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ORIGINAL DATA

Mediterranean Diet and 10-year (2002-2012) Incidence of Diabetes and Cardiovascular Disease in Participants with Prediabetes: The ATTICA Study

Theodosios D. Filippatos[1], Demosthenes B. Panagiotakos[1], Ekavi N. Georgousopoulou[1], Evangelia Pitaraki[1], Georgia-Maria Kouli[1], Christina Chrysohoou[2], Dimitrios Tousoulis[2], Christodoulos Stefanadis[2], Christos Pitsavos[2], and the ATTICA Study Group

[1] *Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, Athens, Greece.* [2] *First Cardiology Clinic, School of Medicine, University of Athens, Greece. Address correspondence to: Demosthenes B. Panagiotakos, Department of Nutrition and Dietetics, School of Health Science and Education, 70 Eleftheriou Venizelou Ave., 17671 Athens, Greece, e-mail: dbpanag@hua.gr*

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■ Abstract

BACKGROUND: Prediabetes has been related to an increased risk of developing diabetes and cardiovascular disease (CVD). **AIM**: The aim of the present study was to examine the effect of the Mediterranean diet on diabetes and CVD risk in subjects with impaired fasting glucose (IFG, i.e. fasting plasma glucose 100-125 mg/dl). **METHODS**: During 2001-2002, 3042 men and women (>18y) were enrolled for the study. The participants showed no clinical evidence of CVD or any other chronic disease, and were living in the greater Athens (Greece) area. In 2011 and 2012, the 10-year follow-up examinations were performed, including a working sample of n = 1875 participants without diabetes at baseline. Adherence to the Mediterranean diet at baseline evaluation was assessed using the MedDietScore (range 0-55). **RESULTS**: The prediabetic subjects (n = 343) had a significantly higher incidence of diabetes (25% vs. 10%, $p < 0.001$) and CVD (17.8% vs. 12.3%, $p = 0.007$) compared with subjects with normal glucose values. A significant trend towards lower diabetes and CVD incidence was observed with medium and high adherence to the Mediterranean diet compared with low adherence ($p < 0.001$). High adherence to the Mediterranean diet (>35/55 score) was associated with lower 10-year incidence of diabetes and CVD. In multiple logistic regression models, participants with high levels of adherence to the Mediterranean diet were significantly less affected by diabetes and CVD than those with low adherence levels. **CONCLUSION**: High adherence to the Mediterranean diet is associated with a low risk of developing diabetes and CVD in prediabetic subjects.

Keywords: prediabetes · Mediterranean diet · impaired fasting glucose · cardiovascular disease · diabetes incidence

1. Introduction

The incidence of diabetes type 2 has reached epidemic dimensions; it affects about 8% of the adult population, and has major health and economic consequences. It has been estimated that healthcare spending on diabetes accounted for 10.8% of total healthcare expenditures worldwide in 2013, amounting to 548 billion dollars in 2013, and is expected to exceed 627 billion dollars in 2035 [1]. These major consequences call for primary prevention strategies, especially the economically favorable dietary interventions, to reduce the incidence of diabetes and its complications [2].

Prediabetes is defined as fasting plasma glucose (FPG) concentration above the highest normal value but below the cut-off level used for the diagnosis of type 2 diabetes. It includes both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). While IGT is defined by a 2-h glucose level of 140-199 mg/dl in response to a 75 g oral glucose tolerance test (OGTT), IFG is defined as FPG concentration between 100 mg/dl and 125 mg/dl according to the ADA definition [3]. Among non-diabetic subjects, those with prediabetes have an increased risk of developing diabetes and its vascular complications [4]. Therefore, it is important to determine the effect of dietary interventions in prediabetic subjects as primary prevention strategies against diabetes and cardiovascular disease (CVD).

The Mediterranean diet is a nutritional model characterized by:

- High consumption of olive oil, whole grain cereals, legumes, vegetables, and fruits.
- Moderate consumption of wine, fish, and dairy products.
- Low consumption of poultry, meat, highly processed foods, refined grains, and sugars [5].

Recently, the 10-year results of the ATTICA cohort study revealed that medium and high adherence to the Mediterranean diet played a positive role in primary prevention of diabetes, mediated by attenuation of inflammation and increased total antioxidant capacity [6]. However, the effect of the Mediterranean diet on the prevention of diabetes and CVD has not been specifically studied in subjects with prediabetes. Therefore, we investigated in this study the effect of adherence to the Mediterranean diet on the 10-year diabetes and CVD risk in Greek prediabetic subjects, an investigation that was carried out within the context of the ATTICA study [7].

