Journal of Thrombosis and Haemostasis, **1**: 1744–1752

ORIGINAL ARTICLE

A high-score Mediterranean dietary pattern is associated with a reduced risk of peripheral arterial disease in Italian patients with Type 2 diabetes

E. CICCARONE,*† A. DI CASTELNUOVO,* M. SALCUNI,‡ A. SIANI,§ A. GIACCO,¶ M. B. DONATI,** G. DE GAETANO,** F. CAPANI† and L. IACOVIELLO* on behalf of the Gendiabe Investigators††

*'Angela Valenti' Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy; †Department of Medicine and Aging and ‡Institute of Radiology, University of Chieti, Chieti, Italy; §Institute of Food Sciences, National Research Council, Avellino, Italy; ¶Department of Clinical and Experimental Medicine, 'Federico II' University of Naples, Naples, Italy; and **Center for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy

To cite this article: Ciccarone E, Di Castelnuovo A, Salcuni M, Siani A, Giacco A, Donati B, De Gaetano G, Capani F, Iacoviello L, on the behalf of the Gendiabe investigators. A high-score Mediterranean dietary pattern is associated with a reduced risk of peripheral arterial disease in Italian patients with Type 2 diabetes. *J Thromb Haemost* 2003; **1**: 1744–52.

Summary. *Background*: The 'Mediterranean diet' is considered to exert protective effects on cardiovascular disease, although a wide range of dietary patterns exists among subjects living even in the same Mediterranean country. *Objective*: To investigate the association between specific dietary patterns and peripheral arterial disease (PAD) in Italian Type 2 diabetes patients. *Design*: From a cohort of 944 patients with Type 2 diabetes, 144 patients with PAD were selected, and matched for age and sex with 288 Type 2 diabetic control patients without macrovascular complications. A dietary score was elaborated from a semiquantitative food frequency questionnaire. The higher the final score, the healthier the eating habit. *Results*: In multivariate analysis, a higher score was independently associated with a significant reduction in PAD risk [odds ratio (OR) =0.44; 95% confidence interval (CI) 0.24, 0.83]. Diabetes duration (OR > 15 years =2.49; 95% CI 1.45, 4.25), hypertension (OR = 2.12; 95% CI 1.31, 3.45) and butter consumption (OR = 2.6; 95% CI 1.15, 3.68) were also significantly associated with PAD. The dietary score significantly improved the predictive value of models based on duration of diabetes and hypertension. (LSR = 2.19, DF = 7, $P < 0.001$). The effect of a high dietary score on the risk of PAD was independent of diabetes duration and hypertension. *Conclusions*: In Italian Type 2 diabetics, a higher dietary score has a protective role against PAD. The use of butter increases the risk of PAD even in patients regularly consuming olive oil. Dietary advice may be helpful for the prevention of PAD in diabetics even in populations traditionally accustomed to a Mediterranean dietary habit.

Keywords: diabetes, Mediterranean diet, peripheral arterial disease.

Correspondence: Licia Iacoviello, 'Angela Valenti' Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Italy.
Tel.: +39 08 7257 0298; fax +39 08 7257 0299; e-mail: iaco@negrisud.it

††The GENDIABE investigators are listed at the end of this paper.

Received 6 December 2002, accepted 23 March 2003

Introduction

Peripheral arterial disease (PAD) has a high social impact, particularly in elderly patients. The prevalence of PAD varies between 12% and 14% in the general population, and increases up to 20% over the age of 75 [1–3]. PAD is the more prevalent expression of vascular arteriosclerosis in Type 2 diabetes patients, predisposing them to recurrent infection or surgical treatment and eventually to the amputation of the affected limb [4,5]. In the Framingham study, PAD was 6.5-fold more frequent in patients with diabetes compared with patients without diabetes [6]. Recognized risk factors for atherosclerotic disease (age, male gender, cigarette smoking, diabetes, hypertension, hypercholesterolemia and hypertriglyceridemia) also apply to PAD [7,8], but other factors might also be involved.

Recent studies suggested that the so-called 'Mediterranean diet' might have protective effects on cardiovascular disease, particularly coronary heart disease [9,10]. Other studies showed that a more favorable saturated/polyunsaturated fat ratio and an increase in the dietary antioxidant potential has beneficial effects also in secondary prevention of myocardial infarction [11,12].

Since atherosclerosis represents the pathogenic mechanism of both PAD and coronary heart disease, similarities in dietary risk factors for these two conditions could be hypothesized. However, only few studies have explored the association between diet and risk of PAD [13,14]. Higher intake of saturated fatty acids was associated with increased risk of PAD, while higher intake of fiber, vitamin C and E were associated with a lower risk. In two US cohorts, moderate alcohol consumption demonstrated a protective effect on the risk of both PAD and intermittent claudication [15,16].

With respect to an operational definition of the Mediterranean diet as 'the dietary pattern of people living in Southern Italy in the 1960s' [9], the dietary profile of the Mediterranean regions appears significantly different from that of other western countries, with particular regard to the use of fat, cereal-derived foods, vegetables and type of alcohol [9,10,17]. In the Mediterranean diet, olive oil (monounsaturated) is the prevalent visible fat, the intake of saturated fats is relatively low, while fish guarantees a substantial provision of polyunsaturated fats (ω-3 polyunsaturated fatty acids). The Mediterranean diet is also characterized by a high amount of vegetables, fruits and whole grain products, which represent a good source of fibers, complex carbohydrates, proteins, potassium, antioxidant substances, and vitamins. Finally, the consumption of red wine is prevalent with respect to other types of alcoholic beverages.

