

A priori-defined Mediterranean-like dietary pattern predicts cardiovascular events better in north Europe than in Mediterranean countries

Fabrizio Veglia^{a,1}, Damiano Baldassarre^{a,b,1}, Ulf de Faire^d, Sudhir Kurl^e, Andries J. Smit^f, Rainer Rauramaa^g, Philippe Giral^h, Mauro Amato^a, Alessandro Di Minno^a, Alessio Ravani^a, Beatrice Frigerio^a, Samuela Castelnovoⁱ, Daniela Sansaro^a, Daniela Coggi^{a,c}, Alice Bonomi^a, Calogero C. Tedesco^a, Elmo Mannarino^j, Steve E. Humphries^k, Anders Hamsten^l, Elena Tremoli^{a,c}, on behalf of the IMPROVE study group.

¹ **These authors contributed equally to this work.**

Author Affiliations:

^aCentro Cardiologico Monzino, IRCCS, Milan - Italy.

^bDepartment of Medical Biotechnology and Translational Medicine, Università di Milano, Milan - Italy.

^cDipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Milan, Italy.

^dDivision of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, and Department of Cardiology, Karolinska University Hospital, Solna, Karolinska Institutet, Stockholm, Sweden.

^eInstitute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio Campus.

^fDepartment of Medicine, University Medical Center Groningen, Groningen, the Netherlands.

^gFoundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland.

^hAssistance Publique - Hopitaux de Paris; Service Endocrinologie-Metabolisme, Groupe Hospitalier Pitie-Salpetriere, Unités de Prévention Cardiovasculaire, Paris, France.

ⁱCentro Dislipidemie E. Grossi Paoletti, Ospedale Ca' Granda di Niguarda, Milan, Italy.

^jInternal Medicine, Angiology and Arteriosclerosis Diseases, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy.

^kCardiovascular Genetics, British Heart Foundation Laboratories, Institute cardiovascular Science, University College of London, Rayne Building, London, United Kingdom.

^lAtherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden.

Corresponding author:

Damiano Baldassarre, PhD,

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano,

Via Balzaretti 9, 20133 - Milan, Italy.

Phone: 39-02-58002253

Fax: +39-02-58002623.

E-mail: damiano.baldassarre@unimi.it

Number of figures and/or tables: 6

Abbreviations used

MD = Mediterranean Diet

CAD = Coronary artery disease

clMT = carotid intima media thickness

VEs = vascular events

VRFs = vascular risk factors

TIA = transient ischemic attack

hs-CRP = C-Reactive Protein

Clinical Trial Registry number:

Contract number: QLG1- CT- 2002- 00896; http://cordis.europa.eu/project/rcn/68775_it.html

Word count : 2509

Abstract

Background and Aim: Mediterranean Diet is a model of healthy eating that contributes to a favorable health status and a better quality of life, but its clinical usefulness is still debated. The aim of this study was to relate the degree of adherence to Mediterranean Diet with the incidence of subsequent cardio- and cerebro-vascular events in north and south European participants of the IMPROVE study.

Methods: The IMPROVE is an observational longitudinal prospective cohort study involving 3,703 high-risk individuals from five European countries (Finland, Sweden, Netherlands, France and Italy). The end-point used in this study was the incidence of the first combined cardio- or cerebro-vascular event occurring during a 36-months median follow-up. At baseline, a dietary questionnaire about the usual intake during the year preceding enrollment was administered. Based on 7 nutritional items, a Mediterranean Diet Score was constructed in which minimal adherence was 0 and maximal adherence was 7.

Results: Latitude was the strongest determinant of Mediterranean Diet score ($p < 0.001$). Vascular events occurred in 215 participants. The incidence of vascular events was the highest in subjects with Mediterranean Diet score 0-1, lower in those with score 2-3 and the lowest in those with score ≥ 4 . Mediterranean Diet score remained significantly associated with subsequent vascular events after adjustment for potential confounders (hazard ratio for one-point increment of the score = 0.75, $p < 0.001$) and the association was stronger in northern than in southern countries ($p = 0.04$ for Mediterranean Diet Score \times latitude interaction).

Conclusions: The Mediterranean Diet adherence score based on a simple dietary questionnaire detects changes of risk of vascular events. North Europeans appear as the best candidates for intervention programs based on Mediterranean Diet.

Key words: Mediterranean Diet score, IMPROVE study, vascular diseases.