Abbreviations:

ADA	American Diabetes Association
ANOVA	analysis of variance
BMI	body mass index
BP	blood pressure
CI	confidence interval
CVD	cardiovascular disease
EPIC	Investigation into Cancer and Nutrition
FPG	fasting plasma glucose
HDL	high-density lipoprotein
HOMA-IR	homeostatic model assessment of insulin resistance
ICD	International Classification of Diseases
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
LDL	low-density lipoprotein
MUFA	monounsaturated fatty acid
NCEP- ATP III	National Cholesterol Education Program Adult Treatment Panel III
OGTT	oral glucose tolerance test
OR	odds ratio
P-P	probability-probability
RIA	radioimmunoassay
SPSS	Statistical Package for Social Sciences

2. Methods

2.1 Baseline sampling procedure and measurements (2001-2002)

The ATTICA study is a large-scale, prospective survey, aimed at evaluating the effects of nutrition on health. It was carried out during 2001-2002 in the province of Attica (Greece), where Athens is the major metropolis [7]. After exclusion of people with CVD (n = 117) and of those with chronic viral infections (n = 107), 3,042 of the 4,056 individuals initially invited were finally included in the study, and agreed to participate (75% participation rate). 1,514 of the participants were men (aged 46 ± 13 years, range 18-87 years) and 1,528 were women (aged 45 ± 13 years, range: 18-89 years).

Trained personnel (i.e., cardiologists, general practitioners, dietitians, and nurses) interviewed the participants, using a standard questionnaire. Baseline assessment included information about socio-demographic characteristics (age, sex, years of school), history of hypertension, hypercholesterolemia, and diabetes, family history of CVD, dietary habits, smoking status, and physical activity. Persons who had smoked at least one cigarette per day during the past year or who had recently stopped smoking (during the past year) were regarded as smokers; the rest of the participants were defined as non-smokers. Nutritional habits were evaluated on the basis of a validated semi-quantitative food-frequency questionnaire, the EPIC-Greek questionnaire, which was kindly provided by the Unit of Nutrition of the Athens Medical School [8].

Adherence to the Mediterranean diet was evaluated on the basis of the MedDietScore (range 0-55, with higher values for greater adherence) [9]. The tertiles of the score were determined, resulting in three categories, i.e., low (<25/55), medium (26-35/55), and high (>35/55) level of adherence. Anthropometric measures, including weight (in kg), height (in m), waist (in cm), and hip (in cm) circumference, and clinical characteristics were determined using standardized procedures. Arterial blood pressure was measured 3 times in the right arm while subjects were in a sitting position for at least 30 min. Patients with mean blood pressure ≥140/90 mmHg and those who were taking antihypertensive medication were classified as hypertensive. Hypercholesterolemia was defined as total serum cholesterol concentrations >200 mg/dl or the use of lipid-lowering agents.

Diagnosis of type 2 diabetes was based on ADA criteria, i.e., fasting blood glucose >125 mg/dl or the use of antidiabetic medication [3]. Diagnosis of IFG was based on fasting glucose levels 100-125 mg/dl according to ADA [3]. In accordance with the definition provided by the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III, participants were diagnosed with the metabolic syndrome if three or more of the following metabolic conditions were present:

- Waist circumference ≥102 cm for men or ≥88 cm for women
- Triglyceride level ≥150 mg/dl
- High-density lipoprotein (HDL) cholesterol level <40 mg/dl for men or <50 mg/dl for women
- Blood pressure ≥130/85 mmHg
- Fasting blood glucose ≥100 mg/dl

Blood samples were collected from the antecubital vein between 8 to 10 am in a sitting position after 12 hours of fasting and abstinence from alcohol. Total serum cholesterol, HDL cholesterol, triglycerides, and glucose concentrations were measured by enzymatic chromatography using a Technicon automatic analyzer RA-1000. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [10]. Serum insulin concentrations were measured by means of radioimmunoassay (RIA100, Pharmacia Co., Erlangen, Germany). The homeostatic model assessment of insulin resistance (HOMA-IR), used to quantify insulin resistance and beta-cell function, was calculated as follows: glucose x insulin/405.