However, uncertainty still remains about a definitive definition of the Mediterranean diet, since it is characterized by a large interindividual variability in type and amount of food items regularly eaten. Moreover, a tendency to adopt westernized food habits even in Southern regions of Europe has increased throughout the last 20 years [18–20]. Therefore, the impact on the cardiovascular risk profile of different regional and/or individual patterns within the general framework of the Mediterranean diet needs to be reconsidered. In this study, we aimed at evaluating the impact of different levels of adherence to a Mediterranean dietary pattern on PAD in Type 2 Italian diabetes patients. We studied a group of patients all born and living in the same region, Abruzzo, located in the Central-Southern region of Italy, where a broadly defined Mediterranean diet is traditionally consumed, in order to investigate if inter-individual differences within the traditional dietary pattern can influence the risk of developing PAD, a disease with a high social impact.

Patients and methods

Study population

The study population consisted of 944 out of 1274 patients with Type 2 diabetes, consecutively seen at the Diabetes Care Center of the General Hospital of Pescara in Italy, from 1996 until 2000 (response rate to the invitation to the study: 74%). Patients were eligible if they were Italian citizens from the Abruzzo region and fulfilled the World Health Organization criteria for Type 2 diabetes. The exclusion criteria consisted of the presence of a concomitant major disease.

Out of 944 participating patients, 144 (cases) were identified as having symptoms and clinical signs of PAD. PAD was defined by either of the following two criteria: persistently recurring ischemic rest pain requiring regular adequate analgesia for more than 2 weeks, with an ankle systolic pressure =50 mmHg and/or a toe systolic pressure =30 mmHg; or ulceration or gangrene of the foot or toes, with an ankle systolic pressure of =50 mmHg or a toe systolic pressure =30 mmHg [21]. Moreover, patients with absence of peripheral pulses were also selected.

Cases were frequency-matched for age and sex with 288 Type 2 diabetes patients (controls) selected from the 588 patients of the survey free of macrovascular complications (myocardial infarction, angina pectoris, stroke, PAD). The clinical diagnosis of PAD was subsequently confirmed by a color-duplex ultrasound examination (low-frequency probes −3.75 to 5 MHz [21]) performed by a trained radiologist [22].

All patients had received dietary advice from a professional dietitian in the Center.

Assessment of clinical features

Patient information was collected by a standardized questionnaire, filled in by a trained monitor.

The prevalence of thrombotic complications was evaluated by a trained monitor who reviewed the medical records of all patients reporting at least one episode of angina pectoris (AP), acute myocardial infarction (AMI), stroke or PAD. Standardized criteria from the MONICA study were used to confirm or reject the diagnosis of each event reported [23].

Assessment of diet

Dietary information was collected by a simple semiquantitative 24-item food-frequency questionnaire (FFQ), structured as a list of different food items: bread, whole-wheat bread, pasta, milk, meat, eggs, raw vegetables, cooked vegetables, carrots, fish, cheese, processed meat, fruit, olive oil, vegetable-non-olive oil, butter, margarine, cream, red, white and rosé wine, beer, spirits and coffee. Patients were asked to rate the frequency of consumption for each item (from 0 to 6/week or once or twice a day) and portion size (small, medium or large). Added fats were classified into five categories (frequency of consumption 1–14 times/week).

Wine consumption was classified as: never drinking, less than two glasses, two to three glasses, or more than three glasses of wine a day (one glass of wine equals about 120 mL). Beer and spirit consumption was classified as never, sometimes, and daily. Coffee consumption was classified as never, sometimes, once a day, twice or more a day.

The validity of dietary data collected by the FFQ was preliminarily assessed against a 7-day food record. Both questionnaires were obtained from 34 volunteers (M $n = 17$, F $n = 17$, age 65 ± 8 years) living in the same region. Total energy intake was underestimated by the FFQ (1557 ± 90 vs. 2024 ± 84, mean $\pm$ SEM), although a significant correlation coefficient was observed between the two measurements ($r = 0.457$, $P = 0.007$). Energy-adjusted correlation coefficients between the FFQ and the 7-day record ranged from 0.118 for protein to 0.919 for alcohol, indicating a satisfactory validity of the FFQ in this study population, similar to that reported in previous studies [24,25].

Although quantification of adherence to a diet is difficult, grouping food items in order to obtain composite scores is a method often used to describe total diet; these scores may be very useful for the evaluation of epidemiological associations, although they require some operational definitions [26]. On the basis of food groups suggested by Davidson and Passmore [27], a dietary score was conceived for all dietary items, by assigning a score 1 to any food (frequency/amount) item with reasonable evidence supporting its beneficial effect on coronary artery disease, but a score 0 for those with potentially harmful effects. Thus, for each individual, the higher the final score, the 'healthier' the eating habit.

