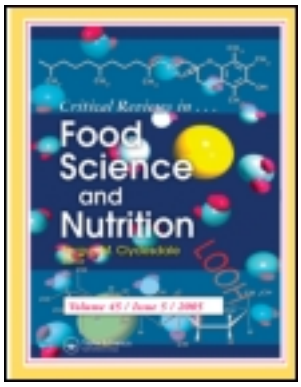


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# Mediterranean Diet and Cardiovascular Risk Factors: A Systematic Review

Giuseppe Grosso [a], Antonio Mistretta [a], Alessandro Frigiola [b], Salvatore Gruttadauria [c], Antonio Biondi [d], Francesco Basile [d], Paola Vitaglione [e], Nicolantonio D'Orazio [f] & Fabio Galvano [g]

[a] Department "G. F. Ingrassia," Section of Hygiene and Public Health, University of Catania, Catania, Italy

[b] Department of Cardiac Surgery, IRCSS Policlinico, S. Donato Milanese, Milan, Italy

[c] Istituto Mediterraneo Trapianti e Terapie ad Alta Specializzazione, University of Pittsburgh Medical Center in Italy, Palermo, Italy

[d] Section of General Surgery and Oncology, Department of General Surgery, University of Catania, Catania, Italy

[e] Department of Food Science, University of Naples, Naples, Italy

[f] Section of Human Nutrition, Department of Biochemical Science, University of Chieti, Chieti, Italy

[g] Section of Biochemistry, Department of Drug Sciences, University of Catania, Catania, Italy

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# Mediterranean Diet and Cardiovascular Risk Factors: A Systematic Review

**GIUSEPPE GROSSO,[1] ANTONIO MISTRETTA,[1] ALESSANDRO FRIGIOLA,[2] SALVATORE GRUTTADAURIA,[3] ANTONIO BIONDI,[4] FRANCESCO BASILE,[4] PAOLA VITAGLIONE,[5] NICOLANTONIO D'ORAZIO,[6] and FABIO GALVANO[7]**

[1]Department "G. F. Ingrassia," Section of Hygiene and Public Health, University of Catania, Catania, Italy
[2]Department of Cardiac Surgery, IRCSS Policlinico, S. Donato Milanese, Milan, Italy
[3]Istituto Mediterraneo Trapianti e Terapie ad Alta Specializzazione, University of Pittsburgh Medical Center in Italy, Palermo, Italy
[4]Section of General Surgery and Oncology, Department of General Surgery, University of Catania, Catania, Italy
[5]Department of Food Science, University of Naples, Naples, Italy
[6]Section of Human Nutrition, Department of Biochemical Science, University of Chieti, Chieti, Italy
[7]Section of Biochemistry, Department of Drug Sciences, University of Catania, Catania, Italy

*The aim of this paper was to systematically review and analyze the epidemiological evidence on the role of Mediterranean diet (MD) in the prevention of cardiovascular diseases (CVD). Fifty-eight studies exploring the relation between MD and lipoprotein concentration, antioxidative capacity and inflammatory markers, hypertension, obesity, diabetes, and metabolic syndrome, were identified and selected. These included 33 cross-sectional, 9 cohort, and 16 intervention studies. Most of the studies showed favorable effects of MD on CVD, although a certain degree of controversy remains in the respect of some issues, as obesity. Important methodological differences and limitations in the studies make difficult to compare results, thus further studies, particularly randomized clinical trials, are needed to finally substantiate the benefits of MD and to shed some lights on mechanisms.*

**Keywords** Dietary pattern, prevention, nutrition



## *1. INTRODUCTION*

Cardiovascular diseases (CVD) are the leading cause of mortality (Murray and Lopez, 1996). A clustering of other pathologies (namely, obesity, type 2 diabetes, hyperlipidemia, and hypertension) are related to metabolic syndrome (MS) (Grundy et al., 2004; Alberti et al., 2006; Tong et al., 2007), and results in a higher risk of CVD occurrence and mortality incidence (Reaven, 1997; Oda, 2008; Bertoni et al., 2007; Noto et al., 2008). Several studies have clearly shown that the epidemiology of such chronic conditions, as well as mortality, widely differs among populations due to genetic and environmental factors. In fact, although genetic polymorphism can play a determinant role in increasing cardiovascular risk (Trichopoulou et al., 2008), some environmental factors such as lifestyle and diet are also involved in the genesis of CVD. Thus, during the last 50 years, a great effort was made to examine the relationship between health and dietary models. Most of the studies assessed the role of different dietary habits in modulating the risk of pathologies: the older ones focused on the effects of single nutrients or foods (Jacobs et al., 1998; Joshipura et al., 2001; Hu et al., 2003), the newer ones examined the contemporary consumption of different foods containing multiple nutrients and nonnutrient compounds (Togo et al., 2001; Kant, 2010) and the role of dietary diversity (Ruel, 2003). The leading concept is that free-living individuals consume complex diets, thus whole dietary patterns play a most important role than specific dietary components in modulating survival (Trichopoulou et al., 2003) and incidence of diseases (Heidemann et al., 2005; Sofi et al., 2008).

On 1986, Ancel Keys observed lower incidence rates of CVD, certain types of cancer and other nutrition-related

Address correspondence to Dr Giuseppe Grosso, Department "G. F. Ingrassia," Section of Hygiene and Public Health, University of Catania, Via Santa Sofia 87, Catania, 95123, Italy. E-mail: giuseppe.grosso@studium.unict.it

diseases in some Mediterranean countries than in other countries, and hypothesized that it was mainly due to dietary habits of those populations (Keys et al., 1986). Since the 90s, food patterns typical of Mediterranean countries were deeply investigated (Helsing and Trichopoulou, 1989; Serra-Majem and Helsing, 1993; Keys, 1995; Nestle, 1995; Willett et al., 1995). On the other hand, although the Mediterranean diet (MD) observed by Keys was generally based on a high consumption of plant foods (such as fruits, vegetables, legumes, cereals, and nuts), on olive oil as the main source of dietary lipids, and on a low consumption of meat and dairy products (mainly cheese and yoghurt), different foods and whole fat content have been found to be consumed in the different regions of the Mediterranean basin. Thus, various indexes and scores have been developed to assess adherence to MD pattern and to link such patterns with several nutrient-related diseases (Trichopoulou et al., 2003; Bach et al., 2006; Panagiotakos et al., 2006b). Using these tools, it was found that a high adherence to MD positively correlates with longevity and delays the onset of some pathologies, even in non-Mediterranean countries (Kouris-Blazos et al., 1999; Haveman-Nies et al., 2003).

In several epidemiological studies, the benefits of MD were mainly related to reduced cardiovascular risk factors (diabetes, hypertension, dyslipidemia, and obesity). However, there is still a certain controversy about some of these associations.

As a general scientific consensus exists on the ability of MD patterns to prevent certain types of cancer, nutritionists agree in extensively recommending MD. However, the consensus mainly derives from observational studies (ecological, cross-sectional, and case-control studies) that only provide association between risk factors and clinical outcomes. Experimental studies (randomized controlled trials) are needed to assess significant causal relationships.

In this framework, the objective of the present review was to explore epidemiological evidence on MD and health-related nutritional biomarkers. Evidence from more recent and rigorous studies focused on MD dietary pattern and CVD were considered.