Introduction

Mediterranean Diet (MD) is a model of healthy eating, known for its contribution to a favorable health status and a better quality of life [1]. MD is based on high consumption of olive oil, legumes, unrefined cereals, fruits, vegetables, moderate/high consumption of fish, moderate consumption of dairy products (mostly cheese and yogurt), wine, especially during meals, and low consumption of meat and meat products [2].

Several studies have documented a positive association between chronic diseases and single foods/nutrients not included into MD such as meat [3], and a negative association with those close to MD (fruit and vegetables [4], diet antioxidants [5]). Based on the results of the Seven Countries Study [6], Trichopoulou *et al.* conducted an observational prospective study in a large Greek cohort investigating the capacity of a 10-point scale MD adherence score to predict the overall and coronary artery disease (CAD) mortalities [7]. The authors showed that the adherence to MD (rather than to some of its individual components) was inversely associated with coronary death. A meta-analysis of prospective cohort studies on 4,172,412 subjects confirmed these findings and the authors proposed a nine item literature-based MD score that may predict the cardiovascular (CV) risk at the individual level [8]. In the present study, we took advantage of the IMPROVE, a large study carried out in 5 European countries with relevant differences in dietary behavior, to investigate the association of a seven item MD adherence score with the incidence of vascular events (VEs) in north and south Europe.

Patients and Methods

A summary of design, objectives, eligibility criteria, methods, baseline characteristics, ultrasonographic variables and type and numbers of VEs in the IMPROVE Study have been reported [9-11]. Briefly, the IMPROVE is an observational longitudinal prospective cohort study that enrolled 3,703 patients (1,774 men, 1,929 women, aged 55-79 years) with ≥ 3 vascular risk factors (VRFs) free from cardio- or cerebro-VEs. Participants were recruited from January 2004 to May 2005 in seven

centers in 5 European countries: Finland (n=1048; 2 centers), Sweden (n=532), the Netherlands (n=527), France (n=501) and Italy (n=553 in Milan and 542 in Perugia). The end of 36 months follow-up was reached by 93.7% of participants. The combined endpoint included angina pectoris, myocardial infarction, cardiovascular death, ischemic stroke, transient ischemic attack (TIA) and revascularizations of coronary or peripheral arteries. The criteria of diagnosis of VEs have been previously described [10, 11]. The study complies with the Declaration of Helsinki. Five local ethics committee have approved the research protocol. Each participant provided two different informed consents, one for general participation in the study and one for genotyping.

The IMPROVE MD score

Dietary intake maintained during the year preceding enrollment was assessed by a semi-quantitative dietary questionnaire, administered by trained personnel. Since the amount and type of vegetables, legumes, nuts and cereals consumed differ among populations [12], the items included in the questionnaire were limited to foods freely available in all the countries involved. We created a MD adherence score analogous to the Greek Mediterranean Index [7], since this index was inversely associated with the incidence of stroke in a recent Italian study [13]. Our scoring is based on intake of 7 items: fruits, fish, wine, olive oil, meat, milk and eggs. For fruit or fish, high consumption (top tertile of their distributions, i.e. fruit ≥ 3 servings/day and fish > 2 times/week) received one point, other intakes received 0 points; for meat, eggs or milk a low intake (bottom tertile of their respective distributions, i.e. meat < 2 times/week, eggs ≤ 1 times/week, milk ≤ 3 dL/day) received 1 point. A predominant consumption of olive oil, rather than of other types of fat, and a moderate consumption of wine (1-2 glasses/day) also received one point. Based on the scale obtained, score 0 indicates minimal adherence and score 7 maximal adherence to MD.

Statistical analysis

Quantitative variables are summarized as means \pm standard deviation (SD), or median (interquartile range) when appropriate. Variables with skewed distributions were log-transformed before analysis. Baseline characteristics of subjects, stratified by MD score categories, were compared by linear regression and chi-square for trend, as appropriate. Multivariable linear regression with stepwise selection was employed to identify independent predictors of MD score; the interaction between latitude and each predictor was also tested. The association between MD score and VEs was assessed by three Cox models: *Model-1*: unadjusted; *Model-2*: adjusted for age, gender and stratified by latitude; *Model-3*: as model 2 plus lifelong exposure to cigarette smoking (pack-years), body mass index (BMI), education (as indexed by years of school), physical activity, occupation (categorized as: 1, white collars; 2, service workers and 3, manual workers), plasma concentrations of LDL and HDL cholesterol, triglycerides, glucose, high sensitive C-Reactive Protein (hs-CRP), creatinine, pulse pressure, antiplatelet and statin treatments. Kaplan-Meier curves stratified by classes of MD score were also computed. In subgroup analysis an interaction term was included in the Cox models. All tests were two-sided. Analyses were carried out by using the SAS statistical package v. 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Compliance with and determinants of the MD Score.