Table 1. Baseline lifestyle and biochemical variables and incidence of diabetes and CVD according to the presence of IFG in n = 1,875 subjects

Baseline characteristic	IFG (n = 343)	Normal glucose values (n = 1532)	p
New diabetes cases, n (%)	71 (25)	120 (10)	<0.001
New CVD cases, n (%)	61 (17.8)	188 (12.3)	0.007
Age (yr)	46.4 ± 12.4	43.5 ± 13.5	<0.001
Male sex, n (%)	222 (64.7)	696 (45.4)	<0.001
Smoking, n (%)	211 (61.5)	822 (53.7)	0.009
Education (years of school)	12.3 ± 3.9	12.4 ± 3.6	0.88
Body mass index (kg/m^2)	27.4 ± 4.7	25.8 ± 4.4	<0.001
Waist circumference (cm)	95 ± 14	88 ± 15	<0.001
Family hist. of diabetes, n (%)	77 (22.4)	308 (20.1)	0.18
Hypertension, n (%)	125 (36.4)	395 (25.8)	<0.001
Hypercholesterolemia, n (%)	185 (53.9)	591 (38.6)	<0.001
Metabolic syndrome, n (%)	137 (39.9)	214 (14)	<0.001
Fasting glucose (mg/dl)	107 ± 6	85 ± 9	<0.001
Fasting insulin (μU/ml)	14.5 ± 1	12.2 ± 2.4	<0.001
HOMA-IR	3.8 ± 0.4	2.6 ± 0.5	<0.001
Systolic BP (mmHg)	127 ± 17	121 ± 18	<0.001
Diastolic BP (mmHg)	82 ± 10	78 ± 12	<0.001
Total cholesterol (mg/dl)	204 ± 40	192 ± 42	<0.001
HDL cholesterol (mg/dl)	46 ± 11	50 ± 15	<0.001
LDL cholesterol (mg/dl)	130 ± 36	121 ± 38	<0.001
Triglyceride (mg/dl)	139 ± 110	110 ± 70	<0.001
Current smokers, n (%)	211 (61.5)	822 (53.7)	0.009
Physically active, n (%)	163 (47.5)	613 (40)	0.011

Legend: Data are presented as mean values and standard deviation or absolute and relative frequencies. P-values are derived from the independent samples test for the normally distributed variables, from the Mann-Whitney test for the non-normally distributed variables (i.e., years of school, triglycerides, fasting glucose, fasting insulin, HOMA-IR), or from chi-square test for the categorical variables. *Abbreviations*: BP - blood pressure, IFG - impaired fasting glucose, CVD - cardiovascular disease, HDL - high-density lipoprotein, LDL - low-density lipoprotein, HOMA-IR - homeostasis model assessment of insulin resistance.

2.2 10-year follow-up evaluation (2011-2012)

During 2011-2012, the 10-year follow-up evaluation was performed. Of the 3,042 participants, 224 could not be reapproached because of changed addresses and telephone numbers, 235 denied being re-examined, and 99 died during the follow-up. Finally, 2,583 were included in the study (85% participation rate), and a detailed evaluation of their medical status was performed. The definition of the outcomes studied was based on the 10th version of International Classification of Diseases (ICD) nomenclature, but it was decided to keep the ICD-9 coding as well because it was used in the intermediate 5-year follow-up. No discordant cases were observed using the two coding systems. Of the 2,583 participants, 210 diagnosed with diabetes at baseline and 498 with no data regarding CVD status at the 10-year follow-up were not included in the present analyses, resulting in a working sample of n = 1,875 participants without diabetes at baseline and with available data on CVD incidence.

Of the 1,875 participants, 343 (18.3%) had IFG and 1,532 (81.7%) were normoglycemic at baseline examinations. To analyze the relationship between diet and diabetes incidence, a working sample of n = 1,485 participants without diabetes at baseline and with available data on diabetes status at 10-

Table 2. Baseline lifestyle and biochemical variables of participants with IFG according to the level of adherence to the Mediterranean diet (n = 343)