We assumed that a diet rich in components scored 1 had beneficial health effects whereas a diet with a prevalence of components scored 0 would be less healthy. This assumption is based on epidemiological and biological evidence as summarized in a report of the National Academy of Science [28] and a critical overview [29]. The frequency of consumption (times/week) for each food item was based on the average consumption in a population of the Abruzzo region. We assigned 1 point to a moderate (3–6/week) and to a high (=7/week) consumption of raw and cooked vegetables, to a high consumption of carrots (=3/week) and fruit (=7/week), and to fish more than once per week. In contrast, 0 was assigned to a high consumption of eggs, meat, processed meat or cheese. We assigned 1 point to the daily (or twice a day) consumption of olive oil, but 0 to the consumption of non-olive vegetable oil, butter or cream. A score of 1 was given to a moderate consumption of wine (less than two glasses or two to three glasses per day), beer (sometimes) or spirits (sometimes). The total dietary score was obtained summing points for each food item. Although cereals (pasta and bread) are important components of the Mediterranean diet, they were considered as neutral in the present analysis, because of their strong relation to metabolic control. Limitation of their use is already strongly advised in Type 2 diabetes patients. Milk is often seen as a potential promoter of atherosclerosis and coronary heart disease because it is a source of cholesterol and saturated fatty acids. Although there is some controversy in the literature, recent studies indicate that milk and milk products may not adversely affect blood lipids as would be predicted from their fat content and fat composition. Therefore, we decided to consider milk as neutral in our analysis [30,31].

The validity of the score was assessed by comparing the results obtained using the FFQ and the 7-day food record. The correlation coefficient between the scores obtained with the two questionnaires was 0.71 ($P = 0.001$).

Statistical analysis

Each case patient was age- and sex-matched with two control patients (three age ranges were used for matching: <60 years; >60 years to <70 years; and >70 years). Data for continuous variables were expressed as mean ± SD; a two-tailed *P*-value <0.05 was chosen as the level of significance. All computations

Table 1 Clinical characteristics of Type 2 diabetes patients with (cases) and without (controls) peripheral arterial disease (PAD)

		Cases % (*n*) (144)	Controls% (*n*) (288)	*P*-value
Patient characteristics				
Age (years)	<60	25% (36)	25% (72)	–
	60–69	42% (60)	42% (120)	
	≥70	33% (48)	33% (96)	
Sex	Men	71.5% (103)	71.5% (206)	–
	Women	28.5% (41)	28.5% (82)	
Diabetes duration (years)	≤5	37% (53)	51% (146)	0.008
	6–14	31% (45)	29% (85)	
	≥15	32% (46)	20% (57)	
HbA_{1c}	<7	30% (43)	34% (99)	
	7–9	57% (82)	52% (149)	
	>9	13% (57)	14% (40)	
Hypertension	No	38% (55)	55% (159)	0.0005
	Yes	62% (89)	45% (128)	
Dyslipidemia	No	68.5% (98)	71% (202)	0.60
	Yes	31.5% (45)	29% (83)	
Body mass index (kg/m^2)	<25	18% (26)	24% (70)	0.13
	≥25	82% (118)	76% (218)	
Smoke	Never	37% (53)	34% (98)	0.74
	Former	39% (56)	44% (126)	
	Current ≤10 cigarettes day^{-1}	9% (13)	8% (24)	
	Current >10 cigarettes day^{-1}	15% (22)	14% (40)	
Physical activity	Minimal	55% (76)	43% (121)	0.049
	Moderate	39% (54)	48% (133)	
	Intense	6% (9)	9% (25)	

HbA_{1c}, Glycated hemoglobin. *P*-value derived from χ^2 tests for proportions. Age and sex were matched between case and control patients.

were carried out using the SAS statistical package (SAS Institute, Cary, NC, USA) [32]. The means were compared by analysis of variance, controlling for matching (procedure GLM for SAS). Odds ratios (and their 95% confidence intervals) were calculated as estimators of relative risk. Odds ratios were obtained by univariate and multivariate conditional to matching logistic regression analysis (procedure PHREG for SAS). Covariates included in multiple analyses were duration of diabetes, smoking habits, history of hyperlipidemia, hypertension, body mass index (BMI), and physical activity.

Finally, we addressed the potential additive value of the dietary score to diabetes duration and hypertension, in a two-step process. Initially, we used likelihood ratio testing to determine whether addition of that marker to classical risk factors for diabetes significantly improved the risk prediction model. Similar logistic regression analyses were then performed after dividing studied subjects into six groups based on diabetes duration longer or shorter than 10 years, presence or absence of hypertension and dietary score. All *P*-values are two-tailed and all confidence intervals computed at 95% level.

Results

Characteristics of patients are shown in Table 1. As expected, no significant difference between cases and controls was found in the distribution of age and sex. Again, no significant difference was found for history of dyslipidemia, smoking habits, BMI and levels of glycated hemoglobin (HbA_{1c}). By contrast, cases had longer diabetes duration, higher prevalence of hypertension and lower physical activity.

Tables 2–4 show the consumption frequency rate of each food item investigated. Cases reported a higher consumption rate of

Table 2 Dietary item distribution, dietary score in cases and controls and univariate and multivariate odds ratio (OR) of peripheral arterial disease (PAD)

