive-growing areas of Crete, Greece and southern Italy in the late 1950s and early 1960s. Its major characteristics are (a) high consumption of non-refined grains, legumes, nuts, fruits and vegetables; (b) relatively high fat consumption (even greater than 40% of total energy intake) mostly from MUFA, which accounts for 20% or more of the total energy intake; (c) olive oil used

Abbreviations: CHO, carbohydrate; Med-Diet, Mediterranean diet; PREDIMED, PREvención con DIeta MEDiterránea.
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to cook and for dressing salads as the principal source of fat; (d) moderate to high consumption of fish; (e) low to moderate consumption of poultry and dairy products (usually as yoghurt or cheese); (f) low consumption of red meats, processed meats or meat products; and (g) moderate alcohol intake, usually in the form of red wine consumed with meals[3,4].

The background of a long and ancient tradition with no evidence of harm makes the Med-Diet very promising for public health. The high fruit and vegetable intake adds other mechanistic benefits provided by their polyphenolic content to its high antioxidant content. Other components of the Med-Diet such as virgin olive oil and red wine gather antioxidant and anti-inflammatory actions that may contribute to the healthy effects of this diet on the heart[5,6]. Accordingly, many scientists agree that the Med-Diet may exert a protective effect on the cardiovascular system. This hypothesis fits well into the current paradigm of studying overall dietary patterns instead of simply assessing isolated nutrients in nutritional epidemiology. The rationale is that food items and nutrients may have synergistic or antagonistic effects when they are consumed in combination. Additionally, overall patterns better represent the dietary practices found in free-living populations and therefore provide more useful epidemiological information[7,8]. Consequently, they have a higher potential for acceptability, palatability and future compliance when they are recommended in behaviour counselling. In spite of its relatively high fat content, or precisely because of it, the use of olive oil or other full-fat salad dressings in the Med-Diet increases vegetable consumption, because it makes vegetables more palatable. The sautéing or stir frying of vegetables with variable amounts of olive oil instead of using low-fat spreads or steaming increases taste and results in long-term maintenance of a vegetable-rich diet. These preparation and cooking techniques are typical of the Med-Diet, where the custom is to cook vegetables in olive oil to enhance flavour. Hence, in health promotion and nutritional education, better compliance with the Med-Diet can be expected. In fact, trials of weight loss reported better adherence to a Med-Diet than to a low-fat diet[9]. Participants viewed this diet as tastier than low-fat regimens, which may explain the increased long-term compliance.

Evidence available on the Mediterranean diet and CVD prevention

Findings from large European cohort studies[10,11] suggest that a high degree of adherence to the Med-Diet is associated with a reduction in both total and CHD mortality. A Med-Diet was inversely associated with mortality from all causes in several small observational cohort studies of elderly people[3,12,13]. Findings from the Greek European Prospective Investigation into Cancer cohort including more than 22 000 participants suggested that a higher adherence to the Med-Diet is associated with a reduction in total mortality and, more specifically, in coronary mortality[10]. Two recent case–control studies also found an inverse association between adherence to the Med-Diet and incidence of non-fatal coronary events[14,15]. A unique secondary prevention trial found a significant reduction in re-infarction or death when coronary patients were assigned to a so-called 'Mediterranean diet'[16]. This trial, the Lyon Diet Heart Study, found a 50–70% relative reduction in the risk of mortality or re-infarction when patients who had had a myocardial infarction were assigned to an experimental diet rich in bread, vegetables, fish and fruit and low in red meat (replaced with poultry). Butter and cream were replaced with a margarine enriched in α-linolenic acid. The 50–70% reduction in cardiac events observed after 46 months in the experimental group of the Lyon trial leads to the consideration that if these results were generalized to non-Mediterranean populations, substantially enhanced and efficient methods to reduce CHD would be available. It would be short-sighted not to acknowledge the vast public health benefit that a Med-Diet could provide with its adoption by the healthy population-at-large if the findings of the secondary trials are also confirmed in primary prevention trials. The American Heart Association has given attention to the Med-Diet as potentially useful for the prevention of CHD, but a cautious recommendation has been issued highlighting that more studies are needed before advising people to follow a Med-Diet. These studies will help to clarify whether the diet itself or other factors (such as more physical activity, a beneficial genetic background or stronger social support systems) account for the lower incidence of CHD in the Mediterranean countries[17].