## 2. MATERIALS AND METHODS

The Pubmed database of the United States National Library of Medicine was used to identify articles focused on MD and incidence/risk of CVD. The search included serum lipids, body mass index (BMI), body weight, blood pressure, fasting plasma glucose, markers of inflammation, insulin resistance, HbA1c, and adiponectin levels as the main biochemical and clinical markers identifying the risk factors of CVD. The keywords used were "Mediterranean diet" alone and in combination with all the biomarkers mentioned above and also with "cardiovascular disease," "cardiovascular risk factors," "diabetes," "metabolic syndrome," "obesity," "hypertension," "body mass index," "intervention program," and "prevention." Studies were considered eligible when examining the effects of the whole MD, while those studies regarding the effects of a single food typical of MD were excluded. The search was restricted to observational studies with a minimum sample size of 500 subjects and intervention studies with a minimum sample of 100 subjects, published from 2001 to August 2010. The literature search was narrowed to articles published in English without any restriction on the availability of full text or only abstract. Additional publications were identified from references provided in the original papers.

## 3. RESULTS

A total of 58 studies meeting all the search criteria were identified. The papers were classified according to study type and resulted in 33 cross-sectional (Scali et al., 2001; Chrysohoou et al., 2004; Panagiotakos et al., 2004, 2005a, 2006a, 2006b, 2007, 2008, 2009; Psaltopoulou et al., 2004; Schroder et al., 2004; Fung et al., 2005; Pitsavos et al., 2005, 2007; Shubair et al., 2005; Trichopoulou et al., 2005; Alvarez Leon et al., 2006 Mantzoros et al., 2006; Thanopoulou et al., 2006; Mozaffarian et al., 2007; Tzima et al., 2007; 2008; Masala et al., 2008; Rossi et al., 2008; Salas-Salvado et al., 2008b; Sanchez-Tainta et al., 2008; Babio et al., 2009; Esposito et al., 2009; Romaguera et al., 2009; Tyrovolas et al., 2009; Yannakoulia et al., 2009; Carter et al., 2010; Fragopoulou et al., 2010), 8 cohort (Romaguera et al., 2010; Rumawas et al., 2009; Nunez-Cordoba et al., 2009; Martinez-Gonzalez et al., 2008; Woo et al., 2008; Tortosa et al., 2007; Sanchez-Villegas et al., 2006; Mendez et al., 2006), and 16 intervention studies (Singh et al., 2002; Esposito et al., 2003, 2004; Sondergaard et al., 2003; Toobert et al., 2003; Bautista-Castano et al., 2004; Flynn and Colquhoun 2004; Vincent-Baudry et al., 2005; Estruch et al., 2006; Michalsen et al., 2006; Fito et al., 2007; Salas-Salvado et al., 2008a; Shai et al., 2008; Mena et al., 2009; Razquin et al., 2009; Elhayany et al., 2010) one of which was without a control group (Bautista-Castano et al., 2004). Information regarding the methodology, outcomes and results are summarized in Table 1.

### 3.1. Characteristics of Study Sample

Forty-three studies were carried out in the Mediterranean countries (Greece, Spain, Italy, and Cyprus) and 17 were from non-Mediterranean countries (USA, France, Germany, UK, China, Israel, Denmark, Australia, India, and Canada). The health status of the subjects varied among the studies depending on the primary objectives. Both observational and intervention studies involved healthy people to examine the role of MD in primary prevention as well as individuals with chronic or acute disease to assess its role in secondary prevention.

**Table 1** Epidemiological studies on the MD and cardiovascular risk factors

| Country | Type of study | Sample characteristic | Methodology | Outcome | Follow-up | Results | Reference |
|---|---|---|---|---|---|---|---|
| | | | | Observational studies $n = 44$ | | | |
| Greece | Cross-sectional | 532 subjects without any clinical evidence of CVD | MD score | Adiponectin | – | Compared to the highest tertile of diet score, participants in the middle or the lowest one had an average $0.99 \pm 0.22$ ($P = .001$) and $1.05 \pm 0.27$ microg/mL ($P = .001$) lower adiponectin levels | Fragopoulou et al., 2010 |
| UK | Cohort | 373,803 subjects (103,455 M and 270,348 F; 25–70 years) | MD score | BMI, obesity incidence | Five years | Individuals with a high adherence to the MD showed a 5-year weight change of −0.16 kg (95% CI: −0.24, −0.07 kg) and were 10% (95% CI: 4%, 18%) less likely to develop overweight or obesity than were individuals with a low adherence to the MD | Romaguera et al., 2010 |
| USA | Cross-sectional | 13,197 subjects (18–90 years) | FFQ + MD score + 24-hour recall | HOMA index, lipids profile, CRP, insulin, HbA1c, fibrinogen, APO B, Hcy | – | For men <45 years of age as MD score increased: TC/HDL-c ratio ($P = .0390$), serum insulin ($P = .0414$), and WBC ($P = .0246$) decreased. For men ≥ 45 years as MD score increased: TC/HDL ratio ($P = .0008$), HbA1c ($P = 0.0001$), HOMA index ($P = 0.0486$), CRP ($P = .0034$), fibrinogen ($P = 0.0028$) decreased and HDL-c levels ($P < .0001$) increased. For premenopausal F, as MD score increased: TC/HDL ratio ($P < .0001$), non-HDL cholesterol ($P = .0012$), APO B ($P = .0112$), HgbA1c ($P = .0001$), decreased and HDL-c levels ($P < .0001$) increased. For postmenopausal F, as MD score increased: TC/HDL ratio ($P = .0005$), TG ($P < .0001$), serum insulin ($P = .0062$), HOMA index ($P = .0063$) and Hcy (.0046) levels decreased and HDL-c levels ($P = .0005$) increased. | Carter et al., 2010 |
| Greece | Prospective cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | Obesity incidence | Five years | No association was detected between adherence to a MD and incidence of obesity in initially normal-weight individuals | Yannakoulia et al., 2009 |
| Greece | Cross-sectional | 1003 MI survivors | FFQ + MD score | CRP, IL-6, fibrinogen | – | For each unit of increasing adherence to the MD score there was a reduction of 3.1% in the average CRP levels (95% CI 0.5–5.7%) and of 1.9% in the average IL-6 levels (95% CI 0.5–3.4%). No significant association was observed between the diet score and fibrinogen levels. | Panagiotakos et al., 2009 |
| Spain | Cross-sectional | 808 subjects at high cardiovascular risk | MD score | MS prevalence | – | Participants with the highest score of adherence to the MD had the lowest OR of having MS (OR [95% CI] of 0.44 [0.27–0.70]) and 47 and 54% lower odds of having low HDL-c and high TG criteria | Babio et al., 2009 |