Patient's characteristics according to the MD adherence score are detailed in **Table 1**. Two third of the patients in Perugia, Milan and Paris scored >2 , whereas 3/4 of patients in Groningen, Stockholm and Kuopio scored 0-2. The lowest average MD adherence score was found in Groningen (mean \pm SD, 1.3 \pm 1.1) and in Kuopio (1.7 \pm 1.1). Intermediate values were in Stockholm (2.1 \pm 1.2) and in Paris (2.9 \pm 1.3), whereas the average MD score in Milano and Perugia was >3 . With univariable analysis (**Table 1**), the MD score was negatively and significantly associated with almost all traditional VRFs. Using multivariable analysis, latitude was the strongest independent determinant of MD score (**Table 2**). Social and behavioral variables, such as education, occupation, physical activity and smoking

habits as well as hs-CRP, HDL-cholesterol, BMI, pulse pressure, statin and insulin treatment were also independently associated with MD score (**Table 2**). A significant interaction between education (a social class index) and latitude was found ($p=0.01$), indicating that the dependence of MD score on social class is stronger in northern than in southern countries, the average MD score change for each year of school being 0.06 in Sweden, 0.03 in Finland and 0.016 in Italy.

MD score and vascular events.

Among the 3,703 subjects enrolled in the IMPROVE Study, 215 (7.96%) developed a first VE within the 36 months follow-up (**Table 3**). Among these, 125 were cardio-VEs; 73 were cerebro-VEs and 17 were peripheral vascular events.

Figure 1 shows the Kaplan-Meier incidence curves of the combined endpoint, and of cardio- and cerebro-VEs, stratified by MD adherence score classes. Regardless of the endpoint considered, the rate of events was the highest in subjects with score 0-1; lower in those with score 2-3 and the lowest in those with score 4-7. The MD score remained significantly associated with the combined endpoint also after stratification by latitude and adjustment for potential confounders (**Table 4**, Model 2 and 3). Similar results were obtained when cardio- and cerebro-VEs were analyzed separately (**Table 4**).

Subgroups analysis (**Figure 2**) showed that the hazard ratios (HRs) associated with one-point increase of MD score were comparable in men and women, and tended to be lower in high-risk subjects (e.g. obese, diabetics etc.) as well as in north European countries. Of note, the MD effect was comparable and statistically significant regardless the use of statins.

Discussion

The high impact of MD on VEs has been previously documented [7, 8, 14, 15] and our data are in line with these findings. We show here, for the first time, that the association of MD with VEs is more

apparent in the north than in the south of Europe and is stronger in some high-risk categories, independent of statin treatments.

To the best of our knowledge, most of the studies addressing the relation between MD scores and VEs were single nation studies (Greece [7], USA [16], Italy [13], north Sweden [17]) and only one [12] was carried out in European countries at different latitudes. Our study, which includes nations with a north to south distribution, shows that latitude is the strongest predictor of the MD score. Another important observation is that MD score is associated with a high social class, as indexed by education level, and that this association is stronger in northern than in southern countries. This latter finding is not easy to explain; one of the possible explanations is that the items of MD are easily available and relatively cheap in southern Europe, whereas they are available only to higher socio-economic classes in northern countries, due to their higher cost [9, 18].

Multiple complex mechanism(s) are likely to be responsible for the protective effects of MD. Indeed, MD influences both atherosclerosis and thrombosis through an effect on low-grade inflammation. Notably, several authors have reported an association between inflammatory processes and the composition of gut microbiota [19] which, in turn, has been related to dietary habits, and specifically to MD [20]. Accordingly, hs-CRP, a widely accepted marker of low-grade inflammation, has been shown to be an important mediator of the effect of MD on VEs [21].

The results of the present study are relevant in terms of public health, and fit well with current guidelines and recommendations that strongly encourage people to consume a MD-like diet for primary and secondary prevention of major chronic diseases [22]. Unfortunately, a progressive shift toward non-MD patterns is developing even in countries bordering the Mediterranean Sea [23] and this emphasizes the need for continuing the search of dietary based preventive programs able to counteract this detrimental tendency.

An important point that needs to be stressed is that our dietary assessment was based on a relatively simple dietary questionnaire with other studies [7, 24], an aspect that, theoretically, could have limited our ability to detect the relevant associations. Nevertheless, the strength and consistency of our data suggest that a MD pattern can be easily extrapolated using a limited number of food items, and that its association with VEs is so strong to be detectable even in the presence of a certain degree of misclassification.