Olive oil, a rich source of MUFA, is a main component of the Med-Diet. Virgin olive oil retains all the lipophilic components of the fruit, small amounts of α-tocopherol and sizeable amounts of phenolic compounds with strong antioxidant properties, while refined olive oil loses most of its antioxidants during the refining process[18]. Tree nuts are also typical Med-Diet foods. Epidemiological studies have consistently shown that frequent nut consumption decreases the risk for CHD[19]. Besides having a favourable fatty acid profile, nuts are a rich source of nutrients and other bioactive compounds that may beneficially influence the risk for CHD, such as fibre, phytosterols, folic acid and antioxidants[19]. Walnuts differ from all other nuts in their high content of PUFA, particularly α-linolenic acid, a vegetable *n*-3 fatty acid[20], which might confer additional antiatherogenic properties[21]. However, the limited capacity for conversion to longer-chain *n*-3 fatty acids, and the lack of efficacy in ameliorating CVD risk factors and inflammatory markers in man, suggests that increased consumption of α-linolenic acid may be of little benefit in altering EPA (20:5*n*-3)+DHA (22:6*n*-3) status or in improving health outcomes compared with other dietary interventions[22].

High-fat diets based on MUFA in diabetics, overweight subjects and high-risk individuals

Traditionally, nutritional advice to diabetics, obese subjects and those with cardiovascular risk factors emphasized the avoiding of animal fat and, preferably, all kinds of dietary fat, and their replacement with carbohydrates

(CHO). The rationale was that fats provided excess energy, thought to promote obesity. However, in the last two decades, scientific evidence has accumulated on the beneficial role of diets with a relatively high MUFA content on cardiovascular risk factors, obesity and diabetes. These beneficial MUFA are provided by the Med-Diet and, specifically, by olive oil and most nuts[23]. In fact, the frequent intake of simple CHO in many otherwise low-fat foods is associated with weight gain. However, when nutritional advice is given to people with obesity or diabetes, a reluctance still exists to recommend high-fat, high-MUFA diets as an alternative to the traditional (and less palatable) low-fat diets. Since obesity is the epidemic of the new century[24], it is important to recognize that there is no evidence that a higher percentage of fat in the diet in the form of MUFA results in increased body weight. The lack of a fattening effect of such MUFA-rich diets has been shown in the context of controlled diets[23], weight-reduction programmes[9] and *ad libitum* diets[25]. Similarly, in a 2-year trial, 322 obese subjects were randomized to one of these three diets: low-fat, restricted-energy; the Med-Diet, restricted-energy; or low-CHO, non-restricted-energy. The authors concluded that the Med-Diet and low-CHO diets may be effective alternatives to low-fat diets. The more favourable effects on lipids (with the low-CHO diet) and on glycemic control (with the Med-Diet) suggest that personal preferences and metabolic considerations might allow individualized tailoring of dietary interventions[26]. Thus, we have compelling evidence that the Med-Diet may be a useful tool in the dietary treatment of obese, diabetic and high-risk subjects.

Protective mechanisms of the Mediterranean diet on CVD

Adoption of healthy lifestyles, such as the Med-Diet, is critical for the prevention and treatment of conventional risk factors, such as blood pressure, serum lipids and insulin resistance[27–30]. Since classical cardiovascular risk factors explain about 50% of the cardiovascular outcomes and multiple studies have demonstrated that about 20–25% of all future events occur in individuals with only one of these factors, some studies have been focused on the analysis of the Med-Diet on other alternative mechanisms such as the anti-inflammatory effects of this diet or its main components[31].