*(Continued on next page)*

**Table 1** Epidemiological studies on the MD and cardiovascular risk factors *(Continued)*

| Country | Type of study | Sample characteristic | Methodology | Outcome | Follow-up | Results | Reference |
|---|---|---|---|---|---|---|---|
| USA | Cohort | I cohort: 2730 subjects (median age: 54 years; 55% F) II cohort: 1918 subjects free of the condition at baseline. | MD score | I cohort: HOMA-insulin resistance, fasting glucose, WC, TG, HDL-c II cohort: MS incidence | Seven years | A higher MD score was associated with lower HOMA-insulin resistance ($P = .02$), WC ($P < .001$), fasting plasma glucose ($P = .03$), and TG ($P < .001$) and higher HDL-c ($P = .02$). Participants in the highest quintile category of the MD score had a lower incidence of MS than those in the lowest quintile category (38.5% compared with 30.1%; $P = .01$). | Rumawas et al., 2009 |
| Italy | Cross-sectional | 901 outpatients with T2DM | MD score | HbA1c, pre- and postprandial glucose | – | Mean HbA1c and 2-hour postmeal glucose concentrations were significantly lower in diabetic patients with high adherence to a MD than those with low adherence [difference: HbA1c 0.9%, 95% CIs (CI) 0.5–1.2%, $P < .001$; 2-hour glucose 2.2 mmol/L, 95% CI 0.8–2.9 mmol/L, $P < .001$] | Esposito et al., 2009 |
| Spain | Cohort | 9,408 university graduates without any clinical evidence of CVD or diabetes | FFQ + MD score | BP | 4.2 years | Adherence to the MD was not associated with hypertension (the HR was 1.10 (95% CI: 0.81, 1.41) for moderate adherence and 1.12 (95% CI: 0.79, 1.60) for high adherence) | Nunez-Cordoba et al., 2009 |
| Greece | Cross-sectional | 1,190 subjects (553 M, 637 F; 74 ± 7 years) | MD score | Obesity prevalence | – | One unit increase in the MD score was associated with 88% ($P = .07$) lower likelihood of being obese | Tyrovolas et al., 2009 |
| UK | Cross-sectional | 497,308 subjects (70.7% F, 25–70 years) | MD score | BMI, WC | – | MD was not significantly associated with BMI. Higher adherence to the MD was significantly associated with lower WC, for a given BMI, in both men (−0.09; 95% CI −0.14 to −0.04) and F (−0.06; 95% CI −0.10 to −0.01). The association was stronger in men (−0.20; 95% CI −0.23 to −0.17) and F (−0.17; 95% CI −0.21 to −0.13) | Romaguera et al., 2009 |
| Greece | Prospective cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | CVD incidence | Five years | Greater adherence to the MD (among 35–65-year-old individuals) were associated with a lower CVD incidence (OR per 1/55 units in diet score = 0.94, $P < .001$) | Panagiotakos et al., 2008 |
| Greece | Prospective cross-sectional | 1,188 subjects, free of CVD, but with defined high BP levels (prehypertension) | MD score | BP | Five years | Greater adherence to MD seems to protect only prehypertensive, with abdominal obesity patients prone to develop hypertension (OR = 0.94, 95% CI 0.90–0.98) | Pitsavos et al., 2008 |
| Spain | Cross-sectional | 772 subjects (339 M, 433 F; 55–80 years) at high cardiovascular risk | FFQ | CRP, IL-6, sICAM-1, sVCAM-1 | – | Participants with higher adherence to the MD did not show significantly lower concentrations of inflammatory markers ($P < .1$ for VCAM-1 and ICAM-1) | Salas-Salvado et al., 2008a, 2008b |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Spain | Cohort | 13,380 university graduates without any clinical evidence of CVD or diabetes | FFQ + MD score | Diabetes incidence | 4.4 years | The incidence rate ratios risk to develop diabetes were 0.41 (95% CI 0.19–0.87) for those with moderate adherence and 0.17 (0.04–0.75) for those with the highest adherence compared with those with low adherence. A two point increase in the score was associated with a 35% relative reduction in the risk of diabetes (incidence rate ratio 0.65, 0.44–0.95), with a significant inverse linear trend ($P = .04$) | Martinez-Gonzalez et al., 2008 |
| Spain | Cross-sectional | 3,204 | MD score | MS criteria | – | The OR to present simultaneously the four risk factors for those above the median value of the MD score was 0.67 (95% CI: 0.53–0.85). The OR for successive categories of adherence to MD were 1 (ref.), 1.03, 0.85, 0.70, and 0.54 ($P$ for trend $<.001$). | Sanchez-Tainta et al., 2008 |
| Italy | Cross-sectional | 6,619 subjects (3,090 M, 3,529 F) admitted to hospital for a wide spectrum of acute conditions | FFQ + MD score | BMI, WHR | – | MD was not related to BMI (beta = 0.05 for men and −0.04 for F) or WHR (beta = 0.000 and 0.001, respectively) | Rossi et al., 2008 |
| China | Cohort | 1,010 subjects | MD score | Obesity incidence | Five to nine years | Inconsistent association between MD and incidence of obesity and overweight | Woo et al., 2008 |
| Italy | Cross-sectional | 10,083 F (35–64 years) | FFQ | BP | – | A high consumption of selected foods resulted inversely associated with systolic (total vegetables, yoghurt, and eggs), diastolic (olive oil) or both systolic and diastolic values (leafy vegetables, milk, coffee) | Masala et al., 2008 |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ | MS criteria | – | Consumption of cereals, fish, legumes, vegetables, and fruits was inversely associated with WC, systolic BP, TG, positively associated with HDL-c levels, and inversely with the likelihood of the MS | Panagiotakos et al., 2007 |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | Fasting glucose, insulin | – | MD adherence was higher in normoglycemic than in IFG subjects, and in IFG than diabetic subjects ($P < .001$). In normoglycemic subjects who were in the upper tertile of the diet score we observed 7% lower glucose ($P < .05$), 5% lower insulin ($P < .05$) and 15% lower HOMA-IR ($P < .01$) levels compared to subjects in the lower tertile of the diet score. No associations between MD and IFG/diabetic subjects | Panagiotakos et al., 2007 |
| Greece | Cross-sectional | 1,762 subjects (1,064 M, 698 F; 20–89 years) overweight and obese | MD score | Insulin, lipids profile, BP, HOMA | – | Individuals in the highest tertile of diet score, were more insulin sensitive than those in the lowest tertile (11.4% lower HOMA, $P = .06$), had 13% lower levels of TC ($P = .001$) and 3 mmHg decrease of systolic BP levels ($P < .001$). After adjusting for confounders, these variables were only modestly correlated with MD | Tzima et al., 2007 |
| Greece | Cross-sectional | 1,337 subjects (625 M, 712 F; 18–89 years) with abdominal obesity | MD score | CRP | – | MD in combination with medium physical activity seems to reduce the likelihood of having high CRP levels by 72% ($P = .018$) | Pitsavos et al., 2007 |
| Italy | Prospective cross-sectional | 8291 patients with a MI within the previous three months, free of diabetes | MD score | Diabetes prevalence | 3.2 years | A lower MD score was a risk factor to develop diabetes or impaired fasting glucose | Mozaffarian et al., 2007 |