Our study has several strengths: 1) it is based on a large cohort of 3,700 participants followed for more than three years by specialized clinical centers; 2) it was conducted across five European countries, with a strong gradient in latitude and incidence of VEs. The study has also some limitations: 1) being an observational study, a causal relation between MD and VEs cannot be demonstrated; 2) as the IMPROVE participants were selected for carrying at least three VRFs, the findings can only be extrapolated cautiously to the general European population or to patients with fewer than 3 VRFs; 3) diet was assessed only at the beginning of the study and dietary changes occurring later may have led to non-differential misclassification and underestimation of the true associations.

In conclusion, we found that a MD score based on a simple dietary questionnaire is able to detect changes of risk of VEs; as the protective effect of MD tend to become stronger with increasing latitude, north Europeans appear as the best candidates for intervention programs based on MD.

Conflict of Interest: All the Authors have nothing to declare

Financial support: This study was supported by the European Commission (Contract number: QLGI-CT- 2002- 00896) (to Elena Tremoli, Damiano Baldassarre, Anders Hamsten, Steve E. Humphries,

Rainer Rauramaa, Ulf de Faire, Andries J. Smit, Philippe Giral, Sudhir Kurl, Elmo Mannarino), Ministero della Salute Ricerca Corrente, Italy (to Elena Tremoli, Damiano Baldassarre), the Swedish Heart-Lung Foundation , the Swedish Research Council - project 8691(to Anders Hamsten) and 0593, (to Ulf de Faire), the Foundation for Strategic Research, the Stockholm County Council - project 562183, (to Anders Hamsten), the Foundation for Strategic Research, the Academy of Finland - Grant #110413, (to Sudhir Kurl) and the British Heart Foundation - RG2008/008, (to Steve E. Humphries). None of the aforementioned funding organizations or sponsors has had a specific role in design or conduct of the study, collection, management, analysis, or interpretation of the data, or preparation, review, or approval of the manuscript.

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Author Contributions:

Elena Tremoli, Damiano Baldassarre, Fabrizio Veglia, Alessandro Di Minno, Steve E. Humphries, Rainer Rauramaa, Ulf de Faire, Andries J. Smit, Philippe Giral, Sudhir Kurl and Elmo Mannarino designed research; Mauro Amato, Beatrice Frigerio, Samuela Castelnovo, Alessio Ravani, Daniela Sansaro and Daniela Coggi conducted research; Fabrizio Veglia and Calogero C. Tedesco performed statistical analysis; Fabrizio Veglia, Damiano Baldassarre wrote the paper; Anders Hamsten, Steve E. Humphries, Rainer Rauramaa, Ulf de Faire, Andries J. Smit, Philippe Giral, Sudhir Kurl and Elmo Mannarino performed a critical revision of the manuscript for important intellectual content; Damiano Baldassarre, Fabrizio Veglia and Elena Tremoli had primary responsibility for final content. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to express their deep and sincere appreciation to all members of the IMPROVE group for their time and extraordinary commitment and Prof. S. Panico and Prof. G. Di Minno for their valuable suggestions.

References

- [1] Panico S, Mattiello A, Panico C, Chiodini P. Mediterranean dietary pattern and chronic diseases. *Cancer Treat Res* 2014;159:69-81.
- [2] Willett WC. The Mediterranean diet: science and practice. *Public Health Nutr* 2006;9:105-10.
- [3] Rohrmann S, Overvad K, Bueno-de-Mesquita HB, et al. Meat consumption and mortality--results from the European Prospective Investigation into Cancer and Nutrition. *BMC Med* 2013;11:63.
- [4] Bendinelli B, Masala G, Saieva C, et al. Fruit, vegetables, and olive oil and risk of coronary heart disease in Italian women: the EPICOR Study. *Am J Clin Nutr* 2011;93:275-83.
- [5] Del Rio D, Agnoli C, Pellegrini N, et al. Total antioxidant capacity of the diet is associated with lower risk of ischemic stroke in a large Italian cohort. *J Nutr* 2011;141:118-23.
- [6] Keys A, Seven countries: a multivariate analysis of death and coronary heart disease, Cambridge, MA, Harvard University Press, 1980.
- [7] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;348:2599-608.
- [8] Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr* 2014;17:2769-82.