The PREvención con DIeta MEDiterránea (PREDIMED) study is a large, parallel group, multi-centre, controlled, randomized 5-year clinical trial aimed at assessing the effects of the Med-Diet on the primary prevention of CVD[32]. Almost 7500 high-risk participants have been recruited and assigned to three interventions: Med-Diet supplemented with virgin olive oil, Med-Diet supplemented with mixed nuts, and low-fat diet. The main outcome is an aggregate of cardiovascular events (cardiovascular death, non-fatal myocardial infarction or non-fatal stroke). The anticipated completion date of the PREDIMED trial is December 2011. In the pilot study of this trial, the 3-month effects of the three interventions on cardiovascular risk factors were assessed in the first 772 high-risk participants included in the trial. Both Med-Diets were associated with significant blood pressure lowering in hypertensive individuals already on anti-hypertensive medication (mean reduction of about 5%; Table 1). Observational studies[33,34] and small feeding trials[35,36] have suggested that increased olive oil consumption helps lower blood pressure. In addition, the OmniHeart study[37] has shown that a healthy diet enriched with MUFA from various sources also has an anti-hypertensive effect. No effects on blood pressure have been reported for diets enriched with nuts in small trials[38]. However, walnuts have favourable effects on endothelial function[39]; thus it is plausible that nut intake had a blood pressure-lowering effect. A likely explanation for the blood pressure reduction observed with the two Med-Diets is the composite dietary change achieved, which was similar to that advocated in the dietary approaches to stop hypertension trial[40], with the exception of the high content of olive oil. Salt intake was not restricted in our study. The blood pressure-lowering effect of the Med-Diets was higher than that obtained by partial substitution of CHO for MUFA in the OmniHeart trial[37], comparable to that of the unrestricted sodium dietary approaches to stop hypertension diets[40] and of lesser magnitude than that of the low-sodium dietary approaches to stop hypertension diet[41].

The Med-Diet with olive oil was associated with decreased blood glucose (about 3%), and the two Med-Diets reduced fasting insulin and insulin resistance in non-diabetic participants (mean reduction of 8% in homeostasis model assessment score; Table 2), thereby extending previous observations on the effects of a Med-Diet on insulin sensitivity in subjects with the metabolic syndrome[42]. Insulin resistance and diabetes are linked to excess energy intake, particularly in the form of saturated fatty acids and simple sugars, and to increased adiposity[43]. Low-fat, high-CHO diets have traditionally been advised for medical nutrition therapy in diabetes. However, such diets may worsen metabolic control, an untoward effect that is not observed when using high-fat diets based on MUFA-rich oils or nuts[23]. Frequent nut consumption has been inversely associated with diabetes risk[44]. Additionally, decreased intake of meat and dairy products and increased fibre intake, as observed in the Med-Diet groups, have been shown, in conjunction with other beneficial lifestyle measures, to prevent the evolution of glucose intolerance to diabetes[45,46]. The results observed in our pilot study further support a beneficial role of healthy diets on insulin resistance.

It is well known that replacement of CHO with dietary fat lowers TAG and raises HDL cholesterol, while substituting MUFA for saturated fatty acids lowers LDL cholesterol[47,48]. Total fat intake was high both at baseline and after 3 months and a similar reduction in saturated fatty acids intake of approximately 1% of energy was observed in the three arms of the study. However, the lipid profile was unchanged in the low-fat diet group, while LDL cholesterol decreased (about 4%) and HDL cholesterol increased (about 6%) in the Med-Diet groups, especially when olive oil was supplemented (Table 3). While diets enriched with a variety of nuts have an established hypocholesterolaemic effect[19,28,39], it is unknown why

Table 1. Changes in physiological and cardiovascular parameters in 772 high-risk subjects included in the PREDIMED study[32]
(Mean values and standard deviations)

	Med-Diet + Virgin Olive Oil					Med-Diet + Mixed Nuts					Control Diet					
Variables	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	*P* Value*
Weight (kg)	76	13	−0·2	−0·5, 0·1	−0·3	76	12	−0·3	−0·6, 0·1	−0·4	76	12	−0·2	−0·5, 0·1	−0·4	0·96
BMI (kg/m^2)	30	4	−0·1	−2·4, 0·1	−0·4	29	4	−0·1	−2·4, 0·1	−0·3	30	4	−0·2	−0·4, −0·1	−0·7	0·56
Waist (cm)	100	12	−0·8	−1·8, 0·1	−0·4	99	10	−0·3	−0·9, 0·4	−0·2	101	11	−0·4	−1·2, 0·4	−0·2	0·52
Systolic BP (mmHg)	152	19	−4·8	−6·7, −2·9	−3	152	19	−6·5	−8·7, −4·3	−4	152	18	0·6	−1·3, 2·3	0·8	<0·001[a,b]
Diastolic BP (mmHg)	83	10	−2·5	−3·5, −1·5	−2	84	10	−3·6	−4·7, −2·5	−4	84	9	−0·8	−1·8, 0·1	−0·7	<0·01[b]