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**Table 1** Epidemiological studies on the MD and cardiovascular risk factors *(Continued)*

| Country | Type of study | Sample characteristic | Methodology | Outcome | Follow-up | Results | Reference |
|---|---|---|---|---|---|---|---|
| Spain | Cohort | 2,563 university graduates without any clinical evidence of CVD or diabetes | FFQ + MD score | MS incidence | Six years | Subjects with the highest adherence to the MD had lower cumulative incidence of the MS than those with the lowest adherence. HDL levels were marginally significantly higher and only WC levels were significantly lower among participants who better adhered to the MD. | Tortosa et al., 2007 |
| Spain | Cross-sectional | 578 subjects (249 M, 329 F; >18 years) | FFQ | MS criteria | – | MD adherence was not related to MS prevalence, but subjects in the third tertile of adherence presented 70% lower prevalence of the BP criteria and 2.5 times more prevalence of the glycemia criteria with respect to the first tertile | Alvarez Leon et al., 2006 |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | Obesity prevalence, BMI | – | An inverse relation was observed between diet score, WHR ($r = -0.31$, $P < .001$), and BMI ($r = -0.4$, $P < .001$). Greater adherence to the MD (i.e., highest tertile) was associated with a 51% lower odds of being obese (OR 0.49, 95% CI: 0.42–0.56) and a 59% lower odds of having central obesity (OR 0.41, 95% CI: 0.35–0.47) compared with a non-MD (i.e., lowest tertile) | Panagiotakos et al., 2006a |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | Anthropometric markers | | A significant inverse association between the MD score and systolic BP ($B$ $-5.1 \pm 0.11$, $P < 0.001$), C reactive protein ($B$ $-0.27 \pm 0.19$, $P < 0.001$), fibrinogen ($B$ $-13.5 \pm 9.15$, $P = 0.02$), total antioxidant capacity ($B$ $1.55 \pm 0.7$, $P < 0.001$), total serum cholesterol ($1.2 \pm 0.12$, $P < 0.001$), BMI ($B - 4.1 \pm 0.25$, $P < 0.001$) was found | |
| USA | Prospective cross-sectional | 987 diabetic F with no history of CVD | MD score | Adiponectin | 10 years | Median plasma adiponectin concentrations were 23% higher in F who most closely followed a Mediterranean-type diet than in low | Mantzoros et al., 2006 |
| Spain | Cohort | 6,319 university graduates without any clinical evidence of CVD or diabetes | FFQ + MD score | BW | 2.4 years | Inverse associations between higher adherence to MD and weight loss did not remain statistically significant after adjusting for relevant confounders | Sanchez-Villegas et al., 2006 |
| Greece | Cross-sectional | 1,833 subjects (916 M, 917 F; 20–74 years) | FFQ | MS criteria | – | The MS is not related to the Mediterranean type of diet | Thanopoulou et al., 2006 |
| Spain | Cohort | 17,827 subjects (10,589 M, 7,238 F; 29–65 years) | FFQ (24-hour recall) + MD score | BMI, obesity incidence | Three years | Among initially overweight subjects, high MD adherence was associated with significantly lower likelihood of becoming obese among overweight subjects (OR 0.69, 0.54–0.89 in F and (0.68, 0.53–0.89 in men). MD adherence was not associated with incidence of overweight in initially normal-weight subjects. | Mendez et al., 2006 |
| USA | Cross-sectional | 690 F (43–69 years) without any clinical evidence of CVD or diabetes | FFQ + MD score | BMI, CRP, IL-6, E-selectin, sICAM-1, sVCAM-1 | – | CRP concentrations were 24% ($P < .05$) lower in the top than in the bottom quintile of the MD index. Higher adherence in MD was associated with reduction in CRP, I-6, E-selectin | Fung et al., 2005 |
| Canada | Cross-sectional | 759 subjects (18–65 years) | FFQ | BMI | – | The MD score was inversely related to BMI ($P = .027$). A higher MD score predicted a lower BMI in the 40–49 year age group | Shubair et al., 2005 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Greece | Prospective cross-sectional | 20,343 subjects (8,685 M, 11 658 F; 50 ± 12 years) free of hypertension | MD score | BP | Five years | Adherence to the MD is inversely associated with arterial BP | Psaltopoulou et al., 2004 |
| Greece | Prospective cross-sectional | 23,597 subjects | FFQ (24-hour recall) + MD score | BMI, WHR | Five years | Adherence to the MD was unrelated to BMI in both sexes and was weakly related to WHR only in F | Trichopoulou et al., 2005 |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 years) without any clinical evidence of CVD | FFQ + MD score | Diabetes prevalence | – | A 10-unit increase in the diet score was associated with 21% lower odds of diabetes ($P < .05$) | Panagiotakos et al., 2005a |
| Greece | Cross-sectional | 3,042 subjects (1,514 M, 1,528 F; 18–89 y) without any clinical evidence of CVD | FFQ + MD score | TAC, oxidized LDL-c | – | The participants in the highest tertile of the diet score had 11% higher TAC levels and 19% lower oxidized LDL-c concentrations than did the participants in the lowest tertile ($P < 0.01$). | Pitsavos et al., 2005 |
| Greece | Cross-sectional | 1,514 men (18–87 years) and 1,528 F (18–89 years) without any clinical evidence of CVD | FFQ + MD score | CRP, WBC counts, IL-6, TNF-alpha, amyloid A, fibrinogen, homocysteine | – | Participants who were in the highest tertile of the diet score had 20% lower CRP levels ($P = .015$), 17% lower IL-6 levels ($P = .025$), 15% lower homocysteine levels ($P = .031$), 14% lower WBC counts ($P = .001$), and 6% lower fibrinogen levels ($P = .025$), as compared with those in the lowest tertile. Borderline associations were found regarding TNF-alpha ($P = .076$) and amyloid A levels ($P = .19$) | Chrysohoou et al., 2004 |
| Greece | Cross-sectional | 2,282 subjects (1,128 M, 1,154 F; 18–89 years) without any clinical evidence of CVD | MD score | MS prevalence | – | The OR of having the MS when the participant consumed the MD was 0.81 (95% CI: 0.68–0.976) | Panagiotakos et al., 2004 |
| Spain | Cross-sectional | 3,162 subjects (1547 M, 1615 F; 25–74 years) | FFQ + MD score | BMI, obesity prevalence | – | An increase of 5 U in the dietary score was associated with a change in the BMI of 0.43 ($P = .030$) and 0.68 ($P = .007$) in men and F, respectively. The obesity risk decreased in men ($P = .010$) and F ($P = .013$) with increasing adherence to the traditional MD pattern. The population in the top tertile of this score were less likely to be obese in both genders [OR (OR) and (95% CI): 0.61 (0.40–0.92) in men; 0.61 (0.40–0.93) in F] | Schroder et al., 2004 |
| France | Cross-sectional | 964 subjects (473 M, 491 F) | MD score | BMI | – | F with a poor MD tended to be obese | Scali et al., 2001 |
| | | | | Intervention studies $n = 16$ | | | |
| Israel | Randomized controlled trial | 259 overweight diabetic patients | Low-carbohydrate MD group versus traditional MD group versus the 2003 ADA diet group | Lipids profile, HbA1c | One year | The reduction in HbA1c was significantly greater in the low-carbohydrate MD group than in the ADA diet group (−2.0 and −1.6%, respectively, $P <$ .022). HDL-c increased (0.1 mmol/L ± 0.02) only on the low-carbohydrate MD group ($P < .002$). The reduction in serum TG was greater in the low-carbohydrate MD group (−1.3 mmol/L) and traditional MD group (−1.5 mmol/L) than in the ADA group (−0.7 mmol/L), $P = 0.001$. | Elhayany et al., 2010 |