- [9] Baldassarre D, Nyssonen K, Rauramaa R, et al. Cross-sectional analysis of baseline data to identify the major determinants of carotid intima-media thickness in a European population: the IMPROVE study. *Eur Heart J* 2010;31:614-22.
- [10] Baldassarre D, Hamsten A, Veglia F, et al. Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study. *J Am Coll Cardiol* 2012;60:1489-99.
- [11] Baldassarre D, Veglia F, Hamsten A, et al. Progression of carotid intima-media thickness as predictor of vascular events: results from the IMPROVE study. *Arterioscler Thromb Vasc Biol* 2013;33:2273-9.
- [12] Knuops KT, de Groot LC, Kromhout D, et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. *JAMA* 2004;292:1433-9.
- [13] Agnoli C, Krogh V, Grioni S, et al. A priori-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. *J Nutr* 2011;141:1552-8.
- [14] de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 1994;343:1454-9.
- [15] Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;368:1279-90.
- [16] Mitrou PN, Kipnis V, Thiebaut AC, et al. Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. *Arch Intern Med* 2007;167:2461-8.

- [17] Tognon G, Nilsson LM, Lissner L, et al. The Mediterranean diet score and mortality are inversely associated in adults living in the subarctic region. *J Nutr* 2012;142:1547-53.
- [18] Trichopoulou A, Naska A, Costacou T. Disparities in food habits across Europe. *Proc Nutr Soc* 2002;61:553-8.
- [19] Bifulco M. Mediterranean diet: the missing link between gut microbiota and inflammatory diseases. *Eur J Clin Nutr* 2015.
- [20] David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559-63.
- [21] Panagiotakos DB, Georgousopoulou EN, Pitsavos C, et al. Exploring the path of Mediterranean diet on 10-year incidence of cardiovascular disease: The ATTICA study (2002-2012). *Nutr Metab Cardiovasc Dis* 2015;25:327-35.
- [22] Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006;114:82-96.
- [23] Sofi F, Vecchio S, Giuliani G, et al. Dietary habits, lifestyle and cardiovascular risk factors in a clinically healthy Italian population: the 'Florence' diet is not Mediterranean. *Eur J Clin Nutr* 2005;59:584-91.
- [24] Gardener H, Wright CB, Cabral D, et al. Mediterranean diet and carotid atherosclerosis in the Northern Manhattan Study. *Atherosclerosis* 2014;234:303-10.

Tables

Table 1. Patient's characteristics according to classes of MD score

| Characteristics | Missing data | MD Score 0-1 (N=1098) | MD Score 2-3 (N=1868) | MD Score 4-7 (N=737) | P-trend |
|-----------------------------|--------------|-----------------------|-----------------------|----------------------|---------|
| | | | | | |
| Latitude | | | | | |
| Kuopio | 0 | 472 (43.0) | 520 (27.8) | 56 (7.6) | <0.0001 |
| Stockholm | 0 | 177 (16.1) | 291 (15.6) | 64 (8.7) | |
| Groningen | 0 | 319 (29.1) | 191 (10.2) | 17 (2.3) | |
| Paris | 0 | 68 (6.2) | 275 (14.7) | 158 (21.4) | |
| Milan | 0 | 37 (3.4) | 282 (15.1) | 234 (31.8) | |
| Perugia | 0 | 25 (2.3) | 309 (16.5) | 208 (28.2) | |
| Vascular Risk Factors | | | | | |
| Framingham Risk Score | 0 | 27 (19, 41) | 22 (14, 33) | 18 (11, 28) | <0.0001 |
| European Score | 0 | 5.4 (3.2, 9.0) | 3.8 (2.3, 6.7) | 2.9 (1.8, 5.1) | <0.0001 |
| Male | 0 | 580 (52.8) | 919 (49.2) | 275 (37.3) | <0.0001 |
| Age | 0 | 64.8 ± 5.4 | 63.9 ± 5.31 | 63.9 ± 5.7 | 0.0001 |
| BMI (kg/m²) | 3 | 28.4 ± 4.43 | 27.18 ± 4.22 | 25.83 ± 3.64 | <0.0001 |
| Waist/hip ratio | 11 | 0.93 ± 0.09 | 0.92 ± 0.09 | 0.9 ± 0.09 | <0.0001 |
| Diastolic BP (mmHg) | 5 | 84 ± 10 | 82 ± 10 | 79 ± 9 | <0.0001 |
| Systolic BP (mmHg) | 5 | 147 ± 18 | 142 ± 18 | 135 ± 18 | <0.0001 |
| Hypertension | 2 | 844 (76.9) | 1288 (69.0) | 420 (57.0) | <0.0001 |
| Diabetes mellitus | 0 | 375 (34.2) | 425 (22.8) | 113 (15.3) | <0.0001 |
| Smoking habits | 72 | | | | |
| Never smokers | | 498 (45.4) | 878 (47.0) | 407 (55.2) | <0.0001 |
| Former smokers | | 398 (36.2) | 727 (38.9) | 246 (33.4) | |
| Current smokers | | 202 (18.4) | 263 (14.1) | 84 (11.4) | |
| Pack-years | 72 | 20 (9.0, 33.2) | 17 (7.8, 28.1) | 16.3 (7.5, 30.0) | 0.03 |
| Family history of: | | | | | |
| Coronary artery Disease | 3 | 738 (70.7) | 1170 (64.7) | 407 (57.8) | <0.0001 |
| Cerebrovascular Disease | 3 | 374 (34.1) | 689 (36.9) | 259 (35.1) | 0.49 |
| Peripheral vascular disease | 3 | 118 (10.7) | 236 (12.6) | 89 (12.1) | 0.3 |
| Social Class | 307 | | | | |
| Office work | | 322 (32.0) | 743 (42.9) | 314 (47.7) | <0.0001 |
| Service work | | 374 (37.2) | 562 (32.4) | 202 (30.7) | |
| Manual work | | 309 (30.7) | 428 (24.7) | 142 (21.6) | |