Dietary items	Category	Score*	Cases % (*n*) (144)	Controls % (*n*) (288)	OR†	CI 95%	OR‡ multi	CI 95%
Food items with score								
Raw vegetables	0–2/week	0	6% (8)	4% (12)	1.0	–	1.0	–
	3–6/week	1	64% (89)	56% (149)	0.88	(0.33, 2.32)	0.84	(0.30, 2.4)
	≥7/week	1	30% (43)	40% (106)	0.61	(0.22, 1.63)	0.56	(0.19, 1.65)
Cooked vegetables	0–2/week	1	6% (9)	12% (31)	1.0	–	1.0	–
	3–6/week	0	67% (93)	58% (154)	2.20	(0.98, 4.93)	2.40	(0.99, 5.76)
	≥7/week	0	27% (38)	30% (81)	1.64	(0.71, 3.82)	1.72	(0.68, 4.34)
Carrots	0–2/week	0	56% (78)	54% (144)	1.0	–	1.0	–
	3–7/week	1	44% (62)	46% (123)	0.97	(0.64, 1.46)	0.98	(0.63, 1.53)
Fruit	0–1/day	0	34% (48)	33% (88)	1.0	–	1.0	–
	>1/day	1	66% (92)	67% (178)	0.58	(0.16, 2.11)	0.72	(0.14, 3.70)
Eggs	0/week	1	13% (18)	16% (41)	1.0	–	1.0	–
	1–2/week	1	67% (94)	69% (185)	1.12	(0.61, 2.05)	0.95	(0.49, 1.83)
	≥3/week	0	20% (28)	15% (40)	1.55	(0.75, 3.21)	1.74	(0.78, 3.87)
Fish	0/week	0	18% (25)	18% (47)	1.0	–	1.0	–
	1–2/week	1	58% (82)	63% (169)	0.99	(0.59, 1.68)	0.88	(0.49, 1.57)
	≥3/week	1	24% (33)	19% (51)	1.6	(0.87, 3.03)	1.65	(0.84, 3.24)
Meat	0–2/week	1	20% (28)	22% (58)	1.0	–	1.0	–
	3–6/week	0	64% (90)	62% (166)	1.18	(0.69, 2.02)	1.27	(0.71, 2.27)
	≥7/week	0	16% (22)	16% (43)	1.14	(0.57, 2.27)	1.17	(0.54, 2.50)
Processed meat	0/week	1	21% (29)	24% (65)	1.0	–	1.0	–
	1–3/week	0	51% (72)	47% (126)	1.24	(0.75, 2.05)	1.19	(0.69, 2.04)
	≥4/week	0	28% (39)	29% (76)	1.13	(0.62, 2.06)	1.30	(0.67, 2.51)
Cheese	0–2/week	1	19% (27)	19% (52)	1.0	–	1.0	–
	3–6/week	0	72% (101)	64% (170)	1.16	(0.69, 1.97)	1.25	(0.71, 2.20)
	≥7/week	0	9% (12)	17% (45)	0.48	(0.22, 1.06)	0.61	(0.26, 1.45)
Food items without a score								
Pasta	0–2/week		7% (10)	8% (22)	1.0	–	1.0	–
	3–6/week		53% (74)	47% (126)	1.31	(0.58, 2.95)	1.70	(0.68, 4.13)
	≥7/week		40% (56)	45% (119)	1.03	(0.45, 2.36)	1.33	(0.54, 3.27)
Bread (white)	0/day		10% (14)	14% (37)	1.0	–	1.0	–
	1/day		19% (26)	24% (64)	1.26	(0.58, 2.72)	1.26	(0.58, 2.72)
	>1/day		71% (100)	62% (166)	1.74	(0.90, 3.37)	1.74	(0.90, 3.37)
Bread (brown)	0/day		69% (97)	70% (186)	1.0	–	1.0	–
	1/day		14% (20)	14% (36)	1.10	(0.60, 1.96)	1.10	(0.60, 2.02)
	>1/day		16% (23)	17% (44)	1.01	(0.58, 1.76)	1.04	(0.56, 1.91)
Milk	0–2/week		26% (37)	22% (59)	1.0	–	1.0	–
	3–6/week		6% (8)	8% (22)	0.57	(0.23, 1.41)	0.52	(0.20, 1.38)
	≥7/week		68% (95)	70% (186)	0.82	(0.50, 1.33)	0.71	(0.42, 1.19)

*1, Healthy; 0, non-healthy. †Univariate analysis. ‡OR calculated from a model including terms for each food item together with all the covariates (duration of diabetes, hypertension, dyslipidemia, body mass index, physical activity, smoking).

Table 3 Distribution of added fats in cases and controls and univariate and multivariate odds ratio (OR) of peripheral arterial disease (PAD)

Added fats		Score*	Cases % (*n*) (144)	Controls % (*n*) (288)	OR† univ	CI 95%	OR‡ multi	CI 95%
Olive oil	0/day	0	0% (0)	0% (0)	–	–	–	–
	≤1/day	1	6% (8)	8% (21)	1.0	–	1.0	–
	>1/day	1	94% (132)	92% (241)	1.43	(0.59, 3.46)	1.78	(0.68, 4.66)
Vegetable oil	0/week	1	39% (52)	49% (120)	1.0	–	1.0	–
	1–2/week	1	29% (39)	26% (64)	1.42	(0.83, 2.44)	1.30	(0.72, 2.32)
	>2/week	0	32% (43)	25% (63)	1.61	(0.96, 2.69)	1.72	(0.99, 3.03)
Butter	No	1	76% (107)	84% (223)	1.0	–	1.0	–
	Yes	0	24% (33)	16% (43)	1.58	(0.95, 2.64)	2.06	(1.15, 3.68)
Cream	No	1	94% (131)	96% (256)	1.0	–	1.0	
	Yes	0	6% (9)	4% (10)	1.90	(0.72, 5.03)	2.79	(0.93, 8.35)
Margarine	No	1	94% (132)	95% (252)	1.0	–	1.0	
	Yes	0	6% (8)	5% (14)	1.10	(0.46, 2.63)	1.19	(0.45, 3.11)

*1, Healthy; 0, non-healthy. †Univariate analysis. ‡OR calculated from a model including terms for each food item together with all the covariates (duration of diabetes, hypertension, dyslipidemia, body mass index, physical activity, smoking).

butter compared with controls. In multivariate analysis for single food items, use of butter more than twice a week resulted in an increase of PAD risk of about double (Table 3). There was no significant difference in the consumption of all other individual food items between patients and controls.