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**Table 1** Epidemiological studies on the MD and cardiovascular risk factors *(Continued)*

| Country | Type of study | Sample characteristic | Methodology | Outcome | Follow-up | Results | Reference |
|---|---|---|---|---|---|---|---|
| Spain | Randomized controlled trial | 106 older subjects with diabetes or ≥ 3 CVD risk factors (43% F; average age 68 years) | Low-fat diet ($n = 257$) control group versus MD + virgin olive oil VOO group versus MD + nuts group | CD49d, CD40, IL-6, sICAM-1, sVCAM-1, and CRP | Three months | Monocyte expression of CD49d and of CD40 decreased ($P < .05$) after both MDs but not after the low-fat diet. Serum IL-6 and sICAM-1 decreased ($P < .05$) in both MD groups. sVCAM-1 and CRP decreased only after the MD with VOO ($P < .05$), whereas IL-6, sICAM-1, and sVCAM-1 increased ($P < .05$) after the low-fat diet. | Mena et al., 2009 |
| Spain | Randomized controlled trial | 187 subjects at high cardiovascular risk | Low-fat diet control group versus MD + virgin olive oil VOO group versus MD + nuts group | TAC, BW | Three years | Plasma TAC in the control, olive oil and nuts groups was 2.01 ± 0.15, 3.51 ± 0.14, and 3.02+/-0.14 mM Trolox ($P < .001$). Higher levels of TAC were significantly associated with a reduction in BW after three years of intervention among subjects allocated to the MD with VOO group ($B = -1.306$; 95% CI = −2.439 to −0.173; $P = .025$) | Razquin et al., 2009 |
| Israel | Randomized controlled trial | 322 obese subjects | Low-fat diet control group versus MD group versus low-carbohydrate group | BW, TC, HDL-c, fasting plasma glucose, insulin | Two years | The mean weight loss was 2.9 kg for the low-fat group, 4.4 kg for the MD group, and 4.7 kg for the low-carbohydrate group ($P < .001$ for the interaction between diet group and time). The relative reduction in the ratio of TC to HDL-c was 20% in the low-carbohydrate group and 12% in the low-fat group ($P = .01$). Participants randomized to the MD group achieved greater significant improvements in fasting plasma glucose and insulin levels. | Shai et al., 2008 |
| Spain | Randomized controlled trial | 1224 asymptomatic subjects at high cardiovascular risk (55–80 years) | Low-fat diet ($n = 408$) control group versus MD + virgin olive oil VOO group versus MD + nuts group | Prevalence and incidence of MS | One year | One-year prevalence was reduced by 6.7%, 13.7%, and 2.0% in the MD + VOO, MD + nuts, and control diet groups, respectively (MD + nuts vs. control groups, $P = .01$; MD + VOO vs. control group, $P = .18$). Incident rates of the MetS were not significantly different among groups (22.9%, 17.9%, and 23.4%, respectively) | Salas-Salvado et al., 2008a, 2008b |
| Spain | Randomized controlled trial | 372 subjects at high cardiovascular risk (162 M, 210 F; 55–80 years) | Low-fat diet ($n = 121$) control group versus MD + virgin olive oil VOO group versus MD + nuts group | Oxidized LDL, MDA, serum glutathione peroxidase activity | Three months | Oxidized LDL levels decreased in the MD + VOO and MD + nuts groups, without changes in the low-fat diet group. Change in oxidized LDL levels in the MD + VOO group reached significance versus that of the low-fat group ($P = .02$). MDA changes in mononuclear cells paralleled those of oxidized LDL. No changes in serum glutathione peroxidase activity | Fito et al., 2007 |

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|---|---|---|---|---|---|---|---|
| Germany | Randomized controlled trial | 101 patients (59.4 ± 8.6 years, 23% F) with established and treated CAD | MD with a one-year program of 100 hours of education intervention group ($n$ = 48) versus general healthy diet control group | hs-CRP, fibrinogen, homocysteine, fasting insuline, lipids profile | One year | Adoption of a MD by patients with medically treated CAD has no effect on markers of inflammation and metabolic risk factors. | Michalsen et al., 2006 |
| Spain | Randomized controlled trial | 772 asymptomatic subjects at high cardiovascular risk (55–80 years) | Low-fat diet ($n$ = 257) control group versus MD + virgin olive oil VOO group versus MD + nuts group | BP, CRP, IL–6, ICAM-1, VCAM-1, HDL-c, plasma glucose | Three months | Compared with the low-fat diet, the mean changes in the MD + VOO group and the MD + nuts group were −0.39 mmol/L (95% CI: −0.70 to −0.07 mmol/L) and −0.30 mmol/L (CI: −0.58 to −0.01 mmol/L), respectively, for plasma glucose levels; −5.9 mm Hg (CI, −8.7 to −3.1 mm Hg) and −7.1 mm Hg (CI: −10.0 to −4.1 mm Hg), respectively, for systolic BP; and −0.38 (CI: −0.55 to −0.22) and −0.26 (CI: −0.42 to −0.10), respectively, for the HDL-c ratio. The MD + VOO reduced CRP levels by 0.54 mg/L (CI: 1.04–0.03 mg/L) compared with the low-fat diet. Both the MDs reduced IL-6, ICAM-1, and VCAM-1 levels. | Estruch et al., 2006 |
| France | Randomized controlled trial | 212 subjects with moderate risk factors for CVD | MD intervention group versus low-fat diet control group | BMI, lipids profile, HOMA, insulin, serum glucose | Three months | BMI, TC, TRL-c, APOs A-I and B, insulinemia, glycemia, and the HOMA score were significantly lower after three months in both group (no significative difference). | Vincent-Baudry et al., 2005 |
| Australia | Randomized controlled trial | 155 subjects (31 M, 124 F) | MD intervention group versus control group | Lipids profile | Three months | Decreased TG (31.6%), increased HDL-c (9.6%), no significant changes on TC and LDL-c | Flynn and Colquhoun 2004 |
| Spain | Randomized trial | 1,018 overweight subjects (788 F, 230 M; 14.8–76.3 years) | hcMD + physical activity | BMI, BW | Three years | Compared with nonadherent subjects, adherent who completed the program had more weight loss (−11.87 ± 6.6kg vs. −6.57 ± 4.9kg, $P < .001$) and BMI (30.1 ± 4.4 kg $m^{-2}$ vs. 32.4 ± 5.4 kg $m^{-2}$, $P < .001$) | Bautista-Castano et al., 2004 |
| Italy | Randomized, single-blind controlled trial | 190 subjects (99 M, 81 F) with MS | MD intervention group versus prudent diet control group | BW, BMI, IL-6, IL-7, IL-18, CRP, endothelial function, insulin resistance | Two years | Mean BW decreased more in patients in the intervention group (−4.0 ± 1.1 kg) than in those in the control group (−1.2 ± 0.6 kg) ($P < .001$), had significantly reduced levels of hs-CRP ($P = .01$), IL-6 ($P = .04$), IL-7 ($P = .4$), and IL-18 ($P = .3$), as well as decreased insulin resistance ($P < .001$). Endothelial function score improved in the intervention group (mean change, +1.9 ± 0.6; $P <$ .001) but remained stable in the control group (+0.2 ± 0.2; $P = .33$). | Esposito et al., 2004 |