Baseline characteristic	Level of adherence to the Mediterranean diet			p (for trend)
	MedDietScore <25 (n = 151)	MedDietScore 26-35 (n = 120)	MedDietScore >35 (n = 72)	
Diabetes cases, n (%)	46 (40)	21 (19)	4 (7)	<0.001
CVD cases, n (%)	43 (29)	15 (13)	3 (4)	<0.001
Death, n (%)	10 (7)	2 (2)	2 (3)	0.10
Age (yr)	52.6 ± 11.3	45.0 ± 10.0**	35.9 ± 10.5**	<0.001
Male sex, n (%)	126 (83)	79 (66)	17 (24)	<0.001
Education (years of school)	11.9 ± 4.2	12.0 ± 3.9	13.3 ± 2.9	0.107
Body mass index (kg/m^2)	30.2 ± 4.6	26.3 ±2.9**	23.3 ± 3.3**	<0.001
Waist circumference (cm)	103 ± 12	92 ± 12**	82 ± 11**	<0.001
Family hist. of diabetes, n (%)	40 (31)	22 (22)	15 (25)	0.34
Hypertension, n (%)	75 (54)	36 (32)	14 (22)	<0.001
Hypercholesterolemia, n (%)	90 (60)	70 (58)	25 (35)	0.001
Metabolic syndrome, n (%)	90 (60)	36 (30)	11 (15)	<0.001
Systolic BP (mmHg)	133 ± 17	126 ± 17*	118 ± 14**	<0.001
Diastolic BP (mmHg)	85 ± 11	81 ± 10*	77 ± 9**	<0.001
Total cholesterol (mg/dl)	210 ± 39	209 ± 41	185 ± 34**	<0.001
HDL cholesterol (mg/dl)	42 ± 10	48± 12**	51 ± 11**	<0.001
LDL cholesterol (mg/dl)	134 ± 37	133 ± 36	115 ± 30*	0.001
Triglycerides (mg/dl)	166 ± 162	133 ± 75*	90 ± 45**	<0.001
Smoking, n (%)	97 (64)	73 (61)	41 (57)	0.56
Physically active, n (%)	68 (45)	60 (50)	35 (49)	0.70
Fasting glucose (mg/dl)	108 ± 6	106 ± 6*	105 ± 5**	<0.001
Fasting insulin (µU/ml)	15.0 ± 0.8	14.4 ± 0.9**	13.4 ± 0.8**	<0.001
HOMA-IR	4.0 ± 0.4	3.8 ± 0.4**	3.5 ± 0.3**	<0.001

Legend: Data are obtained from post-hoc analyses with Bonferroni correction, using low adherence to the Mediterranean diet as reference category, or from the Mann-Whitney test (every two groups) for non-normally distributed variables. * $p < 0.05$, ** $p < 0.001$. *Abbreviations*: BP - blood pressure, IFG - impaired fasting glucose, CVD - cardiovascular disease, HDL - high-density lipoprotein, LDL - low-density lipoprotein, HOMA-IR - homeostasis model assessment insulin resistance.

year follow-up was used. Of these participants without diabetes at baseline, 279 (18.8%) had IFG and 1,206 (81.2%) were normoglycemic.

Details of the aims, baseline procedures, and the 10-year follow-up examinations have been presented elsewhere [7, 11].

2.3 Statistical analysis

The ten-year (2002-2012) incidence of diabetes and CVD was calculated as the ratio of new cases to the number of participants with normal glucose levels or IFG. Ten-year (2002-2012) incidence of diabetes in the Mediterranean diet group was calculated as the ratio of new cases to the number of participants in each tertile of the normal glucose and IFG group. Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies (relative frequencies).

Associations between categorical variables were tested using the chi-squared test. Continuous variables were tested for normality using P-P plots. Student's *t*-test was used to compare mean values of normally distributed variables in individuals with IFG and in the rest of the participants, after ensuring equality of variances using Levene's test. Analysis of variance (ANOVA) was performed to compare the mean values of normally distributed variables per MedDietScore group. Post-hoc analysis was performed using Bonferroni correction to account for the probability of type I errors. For non-normally distributed variables, the Kruskall-Wallis test was applied followed by the Mann-Whitney test for comparisons between the MedDietScore groups.

The relative risk of developing diabetes during the 10-year period according to the participants' baseline characteristics was estimated by the odds ratio (OR) and the 95% corresponding confidence interval, as derived from multiple logistic regression models, since there were no accurate data about diabetes onset, but only diagnosis.

All known confounders were included in the models after testing for colinearity. Interactions with MedDietScore were checked in all steps, and when significant subgroup analyses were performed. The predictive abilities of the components of the Mediterranean diet were ranked by calculating the -2log-likelihood of each model (i.e., the lower the better). A composite outcome was created to explore further the differences in competing risks between prediabetic and normal subjects regarding the development of diabetes or/and CVD adverse events. This variable was used as dependent variable in multiple logistic regression models.

SPSS version 18 (Statistical Package for Social Sciences, IBM Hellas SA, Greece) software was used for all statistical calculations. The level of statistical significance was predefined at a = 0.05.