| | | | | | |
|------------------------------|----|-------------------|-------------------|-----------------|---------|
| Study years | | 10.0 ± 3.4 | 10.6 ± 4.1 | 10.5 ± 4.2 | 0.002 |
| Biochemical variables | | | | | |
| Total Cholesterol, mmol/L | 16 | 5.32 ± 1.11 | 5.47 ± 1.12 | 5.8 ± 1.12 | <0.0001 |
| HDL Cholesterol, mmol/L | 16 | 1.23 ± 0.36 | 1.26 ± 0.36 | 1.32 ± 0.37 | <0.0001 |
| Triglycerides, mmol/L | 16 | 1.31 (0.94, 1.91) | 1.31 (0.94, 1.91) | 1.28 (0.9, 1.8) | 0.09 |
| LDL Cholesterol, mmol/L | 81 | 3.39 ± 0.96 | 3.52 ± 1.02 | 3.83 ± 0.97 | <0.0001 |
| Uric Acid, µmol/L | 16 | 320 (273, 369) | 310 (263, 357) | 298 (253, 352) | <0.0001 |
| hs-CRP, mg/L | 11 | 2.1 (0.9, 3.9) | 1.8 (0.8, 3.5) | 1.6 (0.6, 3.1) | <0.0001 |
| Blood glucose, mmol/L | 12 | 6.26 ± 1.82 | 5.9 ± 1.64 | 5.43 ± 1.15 | <0.0001 |
| Creatinine, µmol/L | 17 | 82 (71, 93) | 79 (69, 90) | 75 (65, 88) | <0.0001 |
| Hypoalphalipoproteinemia | 2 | 160 (14.6) | 248 (13.3) | 80 (10.9) | 0.024 |
| Food items | | | | | |
| Fish (portions/week) | 13 | 1.3 ± 0.8 | 1.8 ± 1.1 | 2.6 ± 1.4 | - |
| Wine (dl/day) | 9 | 0.6 ± 1.6 | 1.2 ± 2 | 1.5 ± 2 | - |
| Meat (portions/week) | 14 | 4.4 ± 1.6 | 3.6 ± 1.8 | 2.7 ± 1.6 | - |
| Fruits (portions/day) | 5 | 1.7 ± 0.9 | 2.2 ± 1.3 | 3.2 ± 1.7 | - |
| Milk (dl/day) | 12 | 3.9 ± 2.8 | 2.4 ± 2.3 | 1.6 ± 2.1 | - |
| Eggs (Number/week) | 14 | 2.2 ± 1.7 | 1.2 ± 1.1 | 0.8 ± 0.7 | - |
| Mostly used fat | | | | | |
| Olive oil | 5 | 109 (9.93) | 942 (50.43) | 663 (89.96) | - |
| Margarine | 5 | 595 (54.19) | 479 (25.64) | 34 (4.61) | - |
| Seed oil | 5 | 235 (21.40) | 299 (16.01) | 26 (3.53) | - |
| Other fat | 5 | 159 (14.48) | 148 (7.92) | 14 (1.90) | - |

Values are mean ± SD, or median (interquartile range) or n (%). P values were calculated by linear regression or by chi square for trend.