*(Continued on next page)*

**Table 1** Epidemiological studies on the MD and cardiovascular risk factors *(Continued)*

| Country | Type of study | Sample characteristic | Methodology | Outcome | Follow-up | Results | Reference |
|---|---|---|---|---|---|---|---|
| Italy | Randomized, single-blind controlled trial | 120 obese F (20–46 years) without diabetes, hypertension, or hyperlipidemia | Detailed advices on lifestyle changing with MD intervention group versus general advices control group | BW, BMI, IL-6, IL-18, CRP, adiponectin, insulin resistance | Two years | BMI decreased more in the intervention group than in controls (−4.2; $P < .001$), as did serum concentrations of IL-6 (−1.1 pg/mL; $P = .009$), IL-18 (−57 pg/mL; $P = .02$), and CRP (−1.6 mg/L; $P = .008$), while adiponectin levels increased significantly (2.2 microg/mL; $P = .01$). In multivariate analyses, changes in free fatty acids ($P = .008$), IL-6 ($P = .02$), and adiponectin ($P = .007$) levels were independently associated with changes in insulin sensitivity. | Esposito et al., 2003 |
| USA | Randomized controlled trial | 279 postmenopausal F with T2DM | MD lifestyle intervention group versus usual care control group | HbA1c, BMI, lipids profile | Six months | Patterns favoring intervention were seen in lipids, BP, and flexibility but did not reach statistical significance | Toobert et al., 2003 |
| Denmark | Randomized controlled trial | 131 patients (70 M, 61 F; 18–80 years) with IHD | MD intervention group ($n = 68$) versus no specific dietary advice control group ($n = 63$) 24-hour recall | FMD, lipids profile, liver transaminases, blood glucose, TSH samples | One year | FMD was significantly higher in the intervention group ($P < .01$). No differences in the arterial diameter or in the nitroglycerin response; LDL-c levels $P < .001$ for the reduction in both of groups; TG levels $P < .05$ only in intervention group; unchanged in both of groups HDL-c | Sondergaard et al., 2003 |
| India | Randomized single-blind controlled trial | 1,000 patients with angina pectoris, MI, or surrogate risk factors for CAD | MD intervention group ($n = 499$) versus National Cholesterol Education Program (NCEP) diet control group | Cardiac events incidence, lipids profile | Two years | Total cardiac end points were significantly fewer in the intervention group than the controls (39 vs. 76 events, $P < .001$). Sudden cardiac deaths were also reduced (6 vs. 16, $P = .015$), as were nonfatal MIs (21 vs. 43, $P < .001$). No statistically significative difference between both groups in serum cholesterol concentration and other risk factors reduction | Singh et al., 2002 |

### *3.2. Definition and Evaluation of MD Adherence*

There was no consensus among the studies in the number and quality of dietary components characterizing the MD. Dietary habits mostly taken into account were: a high consumption of cereals, vegetables, fruits, legumes, fish, nuts, and olive oil (as a source of monounsaturated fatty acids), a moderate consumption of alcohol (from wine), and a low consumption of red meat.

Most of the papers describing cohort and cross-sectional studies reported a priori definitions of MD, and assessed individual levels of adherence to the MD using indexes with linear scoring systems. The studies that belonged to the same project used a common index. More in detail, to define subjects' adherence to MD in the ATTICA study, a scoring based on evaluation of intake frequency of foods included in a MD pyramid was used; in the European Prospective Investigation into Cancer and Nutrition (EPIC) project a 8-point index and a modified version of this, as developed by Trichopoulou (1995), were used; in the Seguimiento Universidad de Navarra (SUN) project a score which included typical healthy foods of the MD was used. Other studies used validated food frequency questionnaires, 24-hour recall, and more detailed methods to derive an a posteriori definition of MD adherence and relative scores. The resulting picture was characterized by a lack of homogeneity of methods used to collect dietary data among different studies.

Among intervention studies, as literature is lacking of multicenter studies, different variations of the MD were recommended, thus resulting in a general lack of consensus in the definition of the diet. Additionally, several MD interventions focused on the role of some MD components (i.e., in the PREDIMED study the two intervention diet groups were consisted in the MD added of nuts or olive oil, but not both) (Estruch et al., 2006; Fito et al., 2007; Salas-Salvado et al., 2008a; Mena et al., 2009; Razquin et al., 2009), on hypo-caloric variation of MD (Elhayany et al., 2010; Bautista-Castano et al., 2004), or advices on lifestyle changing (physical activity programs, cooking classes, and weight loss counseling) were included, thus obtaining results that cannot be compared with those of studies using traditional MD (Esposito et al., 2003; Toobert et al., 2003; Bautista-Castano et al., 2004; Michalsen et al., 2006). Finally, it was found a lack of homogeneity even among the control groups, as they performed prudent diets (Esposito et al., 2004), usual care diets (Toobert et al., 2003), low-fat diets (Vincent-Baudry et al., 2005; Estruch et al., 2006), specific diet programs (Singh et al., 2002), general healthy diet (Esposito et al., 2003; Michalsen et al., 2006), or they did not follow any specific dietary advices (Sondergaard et al., 2003).

### *3.3. Association Between MD and CVD: Observational Studies*

#### *3.3.1. Cross-sectional Studies*

Among the cross-sectional studies, a large number of papers (12 works) were included in the contest of the ATTICA study, a cross-sectional survey conducted in 2001–2002 that included a population of about 3000 participants (18–89 years) from the Attica region in Greece. Authors assessed a protective role of the MD toward several chronic diseases such as MS (Panagiotakos et al., 2004; Panagiotakos et al., 2007), diabetes (Panagiotakos et al., 2005a; Tzima et al., 2007), obesity (Panagiotakos et al., 2006a, 2006b), CVD events (Panagiotakos et al., 2008), as well as improvement of lipid profile (Tzima et al., 2007), hypertension in prehypertensive individuals (Pitsavos et al., 2008), coagulation markers (Chrysohoou et al., 2004), inflammatory markers in subjects with abdominal obesity (Pitsavos et al., 2007), total antioxidant capacity (Pitsavos et al., 2005), and adiponectin concentration both in free-living persons and in patients at high cardiovascular risk (Fragopoulou et al., 2010). Only one work resulted in inconsistent relation between MD and obesity (Yannakoulia et al., 2009).

Contrasting results on association between MD and MS were found in a study involving about 2000 randomly selected non-diabetic subjects (20–74yrs) in five Mediterranean countries (Thanopoulou et al., 2006) and in a study conducted in 500 free-living adults in the Canarian Islands (Spain) and participating to the Canarian Nutrition Survey (ENCA) (Alvarez Leon et al., 2006).

Protective effect of MD on similar outcomes in patients with chronic disease were found (i) within the framework of the multicentre AIRGENE project aimed to study the association of the MD on plasma concentrations of various inflammatory markers, in about 1000 myocardial infarction (MI) survivors from six geographic areas in Europe (Panagiotakos et al., 2009); (ii) in a study as part of the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) regarding about 13,000 adults (18–90 years) living in US (Carter et al., 2010); (iii) in two large prospective cross-sectional studies conducted in USA in about 1000 diabetic women from the Nurses' Health Study who had no history of CVD (Fung et al., 2005; Mantzoros et al., 2006); and (iv) in Italy on about 9000 patients with a MI (Mozaffarian et al., 2007). Another cross-sectional study focused on relation between MD and glycated hemoglobin was conducted in Italy among 901 outpatients with Type 2 diabetes (Esposito et al., 2009).

Regarding obesity, several studies found an inverse relation between MD and BMI and waist-to-hip ratio. In particular, it was found that: in 1000 subjects living in Mediterranean southern France those who were more adherent to the MD model were less likely to be obese (Scali et al., 2001); in a group of about 3000 free-living Spanish subjects aged 25–74 years, the obesity risk decreased in both men and women with increasing adherence to the traditional MD dietary pattern (Schroder et al., 2004). The same relation was reported in the MEDIS study, during the period 2005–2007, in a sample of about 1000 elderly people from eight Mediterranean Islands in Greece and Cyprus (Tyrovolas et al., 2009). On the other hand, a study conducted in Italy on 6000 subjects resulted in inconsistent association (Rossi et al., 2008).