Wine consumption was assessed by grouping the consumption of the three different types of wine (red, white and rosé wine). About 49% of Type 2 diabetes patients never drank any type of wine, while 81% never consumed beer or spirits. No association was found when total alcohol consumption was used for analysis (Table 4).

To assess the role of a global 'healthy' dietary pattern, individual dietary scores were grouped into three categories, according to approximated tertiles of distribution of the diet score in controls (Table 5). (first category: score 0–8 points; second category: 9–10; third category: 11 or more points). We assumed that higher scores are associated with healthier dietary habits. Thirty-eight percent of controls ($n = 102$) scored ≥ 11 compared with 29% of PAD cases ($n = 40$), 41% of cases ($n = 58$) scored between 9 and 10 compared with 39% of controls ($n = 105$) and 30% of the cases ($n = 42$) scored from 0 to 8 compared with 23% of controls ($n = 62$, $P = 0.043$).

In multivariate analysis, dietary score, diabetes duration and hypertension were the only variables significantly and independently associated with the risk of PAD (Table 5). The highest dietary score category was associated with a significant 56% reduction in the risk of PAD. The risk progressively increased with the duration of diabetes: a diabetes duration >15 years increased 2.5 times the risk of PAD; finally, the presence of hypertension increased the risk by more than double.

To explore whether the dietary score added to the predictive value of diabetes duration and hypertension, a series of additional analyses were undertaken. First, likelihood ratio tests were used to compare the fit of risk prediction model based on

Table 4 Drink distribution and dietary score in cases and controls and univariate and multivariate odds ratio (OR) of peripheral arterial disease (PAD)

Type of alcohol	Score*	Cases % (*n*) (144)	Controls % (*n*) (288)	OR† univ	CI 95%	OR‡ multi	CI 95%
Wine (white, red, rosé)							
Never	0	49% (64)	44% (114)	1.0	–	1.0	–
<2 glasses/day (<200 mL day^{-1})	1	18% (24)	23% (58)	0.74	(0.42, 1.30)	0.78	(0.42, 1.46)
2–3 glasses/day (200–400 mL day^{-1})	1	27% (36)	28% (72)	0.86	(0.50, 1.49)	1.0	(0.55, 1.80)
>3 glasses/day (>400 mL day^{-1})	0	6% (8)	5% (12)	1.12	(0.42, 2.96)	1.05	(0.37, 2.96)
Beer							
Never	0	70% (98)	67% (177)	1.0	–	1.0	–
Sometimes	1	29% (40)	32% (85)	0.83	(0.51, 1.33)	0.90	(0.53, 1.54)
Daily	0	1% (1)	1% (3)	0.57	(0.06, 5.56)	0.34	(0.02, 5.41)
Spirits							
Never	0	81% (112)	76% (201)	1.0	–	1.0	–
Sometimes	1	19% (27)	23% (61)	0.76	(0.44, 1.29)	0.77	(0.43, 1.36)
Daily	0	0% (0)	3% (1)	–	–	–	–
Grams of alcohol							
Never	0	39% (56)	34% (98)	1.0	–	1.0	–
≤12 g day^{-1}	1	27% (39)	29% (82)	0.79	(0.46, 1.36)	0.92	(0.51, 1.67)
13–36 g day^{-1}	1	26% (37)	26% (76)	0.80	(0.45, 1.40)	1.0	(0.33, 2.76)
≥36 g day^{-1}	0	6% (8)	4% (12)	1.0	(0.37, 2.72)	0.95	(0.33, 2.76)

*1, Healthy; 0, non-healthy. †Univariate analysis. ‡OR calculated from a model including terms for each food item together with all the covariates (duration of diabetes, hypertension, dyslipidemia, body mass index, physical activity, smoking).

Table 5 Adjusted odds ratios (OR) of peripheral arterial disease (PAD) according to the dietary score and common risk factors

Characteristics	OR	CI 95%
Dietary score		
0–8	1.0	–
9–10	0.73	(0.42, 1.26)
≥11	0.44	(0.24, 0.83)
Diabetes duration (years)		
≤5	1.0	–
6–14	1.75	(1.05, 2.90)
≥15	2.52	(1.45, 4.39)
Hypertension		
No	1.0	–
Yes	2.30	(1.39, 3.79)
Dyslipidemia		
No	1.0	–
Yes	0.89	(0.54, 1.45)
Body mass index		
<25	1.0	–
≥25	1.50	(0.82, 2.74)
Smoking habit		
Never	1.0	–
Former	0.70	(0.37, 1.33)
Current ≤10 cigarettes day^{-1}	1.16	(0.47, 2.86)
Current >10 cigarettes day^{-1}	1.20	(0.54, 2.65)
Physical activity		
Minimal	1.0	–
Moderate	0.58	(0.27, 1.25)
Intense	0.45	(0.14, 1.44)

All models conditioned on age and sex, and additionally adjusted for dietary score, diabetes duration, hypertension, dyslipidemia, body mass index, smoking habit and physical activity.

the measurement of dietary score in combination with classical risk factors. Second, we computed the odds ratio of developing PAD in analyses that stratified study participants into six groups based on diabetes duration or hypertension for dietary score. Dietary score significantly improved the predictive value of models based on duration of diabetes and hypertension ($LSR = 2.19$; $DF = 7$, $P < 0.001$).