Med-Diet, Mediterranean-style diet; %, mean percent change from baseline; BP, blood pressure.
Three-month changes, including centre as a stratification factor, were analysed using a multivariate model, controlled for potential confounding by age, gender and baseline body weight. [a], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Control; [b], significant ($P<0·05$) differences between Med-Diet + Mixed Nuts and Control; [c], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Med-Diet + Mixed Nuts.

Table 2. Changes in plasma glucose and insulin concentrations in 772 high-risk subjects included in the PREDIMED study[32]
(Mean values and standard deviations)

	Med-Diet + Virgin Olive Oil					Med-Diet + Mixed Nuts					Control Diet					
Variables	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	*P* Value*
Fasting glucose (mg/dl)	118	38	−3·8	−7·4, −0·2	−3	118	34	−2·5	−5·5, 0·5	−2	125	45	3·5	−1·0, 8·0	4	<0·01[a,b]
Fasting insulin (units/ml)†	18	6	−1·4	−2·2, −0·6	−6	18	7	−1·4	−2·3, −0·5	−6	18	7	0·9	−0·5, 2·4	5	<0·01[a,b]
HOMA score†	4·2	1·9	−0·5	−0·8, −0·2	−8	4·1	1·6	−0·5	−0·8, −0·2	−9	4·2	2·7	0·3	−0·1, 0·7	7	<0·001[a,b]

Med-Diet, Mediterranean-style diet; %, Mean percent change from baseline; HOMA, homeostasis model assessment.
Three-month changes, including centre as a stratification factor, were analysed using a multivariate model, controlled for potential confounding by age and gender.
[a], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Control; [b], significant ($P<0·05$) differences between Med-Diet + Mixed Nuts and Control; [c], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Med-Diet + Mixed Nuts.

substituting virgin for refined olive oil has such beneficial lipid effects. Minor olive oil constituents that are enriched in virgin oils[18] might explain these effects and merit further study. Since low-fat diets usually lower both LDL and HDL cholesterol concentrations[49–51], a fat-rich Med-Diet may be a better nutritional option for high-risk individuals.

In conclusion, our results suggest that the salutary health effects of the Med-Diet observed in epidemiological studies are exerted in part through plausible mechanisms: improved lipid profiles and reductions in blood pressure, and insulin resistance.

Anti-inflammatory effects of the Mediterranean diet

Up to now, the beneficial effect of the Med-Diet against CVD has been attributed to its effects controlling classical atherosclerosis risk factors, but, recently, some authors have suggested that an anti-inflammatory effect in the vascular wall may be another important mechanism to explain the link between the Med-Diet and low cardiovascular mortality[42]. Indeed, atherosclerosis has long been considered the result of lipid accumulation in the artery wall, but there is currently compelling evidence that inflammation plays a key role at all stages of the disease[52]. Early phases of atherosclerosis involve the recruitment of inflammatory cells from the circulation, their adhesion to endothelium and finally migration to subendothelial space, a complex process mediated by inflammatory stimuli, which involves cytokine production and up-regulation of adhesion molecules on endothelial cells and leucocytes[53]. Ongoing inflammation is also crucial in the development of instability and rupture of atheromatous plaques and the subsequent appearance of ischaemic events in advanced stages of the disease[52,53].

The important role of inflammation in the pathogenesis of atherosclerosis has led to the belief that dietary preventive measures act in part by modifying related inflammatory pathways[31]. Indeed, results from cross-sectional studies[54,55] and a previous feeding trial[42] in Mediterranean populations suggest that the Med-Diet has anti-inflammatory effects, as was also ascertained in the US population[56].