Studies assessing the relation between MD and obesity have been conducted also far from the Mediterranean basin, i.e., a study on more than 700 subjects living in Canada (Shubair

et al., 2005) and another one on about 1000 subjects from China (Woo et al., 2008). However, the results of those studies were contrasting. Basing on the lack of consistent evidence of a relationship between MD and body fat, Romaguera et al. (2009) assessed the cross-sectional association between adherence to a modified MD, BMI, and waist circumference in a total of 497,308 individuals (70.7% women) aged 25–70 years from 10 European countries. The findings indicated that adherence to a modified MD, high in vegetables and unsaturated fatty acids, is associated with lower abdominal adiposity measured by waist circumference without resulting significantly associated with BMI (Romaguera et al., 2009).

#### *3.3.2. Cohort Studies*

Among the prospective cohort studies, the three largest multicenter works were the EPIC, the PREDIMED and the SUN.

The EPIC is the largest study focused on diet and health ever undertaken, having recruited over half a million (520,000) of people in 10 European countries. In this contest, studies from the Greek, the Italian, and the Spanish arms were examined and inverse associations were found between MD and blood pressure (Psaltopoulou et al., 2004; Masala et al., 2008), BMI (Trichopoulou et al., 2005) and likelihood of becoming obese (Mendez et al., 2006; Romaguera et al., 2010).

A substudy of the PREDIMED (see below) cohort, in a total of about 800 asymptomatic subjects (55–80 years) at high cardiovascular risk, showed that only the subjects with the highest consumption of nuts and virgin olive oil (but not those with the highest adherence to the MD) had the lowest serum concentrations of inflammatory markers (Salas-Salvado et al., 2008a, 2008b) and a significantly lower odds ratio of having MS (Babio et al., 2009), thus confirming a previous study conducted on 3000 subjects (Sanchez-Tainta et al., 2008).

The SUN prospective cohort examined more than 15,000 Spanish university graduates without any particular disease risk factors and found inverse association between MD and the incidence of several chronic diseases and the cumulative incidence of MS (Tortosa et al., 2007), diabetes (Martinez-Gonzalez et al., 2008), and blood pressure (Nunez-Cordoba et al., 2009); while no association with BMI was found (Sanchez-Villegas et al., 2006).

Finally, two other cohorts were examined in the contest of the Framingham Heart Study Offspring Cohort, which confirmed the inverse relation between MD and MS traits (Rumawas et al., 2009).

### *3.4. Association Between the MD and CVD: Intervention Studies*

The PREDIMED Study was the largest, multicenter, randomized, controlled, parallel-group clinical trial aiming at testing the efficacy of the traditional MD on the primary prevention of coronary heart disease. It was performed on 2003–2004 in 9000 subjects (55–80 years) with high cardiovascular risk. Participants were assigned to a low-fat diet or to one of two traditional MDs supplemented with extra olive oil or nuts. Results showed that in subjects following MD, cellular lipids (Fito et al., 2007), and inflammatory markers (Mena et al., 2009) were significantly reduced, while plasma antioxidant capacity was increased (Razquin et al., 2009). In parallel, a decreased prevalence and incidence of MS (Salas-Salvado et al., 2008a), and beneficial effects on cardiovascular risk factors (Estruch et al., 2006) were also found.

The Medi-RIVAGE study (MD, cardiovascular risks, and gene polymorphisms) was a randomized, parallel trial comparing a new nutritional pattern with a conventional one. It was performed in France in 212 subjects with at least one cardiovascular risk factor. The data obtained at three-month follow-up showed that changing to a MD pattern improves blood biochemical markers (Esposito et al., 2003, 2004; Vincent-Baudry et al., 2005).

In the Dietary Intervention Randomized Controlled Trial 322 moderately obese participants were randomized to follow for 2 years one of the three diet groups: low-fat, Mediterranean, and low carbohydrate. Significant improvements in fasting plasma glucose and insulin levels were found among subjects in MD diet group (Shai et al., 2008).

Similar protective effects of MD were found also in a 12-month trial in which about 200 diabetic patients were randomized to follow a low carbohydrate Mediterranean, or a traditional Mediterranean, or the 2003 American Diabetic Association diet (Elhayany et al., 2010). An improvement of lipidemia by MD was shown in a study on 155 patients (Flynn and Colquhoun 2004), whereas in an intervention program conducted in Spain on more than 1000 subjects following hypocaloric MD and recommendations for free-time exercise and day-to-day activity, was found that the highest adherence levels to MD were associated to lower BMI and higher weight loss (Bautista-Castano et al., 2004).

A positive impact in secondary prevention was established in two studies conducted respectively on 1000 and 131 patients with CVD and finding that dietary intervention with the MD might be more effective in primary and secondary prevention of coronary artery disease than the conventional prudent diet (Singh et al., 2002; Sondergaard et al., 2003).

On the other hand, a study on 101 patients with established and treated coronary artery disease and another on 279 postmenopausal women with type 2 diabetes, both aimed at reducing markers of inflammation and metabolic risk factors, failed to reach statistical significance (Toobert et al., 2003; Michalsen et al., 2006).

## *4. DISCUSSION*

Over the last decades, in parallel to the growing amount of researches focused on the correlation between diet and health, the

scientific community showed a great interest to the relationships between MD and chronic diseases risk.

In this paper, the most recent evidence on the healthy value of the MD in the respect of CVD and its relation with cardiovascular risk factors were reviewed. Results from the dynamic SUN cohort, the EPIC study, the ATTICA study, as well as from several nutrition surveys as ENCA, NHANES III, and monocenter studies, totally examining more than 1,000,000 subjects living in countries of Mediterranean basin and not, were considered. In addition, several experimental studies such as the PREDIMED study, that focused their attention on the role of the MD as a predictor of good health or that used this dietary pattern in interventions programs, were examined.