The effect of dietary score on the risk of PAD was independent of diabetes duration; indeed, it affected the risk of PAD in the presence of a diabetes duration either lower or higher than 10 years (Fig. 1a). Similar results were observed for the presence or absence of hypertension (Fig. 1b).

Discussion

A high-score Mediterranean dietary pattern was associated with a reduced risk of PAD in patients with Type 2 diabetes. The patients recruited in this study were all from at least two generations living in Abruzzo, a region of Central-Southern Italy where the consumption of the Mediterranean diet is widely diffused, although with large interindividual variation. All patients attended a diabetes outpatient clinic and since the time of the diagnosis routinely received appropriate dietary counseling for the treatment of diabetes. Ten percent of the PAD patients and 20.5% of control patients had diabetes controlled only with a 55–60% carbohydrate diet, while the others required either hypoglycemic agents or intensive insulin treatment.

To evaluate the dietary patterns of patients with Type 2 diabetes, a dietary score was elaborated in order to assess the number of food items consumed with proved or supposed protective effect against coronary artery disease. A progressive decrease in the risk of PAD in patients with Type 2 diabetes was observed along with the increase in dietary score. Patients in the highest score category had a strong reduction in the risk of PAD (average 56%), independently of the duration of diabetes, its metabolic control and other recognized risk factors for ischemic vascular disease.

Classic Mediterranean diet scores are useful when Mediterranean diet is compared with other diets, such as western or eastern diets; thus, we introduced a new score to assess different levels of adherence to Mediterranean diet within a population living in a Mediterranean region [33].

None of the individual dietary items considered as healthy was by itself significantly associated with the risk of PAD,

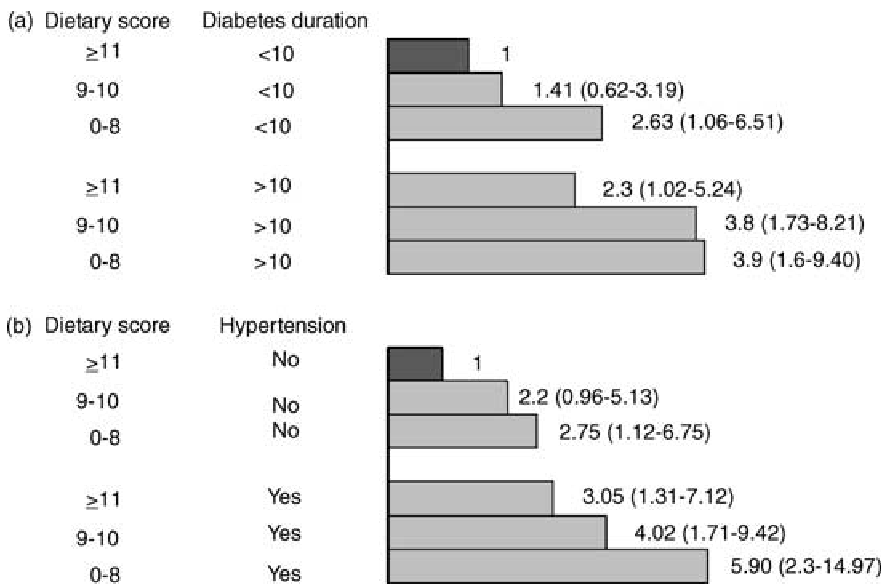

Fig. 1. Odds ratios of peripheral arterial disease among Type 2 diabetes patients, stratified by (a) diabetes duration and (b) hypertension. Reference group: dietary score ≥11 and diabetes duration ≤10 years or absence of hypertension.

suggesting that rather than single foods or nutrients, a global healthy dietary pattern could exert a protective effect against atherothrombotic disease, such as PAD. This hypothesis is in agreement with the negative results of several recent clinical trials supplementing only specific nutrients, which failed to demonstrate any reduction in the risk of ischemic vascular disease [12,34,35].

In our study, almost all patients with Type 2 diabetes consumed olive oil at least once a day as main added fat and no difference was found in the frequency of its consumption between cases and controls. In contrast, the consumption of butter and non-olive vegetable oil was very low (Table 3). Butter was occasionally consumed as an added fat in 20% of the population, while non-olive vegetable oil was used, essentially for frying, more than twice a week by about 40% of patients with Type 2 diabetes. Nevertheless, the use of butter, even with a low frequency, was associated with higher risk of PAD, despite a daily consumption of olive oil.

Fish, which contains a high amount of polyunsaturated fatty acids, is another important component of the Mediterranean diet. The consumption of fish or of its derivatives once or twice a week is associated with a significant reduction in mortality from coronary heart disease [13,36,37]. However, this effect might be related to causes other than a reduction in the atherosclerotic process, which is the main pathogenic feature of PAD. Recent studies of fish-derived ω-3 fatty acid supplementation in secondary prevention have demonstrated that the effect of fish oil on cardiovascular mortality is mainly attributable to a reduction in sudden death [12,37]. These findings may explain the absence of association between fish consumption and PAD.

Red wine is consumed largely during meals in Mediterranean countries and may contribute to lower rates of cardiovascular disease observed among Mediterranean populations [38,39]. However, there was no significant difference between cases and controls in the consumption of wine, other types of alcohol or total alcohol, although a non-significant trend towards a reduction in PAD risk was associated with moderate intake compared with low or no intake. The consumption of wine and total alcohol, in our population of patients with Type 2 diabetes, was lower than that expected in an aged Italian population. Indeed, only half of the patients (51% cases, 56% controls) reported drinking wine. Several of them had stopped drinking because of gastric problems and flushing phenomena after treatment with hypoglycemic agents.