In the pilot study of the PREDIMED trial[32], we also analysed the effects of the three mentioned interventions on soluble adhesion molecules and cytokines related to atherosclerosis. Recent epidemiological and clinical studies have shown that the Med-Diet or its main components are associated with a lower inflammatory status and/or improved endothelial function[10–12,39,52]. Similar findings have recently been reported for other healthy dietary patterns[53]. Our findings of reduced circulating levels of cell adhesion molecules support the anti-inflammatory effects of Med-Diets.

In another sub-study of the PREDIMED trial[57], we analysed the effects of the three mentioned interventions on immune cell activation and soluble inflammatory biomarkers related to atherogenesis in 106 subjects at high risk for CVD. Changes from baseline in cellular and serum inflammatory biomarkers were assessed (Table 4). At 3 months, monocyte expression of CD49d, an adhesion

Table 3. Changes in plasma lipid parameters in 772 high-risk subjects included in the PREDIMED study (Modified from Estruch *et al.*[32])
(Mean values and standard deviations)

	Med-Diet + Virgin Olive Oil					Med-Diet + Mixed Nuts					Control Diet					
Variables	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	Baseline	SD	Mean change	95% CI	%	*P* Value*
Cholesterol (mg/dl)	220	39	−3·9	−8·1, 0·4	−1	215	38	−5·0	−8·6, −1·4	−2	219	40	0·7	−3·8, 5·3	0·3	0·04[b]
LDL cholesterol (mg/dl)	147	35	−5·8	−9·8, −1·8	−4	141	35	−3·8	−7·3, −0·4	−3	142	33	−0·6	−4·6, 3·5	−0·4	0·22
HDL cholesterol (mg/dl)	45	10	2·4	1·6, 3·1	6	46	10	0·9	0·1, 1·7	3	47	10	−0·4	−1·2, 0·4	−0·1	<0·001[a,b,c]
TAG (mg/dl)	138	64	−3·0	−11·8, 5·9	−2	138	62	−7·6	−14·2, −1·1	−5	149	69	2·4	−4·4, 9·1	1	0·03[b]
Apo AI (mg/dl)	134	21	2·8	1·0, 4·6	2	134	24	0·2	−1·6, 2·0	1	135	25	−0·9	−2·8, 0·9	−0·6	0·02[a]
Apo B (mg/dl)	102	20	−2·8	−5·1, 0·5	−2	101	21	−1·7	−3·5, 0·2	−1	101	21	0·0	−2·1, 2·1	0·0	0·12
Cholesterol : HDL	5·0	1·2	−0·3	−0·4, −0·2	−5	4·9	1·1	−0·2	−0·3, −0·1	−3	4·9	1·2	0·0	−0·1, 0·1	1	<0·001[a,b]
LDL : HDL	3·4	1·0	−0·3	−0·4, −0·2	−7	3·2	0·9	−0·1	−0·2, 0·0	−2	3·2	0·8	0·0	−0·1, 0·1	0·9	<0·001[a]

Med-Diet, Mediterranean-style diet; %, Mean percent change from baseline.
Three-month changes, including centre as a stratification factor, were analysed using a multivariate model, controlled for potential confounding by age and gender.
[a], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Control; [b], significant ($P<0·05$) differences between Med-Diet + Mixed Nuts and Control; [c], significant ($P<0·05$) differences between Med-Diet + Virgin Olive Oil and Med-Diet + Mixed Nuts.

Table 4. Changes in peripheral blood mononuclear cells expression of cell surface inflammatory mediators (adhesion molecules and CD40) after 3 months of intervention (PREDIMED Study)[57]
(Mean values and standard deviations)

	Med-Diet + Olive oil				Med-Diet + Nuts				Low-fat diet			
	Baseline	SD	3-month	SD	Baseline	SD	3-month	SD	Baseline	SD	3-month	SD
T-lymphocytes (mean fluorescence intensity)												
CD-11a	99·8	4·8	99·5	4·6	99·1	4·1	99·5	4·3	99·2	3·2	98·0	5·5
CD49d	77·3	4·5	70·2*	3·8	77·3	4·4	70·2*	4·5	75·6	5·8	73·1	5·2
Monocytes (mean fluorescence intensity)												
CD11a	98·7	6·0	98·2	5·6	99·8	2·4	98·1	6·6	97·6	4·9	95·6	5·4
CD11b	94·4	3·3	90·1*	3·8	97·2	5·5	89·4*	5·5	92·2	8·2	89·7	5·5
CD49d	81·4	5·4	70·0*	5·6	82·5	5·5	51·5*	6·6	82·1	7·8	80·2	7·3
CD40	80·9	9·5	56·9*	9·1	70·6	9·7	34·7*	9·4	75·2	9·4	80·3	9·4