Table 2. Variables independently associated with MD score by multivariable linear regression with stepwise selection.

| Variables | Beta ^a | SE | P value |
|--------------------------------------|-------------------|-------|---------|
| Latitude (degrees) | -0.09 | 0.003 | <0.0001 |
| Education (study years) | 0.03 | 0.01 | <0.0001 |
| Physical activity (1 step) | 0.11 | 0.03 | 0.0003 |
| Use of antiplatelet agents | 0.19 | 0.06 | 0.0007 |
| Log Hs-CRP (mg/L) | -0.14 | 0.04 | 0.0009 |
| HDL-cholesterol (mmol/L) | 0.20 | 0.06 | 0.002 |
| Body mass index (Kg/m ²) | -0.02 | 0.01 | 0.002 |
| Statin treatment | 0.12 | 0.04 | 0.006 |
| Pack-years of cigarette smoking | -0.003 | 0.001 | 0.009 |
| Creatinine (μmol/L) | -0.003 | 0.001 | 0.02 |
| Pulse pressure (mmHg) | -0.004 | 0.002 | 0.02 |
| Insulin treatment | -0.25 | 0.11 | 0.03 |
| Occupation (office work) | 0.10 | 0.05 | 0.03 |

^aBeta values indicate change in MD score associated with a unit increment of the predictor. Variables not significantly associated were: sex, age, blood glucose, triglycerides, uric acid, WBC count, family history of PVD, CVD and CHD and other pharmacological treatments (Beta blockers, calcium antagonists, ACE inhibitors, sartans, diuretics, insulin, estrogens, statin, fibrates and fish-oil).

Table 3. Vascular events stratified according to the MD score.

| MD Score | 0-1 | 2-3 | 4-7 |
|--|-----------|----------|----------|
| | n (%) | n (%) | n (%) |
| Combined Events ^a (n=215) | 101 (9.2) | 94 (5.0) | 20 (2.7) |
| Cardiovascular Events ^b (n=125) | 58 (5.3) | 56 (3.0) | 11 (1.5) |
| Cerebrovascular Events ^c (n=73) | 32 (2.9) | 33 (1.8) | 8 (1.1) |

^aCombined events: Ischemic stroke, transitory ischemic attack, acute myocardial infarction, angina pectoris, angioplasty, coronary bypass grafting and sudden cardiac death or any revascularization or peripheral arterial territories and new diagnosis of intermittent claudication.

^bCardiovascular events: acute myocardial infarction, angina pectoris, coronary angioplasty or bypass grafting and sudden cardiac death.

^cCerebrovascular events: Ischemic stroke, transitory ischemic attack.

Table 4. Association between MD score and vascular events: multivariable adjusted Cox models.

| | Combined Events (n=215) | | | | Cardiovascular Events (n=125) | | | Cerebrovascular Events (n=73) | | |
|----------------|----------------------------|-----------------|--------------------------|-------------------|----------------------------------|--------------------------|-------------------|----------------------------------|--------------------------|---------------|
| | MD-score | Events/Subjects | HR (95% CI) | p-value | Events/Subjects | HR (95% CI) | p-value | Events/Subjects | HR (95% CI) | p-value |
| Model 1 | 0-1 | 101/1098 | 1 | | 58/1098 | 1 | | 32/1098 | 1 | |
| | 2-3 | 94/1868 | 0.55 (0.41, 0.73) | <0.0001 | 56/1868 | 0.58 (0.40, 0.84) | 0.004 | 33/1868 | 0.59 (0.36, 0.96) | 0.03 |
| | 4-7 | 20/737 | 0.29 (0.18, 0.47) | <0.0001 | 11/737 | 0.28 (0.15, 0.53) | 0.0001 | 8/737 | 0.35 (0.16, 0.75) | 0.007 |
| | Trend | | 0.74 (0.67, 0.82) | <0.0001 | | 0.76 (0.66, 0.87) | <0.0001 | | 0.72 (0.61, 0.87) | 0.0004 |
| Model 2 | 0-1 | 101/1098 | 1 | | 58/1098 | 1 | | 32/1098 | 1 | |
| | 2-3 | 94/1868 | 0.62 (0.46, 0.84) | 0.0023 | 56/1868 | 0.65 (0.44, 0.97) | 0.04 | 33/1868 | 0.66 (0.39, 1.13) | 0.13 |
| | 4-7 | 20/737 | 0.36 (0.21, 0.62) | 0.0002 | 11/737 | 0.35 (0.17, 0.73) | 0.005 | 8/737 | 0.43 (0.18, 1.04) | 0.06 |
| | Trend | | 0.79 (0.70, 0.89) | 0.0002 | | 0.81 (0.69, 0.96) | 0.012 | | 0.75 (0.61, 0.93) | 0.009 |
| Model 3 | 0-1 | 101/1098 | 1 | | 58/1098 | 1 | | 32/1098 | 1 | |
| | 2-3 | 94/1868 | 0.53 (0.38, 0.74) | 0.0002 | 56/1868 | 0.51 (0.33, 0.80) | 0.003 | 33/1868 | 0.65 (0.37, 1.15) | 0.14 |
| | 4-7 | 20/737 | 0.31 (0.17, 0.56) | 0.0001 | 11/737 | 0.27 (0.12, 0.63) | 0.002 | 8/737 | 0.41 (0.16, 1.1) | 0.065 |
| | Trend | | 0.75 (0.67, 0.87) | <0.0001 | | 0.75 (0.62, 0.90) | 0.002 | | 0.76 (0.61, 0.96) | 0.021 |