Surprisingly, no association could be detected between PAD and cigarette smoking, possibly due to the relatively low prevalence of smoking in our population. Although the latter is a strong risk factor for PAD in both diabetic and non-diabetic patients, the lack of association between smoking, PAD and its complications has already been reported in several studies of patients with Type 2 diabetes [40–42]. In consequence, the latter finding cannot be extended to other high-risk populations.

In this study, a significant difference between cases and controls was found in the distribution of diabetes duration. In particular, the risk of PAD progressively increased with the duration of diabetes, and was 2.5 times higher when the disease lasted >15 years. The presence of hypertension also independently increased the risk of PAD by more than double. The effect of the dietary score on the risk of PAD was independent of diabetes duration; indeed, it influenced the risk of PAD in the presence of a diabetes duration both shorter and longer than 10 years. Similar results were observed for the presence or absence of hypertension. Moreover, a high dietary score improved the individual predictive value of these two variables.

In conclusion, this study shows that—rather than individual items—a high-score dietary pattern may have a protective role on PAD in Italian patients with Type 2 diabetes, living in a traditionally Mediterranean region. The combination of different types of food with healthy characteristics might be necessary to express their protective effect on atherosclerosis development. Therefore, in order to prevent PAD, repeated dietary advice should be given, together with careful blood pressure and other risk factor control, to Type 2 diabetic patients, also in populations traditionally consuming a 'Mediterranean diet'.

Acknowledgements

The authors thank Miss Alexandra Cianci for her help in the manuscript's preparation. This work was partially supported by a grant from Bayer SpA, Milan, Italy and by the Italian Ministry of Health Rome, Italy (ICS 110.1/RF98.97 and 99.108, ICS 120.4/RF 99.11).

Contribution of authors

E.C.: Co-ordination of the study and drafting of the manuscript; A.D.C.: statistical analysis and interpretation of data; M.S.: execution and interpretation of color-duplex ultrasound examination; A.S. and A.G.: validation of the food frequency questionnaire; M.B.D., G.d.G. and F.C.: conception and design, critical revision of the manuscript and of all the study phases, L.I.: conception and design, interpretation of data, drafting of the manuscript and its final approval.

GENDIABE Investigators

Project Leader: L. Iacoviello*

Scientific Committee: F. Capani,† A. Consoli,† G. de Gaetano,‡ M. B. Donati‡

Organizing Committee: E. Ciccarone,*† K. Krekora,*§ A. Pandolfi,† E. Vitacolonna,† F. Zito*

Data Management and Statistics: A. Cianci,* A. Di Castelnuovo*

Laboratory Evaluations: C. Amore,* A. D'Orazio*

Dietary assessment: E. Pece*

Radiological evaluation: L. Bonomo,¶ S. Matteo¶

*'Angela Valenti' Laboratory of Genetic and Environmental Risk Factors for Thrombotic Disease, Consorzio Mario

Negri Sud, Santa Maria Imbaro, Italy; †Department of Medicine and Aging, University of Chieti, Chieti, Italy; ‡Center for High Technology Research and Education in Biomedical Sciences, Catholic University, Campobasso, Italy; §Department of Endocrinology and Iodotherapy, Holy Cross Cancer Center, ul. Artwinskiego 3, 25–734 Kielce, Poland (present address); and ¶Institute of Radiology, University of Chieti, Chieti, Italy.

References

1 Hooi JD, Stoffers HE, Knottnerus JA, van Ree JW. The prognosis of non-critical limb ischaemia: a systematic review of population-based evidence. *Br J General Pract* 1999; **49**: 49–55.

2 Fowkes FG, Housley E, Cawood EH, Macintyre CC, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991; **20**: 384–92.

3 Golledge J. Lower-limb arterial disease. *Lancet* 1997; **350**: 1459–65.

4 Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care* 1990; **13**: 513–21.

5 Ghirlanda G, Mancini L, Castagneto M, Citterio F, Serra F, Cotroneo AR, Marano P. The foot clinic. Multidisciplinary management of the patient with diabetic foot. *Rays* 1997; **22**: 638–43.

6 Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. *Am J Cardiol* 1976; **38**: 46–51.

7 Leng GC, Lee AJ, Fowkes FG, Whiteman M, Dunbar J, Housley E, Ruckley CV. Incidence, natural history and cardiovascular events in symptomatic and asymptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1996; **25**: 1172–81.

8 Fowkes FG, Housley E, Riemersma RA, Macintyre CC, Cawood EH, Prescott RJ, Ruckley CV. Smoking, lipids, glucose intolerance, and blood pressure as risk factors for peripheral atherosclerosis compared with ischemic heart disease in the Edinburgh Artery Study. *Am J Epidemiol* 1992; **135**: 331–40.

9 Ferro-Luzzi A, Branca F. Mediterranean diet, Italian-style: prototype of a healthy diet. *Am J Clin Nutr* 1995; **61** (6 Suppl.): 1338S–1345S.

10 Kushi LH, Lenart EB, Willett WC. Health implications of Mediterranean diets in light of contemporary knowledge. 1. Plant foods and dairy products. 2. Meat, wine, fats, and oils. *Am J Clin Nutr* 1995; **61** (6 Suppl.): 1407S–1415S.

11 de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999; **99**: 779–85.

12 GISSI Prevenzione investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999; **354**: 447–55.

13 Katsouyanni K, Skalkidis Y, Petridou E, Polychronopoulou-Trichopoulou A, Willett W, Trichopoulos D. Diet and peripheral arterial occlusive disease: the role of poly-, mono-, and saturated fatty acids. *Am J Epidemiol* 1991; **133**: 24–31.