Med-Diet: Mediterranean diet. *$P<0·05$ compared to baseline.
Significant interactions between diets were analysed by the simple effects test with multiple contrasts of Bonferroni. Med-Diets supplemented with olive oil and nuts down-regulate cellular (inflammatory biomarkers related to atherogenesis: CD49d molecules in peripheral lymphocyte and CD11b, CD49d and CD40 in monocytes.

molecule crucial for leucocyte homing, and of CD40, a pro-inflammatory ligand, decreased ($P<0·05$) after both Med-Diets but not after the low-fat diet. Serum IL-6 and soluble intercellular adhesion molecule-1 decreased in both Med-Diet groups ($P<0·05$). Soluble vascular cellular adhesion molecule-1 and C-reactive protein decreased only after the Med-Diet with virgin olive oil ($P<0·05$), whereas IL-6, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1 increased ($P<0·05$) after the low-fat diet. The effects of the Med-Diet on adhesion molecule expression on circulating peripheral blood mononuclear cells, a crucial step for their firm adhesion to endothelial cells during the inflammatory wall reaction, linked to atherosclerosis development[52,53] has not been previously investigated.

Experimental and clinical studies have shown that olive oil down-regulates vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin expression in the endothelium[58] and decreases the plasma levels of soluble intercellular adhesion molecule-1, soluble E-selectin, IL-6 and high-sensitive C-reactive protein in high-risk patients[59–61]. Another diet was with mixed nuts, a classical Mediterranean food rich in antioxidants, with walnuts as the major component. A frequent intake of nuts has been associated with decreased levels of IL-6, C-reactive protein and fibrinogen in a population free of CVD[62], while a Med-Diet supplemented with walnuts decreased the levels of soluble vascular cell adhesion molecule-1 in hypercholesterolaemic and healthy subjects[63].

The recruitment and adhesion of peripheral blood mononuclear cells to the endothelium is an early event in fatty streak formation in which adhesion molecules and IL have a key role, but thereafter resident macrophages and lymphocytes become activated and secrete abundant amounts of cytokines that in turn can activate other cell types leading to a self-perpetuating inflammatory process in the vascular wall that is instrumental in more advanced stages of the disease: plaque formation, vulnerability and rupture leading to thrombosis and acute ischaemic episodes[52,53]. Inhibition of both cell-mediated and humoral inflammatory pathways, as shown in our study, provides a molecular mechanism for an anti-atherosclerotic effect of the Med-Diet. Interestingly, these changes were opposite to those observed in our study after the recommended low-fat diet, which, given the little changes in nutrients from baseline, was essentially a control diet, similar to the Med-Diets except for the olive oil and nut supplements. Noticeably, the beneficial effects of the Med-Diet on inflammatory markers were essentially similar in subjects under stable treatment with risk-reducing agents that have demonstrable anti-inflammatory properties, namely angiotensin-converting enzyme inhibitors and statins, and in those not treated with these drugs[63]. Thus, the anti-inflammatory effect of the Med-Diet appears to be complementary to that of pharmacological treatment. Moreover, the benefit of dietary change occurred in older individuals with a sizeable burden of risk factors for CVD. This suggests that the potential anti-atherosclerotic effect of healthy foods is not limited to early disease.

In conclusion, Med-Diet supplemented with olive oil or nuts reduce the potency of cardiovascular risk factors and down-regulate cellular and humoral inflammatory pathways related to atherosclerosis. The fact that these beneficial effects are observed in older subjects at high cardiovascular risk suggests that it is never too late to change dietary habits for improving health status. These results support the recommendation of the Med-Diet as a useful tool against CVD in all stages of the disease.

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