The data reviewed suggest that the health benefits of MD pattern are mainly due to the existence of biologic interactions between different components of it rather than to the effect of a single food group or nutrients. In the respect of CVD plausible mechanisms underlying the protective effects include the improvement of blood lipid profile (Rossi et al., 2008; Shai et al., 2008; Carter et al., 2010; Elhayany et al., 2010) as well as the reduction of blood pressure (Psaltopoulou et al., 2004; Masala et al., 2008), insulin resistance (Esposito et al., 2003, 2004; Shai et al., 2008; Rumawas et al., 2009; Carter et al., 2010), and serum markers of inflammation (Esposito et al., 2003, 2004; Chrysohoou et al., 2004; Fung et al., 2005; Pitsavos et al., 2005, 2007; Estruch et al., 2006; Fito et al., 2007; Mena et al., 2009; Panagiotakos et al., 2009; Carter et al., 2010) even in individuals with diabetes (Esposito et al., 2009; Mena et al., 2009; Elhayany et al., 2010) or MS (Esposito et al., 2004). These results were in accordance with older findings reported by de Lorgeril et al. (1999) in the Lyon Heart Study (de Lorgeril M. et al., 1999; Barzi et al., 2003). Additionally, observational studies showed that among people more adherent to the MD there was a lower prevalence and incidence of diabetes (Panagiotakos et al., 2005a; Mozaffarian et al., 2007; Buckland et al., 2009), MS (Panagiotakos et al., 2004, 2007; Sanchez-Tainta et al., 2008; Woo et al., 2008; Salas-Salvado et al., 2008a; Babio et al., 2009; Buckland et al., 2009) and obesity (Esposito et al., 2003, 2004; Schroder et al., 2004; Shubair et al., 2005; Vincent-Baudry et al., 2005; Panagiotakos et al., 2006a, 2006b; Tyrovolas et al., 2009; Romaguera et al., 2010). Notwithstanding, in some studies conducted in the contest of the SUN study (Sanchez-Villegas et al., 2006), the ATTICA study (Yannakoulia et al., 2009), the EPIC prospective cohort (Trichopoulou et al., 2005; Mendez et al., 2006), as well as in other works (Scali et al., 2001; Toobert et al., 2003; Sanchez-Villegas et al., 2006; Rossi et al., 2008; Woo et al., 2008; Romaguera et al., 2009), the relationship between MD and obesity was not confirmed. A certain degree of controversy was also shown in the contest of the ENCA (Alvarez Leon et al., 2006) and in a multicenter study conducted by Thanopoulou et al. (Thanopoulou et al., 2006) about the association between MD and MS. Similarly, findings on the association of MD with blood pressure (Pitsavos et al., 2008; Nunez-Cordoba et al., 2009), or markers of inflammation (Michalsen et al., 2006), or lipid profile (Singh et al., 2002), or fasting insulin and glucose (Estruch et al., 2006; Panagiotakos et al., 2007; Tzima et al., 2007) were not conclusive.

However, robust scientific evidences support the promotion of MD for both primary and secondary prevention of chronic disease and, specifically, of CVD. In particular, as regards the latter issue, inside the CARDIO2000 study (a project realized in 2000–2002), many authors showed that the adoption of MD attenuated the coronary risk both in the whole subjects sample (Panagiotakos et al., 2005a, 2005b), and in subjects with MS (Pitsavos et al., 2003), hypercholesterolemia (Pitsavos et al., 2002b), or hypertension (Panagiotakos et al., 2002; Pitsavos et al., 2002a. Similar findings were found in the contest of the Greek Acute Coronary Syndromes (GREECS) study in 2006 (Panagiotakos et al., 2006c, 2006d) while in a more recent study by Chrysohoou et al. the role of MD in preserving left ventricular systolic function and its association with better long-term prognosis of patients was confirmed (Chrysohoou et al., 2010). Another recent work assessed that MD pattern was associated with improved cardiac autonomic function among middle-aged twins (Dai et al., 2010).

Mechanisms underlying the health benefits of MD against CVD may depends on increased intake of dietary fiber, antioxidant vitamins (A, C, and E) (Riccioni et al., 2007a, 2007b) and carotenoids (beta-carotene, lycopene, lutein, beta-cryptoxanthin, zeaxanthin, and astaxanthin) from fruits and vegetables (Riccioni et al., 2007a, 2007b; Riccioni et al., 2008a, 2008b, 2009, 2010), monounsaturated oleic acid from virgin olive oil, omega-3 fatty acids from fish, and reduced consumption of dietary cholesterol and saturated fatty acids. The fascinating putative role of non-nutrients with an antioxidant activity, such as polyphenols, still needs to be corroborated by clinical evidences.

In an attempt to understand mechanisms underlying the health benefits of MD also nutrigenomic studies were performed. The few studies on the in vivo effect of the MD on human gene expression agree with a protective role of MD (and specifically of olive oil) on expression of proatherogenic genes (Khymenets et al., 2009; Konstantinidou et al., 2010; Llorente-Cortes et al., 2010), insulin sensitivity-related genes (Konstantinidou et al., 2009a, 2009b), postprandial adiponectin (Paniagua et al., 2007), and in the expression of atheriosclerosis-related genes (Konstantinidou et al., 2009a, 2009b).

Despite randomized trials represents the best tool to ascertain the association between a dietary pattern and a disease, scientific evidence for the MD is primarily sustained by observational studies and personal review. Serra-Majem et al. examined the publication trend focused on MD from 1985 up to 2005, and showed that over the last years the number of original articles was similar to the number of reviews, most of them being lacking of a systematic methodology and of an objective analysis of robust evidence (Serra-Majem et al., 2006). Some recent systematic review (Kastorini et al., 2010) and metanalysis (Sofi et al., 2010) confirmed the significant and consistent protection provided by adherence to the MD in relation to some chronic degenerative diseases.

On the other hand, the scientific community recognized the need for further experimental studies to achieve sound, evidence-based recommendations. Indeed, scarcity of reliable experimental research put into evidence a limited and conflicting epidemiological evidence and lack of consensus about the role of the MD in some clinical conditions as the case of obesity (Ferro-Luzzi et al., 2002; Trichopoulos, 2002).

Between the cohort and cross-sectional studies, the inconsistency may be due to the use of different scoring for evaluation of adherence to MD and in general to different methodology at the basis of different indexes. For example, the use of cutoffs such as medians to indicate the adherence to diet could not reflect a traditional MD because the consumption of certain components, such as olive oil or legumes, may be lower thus resulting in an uncharacteristically low cutoffs to define MD adherence. Additionally, recent analyses have shown a lower spreading of the traditional MD and a fewer consumption of its components also in the Mediterranean countries (Serra-Majem et al., 2004). Thus, using intake medians to define a high adherence to the MD would again be influenced by current trend. A common issue biasing results of prospective studies is the frequent choice to exclude at baseline (or lose at follow-up) individuals poorly prone to report data (or to remain in the study). On the other hand, cross-sectional studies have limitation in exploring the causality among the variables of interest.

Several issues affect also the nutritional interventional studies. Firstly, most of the studies had a limited number of subjects. As the sample size varied within the different studies, this could alter the statistical power to detect significant associations. Another, but probably the most important, methodological issue was the use of inconsistent definition of the MD. In fact, several works did not include in their definition some key components of the diet such as olive oil, nuts, or wholegrain cereals (Singh et al., 2002), or they focused the intervention only on one of these components (Estruch et al., 2006; Fito et al., 2007; Salas-Salvado et al., 2008a, 2008b; Mena et al., 2009; Razquin et al., 2009). Finally, assessment of compliance to the diet in some specific cohort subjects, such as in obese participants, may result in methodological biases due to the known under-reporting of obese compared to lean subjects (Mendez et al., 2004). Moreover, changing dietary habits and other factors (such as participants' health status, type of MD, duration of intervention, and additional lifestyle intervention) may affect the level of adherence and effectiveness of the diet. All these issues make it difficult to compare the results between different studies and to establish homogeneous evidence-based recommendations.

The results here reviewed support the healthy role of MD in both primary and secondary prevention of CVD diseases. However, further research is needed to substantiate these findings. MD recommendations need to be evidence based and supported by the development of more homogeneous observational studies and stronger randomized clinical trials. Cross-sectional and cohort studies able to provide better evidence of causality, together with the use of a consistent universal definition of the MD and method to measure the adherence, are necessary. The intervention studies with MD should be more controlled, as regards the extra-diet factors among the treatment groups, and should provide longer interventions to evaluate the long-term efficacy of the MD.

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