14 Donnan PT, Thomson M, Fowkes FG, Prescott RJ, Housley E. Diet as a risk factor for peripheral arterial disease in the general population: the Edinburgh Artery Study. *Am J Clin Nutr* 1993; **57**: 917–21.

15 Camargo CA Jr, Stampfer MJ, Glynn RJ, Gaziano JM, Manson JE, Goldhaber SZ, Hennekens CH. Prospective study of moderate alcohol consumption and risk of peripheral arterial disease in US male physicians. *Circulation* 1997; **95**: 577–80.

16 Djousse L, Levy D, Murabito JM, Cupples LA, Ellison RC. Alcohol consumption and risk of intermittent claudication in the Framingham Heart Study. *Circulation* 2000; **102**: 3092–7.

17 Assmann G, de Backer G, Bagnara S, Betteridge J, Crepaldi G, Fernandez-Cruz A, Godtfredsen J, Jacotot B, Paoletti R, Renaud S, Ricci G, Rocha E, Trautwein E, Urbinati GC, Varela G, Williams C. Olive oil and the Mediterranean diet: implications for health in Europe. *Br J Nurs* 1997; **6**: 675–7.

18 Keys A. Mediterranean diet and public health: personal reflections. *Am J Clin Nutr* 1995; **61** (6 Suppl.): 1321S–1323S.

19 Laurenzi M, Stamler R, Trevisan M, Dyer A, Stamler J. Is Italy losing the 'Mediterranean advantage?' Report on the Gubbio population study: cardiovascular risk factors at baseline. Gubbio Collaborative Study Group. *Prev Med* 1989; **18**: 35–44.

20 Lupo A. Nutrition in general practice in Italy. *Am J Clin Nutr* 1997; **65** (6 Suppl.): 1963S–1966S.

21 Second European Consensus Document. *Circulation* 1991; **84** (4 Suppl.): iv 1–26.

22 Langholz J. Investigation of peripheral arterial disease—the expanding role of echo-enhanced color flow Doppler and duplex sonography. *Eur J Ultrasound* 1998; **7** (3 Suppl.): S53–61.

23 WHO MONICA Project. *MONICA Manual 1998–1999.* Available from URL: http://www.ktl.fi/publications/monica/manual/index.htm

24 Bonifacj C, Gerber M, Scali J, Daures JP. Comparison of dietary assessment methods in a southern French population: use of weighed records, estimated-diet records and a food-frequency questionnaire. *Eur J Clin Nutr* 1997; **51**: 217–31.

25 Decarli A, Franceschi S, Ferraroni M, Gnagnarella P, Parpinel MT, La Vecchia C, Negri E, Salvini S, Falcini F, Giacosa A. Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol* 1996; **6**: 110–8.

26 Manousos O, Day NE, Trichopoulos D, Gerovassilis F, Tzonou A, Polychronopoulou A. Diet and colorectal cancer: a case–control study in Greece. *Int J Cancer* 1983; **32**: 1–5.

27 Davidson S, Passmore R, Brock JF, Truswell AS. *Human Nutrition and Dietetics*, 7th edn. Edinburgh: Churchill Livingstone, 1979.

28 National Research Council (U.S.). Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences. *Diet and Health: Implications for Reducing Chronic Disease Risk*. Washington DC: National Academy Press, 1989.

29 Willett WC. Diet and health: what should we eat? *Science* 1994; **264**: 532–7.

30 Massey LK. Dairy food consumption, blood pressure and stroke. *J Nutr* 2001; **131**: 1875–8.

31 Pfeuffer M, Schrezenmeir J. Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. *Br J Nutr* 2000; **84** (1 Suppl.): S155–9.

32 SAS Institute Inc. *SAS/STAT User's Guide*, Version 8.1. Cary, NC: SAS Institute Inc., 1989.

33 Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L, Trichopoulos D. Diet and overall survival in elderly people. *BMJ* 1995; **311**: 1457–60.

34 Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000; **342**: 154–60.

35 Jha P, Flather M, Lonn E, Farkouh M, Yusuf S. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Ann Intern Med* 1995; **123**: 860–72.

36 Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985; **312**: 1205–9.

37 Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by dietary pure omega-3 polyunsaturated fatty acids in dogs. *Circulation* 1999; **99**: 2452–7.

38 Rotondo S, Di Castelnuovo A, de Gaetano G. The relationship between wine consumption and cardiovascular risk: from epidemiological evidence to biological plausibility. *Ital Heart J* 2001; **2**: 1–8.

39 Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, De Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. *Circulation* 2002; **105**: 2836–44.

40 el-Shazly M, Abdel-Fattah M, Scorpiglione N, Benedetti MM, Capani F, Carinci F, Carta Q, Cavaliere D, De Feo EM, Taboga C, Tognoni G, Nicolucci A. Risk factors for lower limb complications in diabetic patients. The Italian Study Group for the Implementation of the St. Vincent Declaration. *J Diabetes Complications* 1998; **12**: 10–7.

41 Reiber GE, Pecoraro RE, Koepsell TD. Risk factors for amputation in patients with diabetes mellitus. A case–control study. *Ann Intern Med* 1992; **117**: 97–105.

42 Apelqvist J, Agardh CD. The association between clinical risk factors and outcome of diabetic foot ulcers. *Diabetes Res Clin Pract* 1992; **18**: 43–53.