Model 1: unadjusted; **Model 2:** adjusted for age, sex and stratified by latitude; **Model 3:** as model 2 plus smoking, body mass index, education, physical activity, occupation, LDL and HDL cholesterol, triglycerides, hs-CRP, creatinine, plasma glucose, pulse pressure, antiplatelet and statin treatments.

Figure legends

Figure 1. Kaplan-Meier incidence curves of combined, cardio and cerebro vascular events stratified by MD adherence score classes.

Combined endpoint is a composite of myocardial infarction, sudden cardiac death, angina pectoris, ischemic stroke, transient ischemic attack, new diagnosis of intermittent claudication or any surgical intervention or revascularization of coronary or peripheral arteries.

Cardiovascular events include acute myocardial infarction, angina pectoris, coronary angioplasty or bypass grafting and sudden cardiac death. Cerebrovascular events include ischemic stroke, transitory ischemic attack. HR = hazard ratio, MD = Mediterranean diet, VEs = vascular events.

Covariates for adjusted HRs are the same of those reported in Table 2 model 3.

Figure 2. Hazard ratios for combined cardiovascular events associated with one point increase of Mediterranean diet score: subgroups analysis.

*Number of vascular risk factors (age>64, diabetes, hypertension and hypercholesterolemia).

Horizontal lines represents 95%CI, adjusted for covariates in Model 3 (see Methods), excluding the respective stratification variables.

Appendix

The IMPROVE study group

- *Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Milan, Italy:* E. Tremoli, D. Baldassarre, Laura Calabresi, C.R. Sirtori.
- *Centro Dislipidemie E. Grossi Paoletti, Ospedale Ca' Granda di Niguarda. S. Castelnovo.*
- *Centro Cardiologico Monzino, IRCCS, Milan Italy:* E. Tremoli, D. Baldassarre, M. Amato, B. Frigerio, A. Ravani, D. Sansaro, D. Coggi, F. Veglia, C. Tedesco, A. Bonomi.
- *Atherosclerosis Research Unit, Departments of Medicine and Cardiology, Karolinska University Hospital Solna, & Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden:* M. Ahl, G. Blomgren, M.J. Eriksson, P. Fahlstadius, M. Heinonen, L. Nilson.
- *University College of London, Department of Medicine, Rayne Institute, London, United Kingdom:* J. Cooper, J. Acharya.
- *Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland:* K. Huttunen, E. Rauramaa, H Pekkarinen, I.M. Penttila, J. Törrönen.
- *Department of Medicine, University Medical Center Groningen, Groningen & Isala Clinics Zwolle, Department of Medicine; the Netherlands:* A.I. van Gessel, A.M van Roon, G.C. Teune, W.D. Kuipers, M. Bruin, A. Nicolai, P. Haarsma-Jorritsma, D.J. Mulder, H.J.G. Bilo, G.H. Smeets,
- *Assistance Publique - Hôpitaux de Paris; Service Endocrinologie-Metabolisme, Groupe Hôpitalier Pitié-Salpêtrière, Unités de Prévention Cardiovasculaire, Paris, France:* J.L. Beaudoux, J.F. Kahn, V. Carreau, A. Kontush.
- *Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio Campus:* J. Karppi, T. Nurmi, K. Nyysönen, R. Salonen, T.P. Tuomainen, J. Tuomainen, J. Kauhanen.
Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy: G. Vaudo, A. Alaeddin, D. Siepi, G. Lupattelli.