3. Results

3.1 10-year diabetes and CVD incidence among participants with prediabetes

During the 10-year follow-up period, 191 diabetes cases were documented (12.9%); 97 men and 94 women (13.4% vs. 12.4% respectively, $p = 0.79$ for gender difference) [11]. Additionally, 249 CVD cases were documented (13.8%); 153 men and 96 women (16.7% vs. 10.0% respectively, $p < 0.001$ for gender difference). Among subjects with IFG, n = 71 (25.4%) new diabetes cases and n = 61 (17.8%) new CVD cases were identified, whereas among subjects with normal glucose levels n = 120 (10%) new diabetes cases ($p < 0.001$ vs. IFG group) and n = 188 (12.3%) new CVD cases ($p = 0.007$ vs. IFG group) were documented (**Table 1**).

3.2 Participants' baseline characteristics by prediabetes status

Subjects with IFG were more likely to be male, elderly, smokers, hypertensive, and hypercholesterolemic and to have increased body mass index (BMI) and waist circumference compared with subjects with normal glucose values at baseline (**Table 1**). IFG status at baseline was associated with increased blood pressure, total cholesterol, LDL cholesterol, and triglycerides, as well as with decreased HDL cholesterol levels (**Table 1**).

3.3 Baseline characteristics of participants with IFG by level of adherence to the Mediterranean diet

Demographic and clinical characteristics of the participants with IFG are presented by adherence to Mediterranean diet status in **Table 2**. As the level of adherence to the Mediterranean diet increased, participants were more likely to be females and younger and to have decreased BMI and waist circumference. Hypertension and hypercholesterolemia were less frequent. Furthermore, systolic/diastolic blood pressure, total cholesterol, LDL cholesterol, and triglycerides were lower, whereas HDL cholesterol was higher in persons with high adherence to the diet (**Table 2**).

3.4 10-year diabetes incidence and adherence to the Mediterranean diet

The 10-year incidence of diabetes in subjects with IFG at baseline was (**Table 2**):

- N = 46 cases (40%) among participants with little adherence to the Mediterranean diet.
- N = 21 cases (19%) among participants with medium adherence to the Mediterranean diet.
- N = 4 cases (7%) in participants with high adherence to the Mediterranean diet.

Thus, the incidence of diabetes in patients with IFG with high adherence to the Mediterranean diet was similar to that observed in subjects with normal glucose levels. Participants who did not develop diabetes within the 10-year follow-up period were similarly distributed among the three Mediterranean diet groups (33%, 42%, and 25% for low, medium, and high adherence, respectively), whereas participants who developed diabetes were mostly non-followers of the dietary pattern (65%, 30%, and 5% for the three groups, respectively).

To eliminate the impact of potentially confounding variables, multi-adjusted analysis was performed by the use of nested models. In an age- and sex-adjusted model (**Table 3**, model 1), a significant inverse association in relation to the 10-year incidence of diabetes was observed for participants with both medium and high, compared to low, adherence to the Mediterranean diet (OR: 0.33, 95% CI: 0.16-0.87 and OR: 0.10, 95% CI: 0.03-0.37, respectively). This finding remained significant even after controlling for family history of diabetes and cardiovascular risk factors (i.e., hypertension, hypercholesterolemia, and smoking status) (**Table 3**, model 2), educational status and physical activity status (**Table 3**, model 3), and waist circumference category (**Table 3**, model 4).

To determine which components of the Mediterranean diet contributed most to the observed association between high adherence and low risk of diabetes, all Mediterranean diet components were added sequentially to model 4. The three components with the lowest values were fruits, poultry, and monounsaturated fatty acids (MUFAs) (with -2LogLikelihood values equal to 66.6, 68.2, and 68.6, respectively).

3.5 10-year CVD incidence and adherence to the Mediterranean diet

The 10-year incidence of CVD in subjects with IFG at baseline was (**Table 2**):

Table 3. Results from multiple logistic regression models applied to evaluate the adherence to the Mediterranean diet and other characteristics of participants with impaired fasting glucose in relation to the 10-year incidence of diabetes (n = 279)

Model	**OR for MedDietScore (per 1 unit)**	**Level of adherence to the Mediterranean diet**		
		MedDietScore <25 (n = 114)	MedDietScore 26-35 (n = 108)	MedDietScore >35 (n = 57)
Model 1	0.98 (0.93-1.03)	Reference	0.33 (0.16-0.67)	0.10 (0.03-0.37)
Model 2	0.99 (0.93-1.04)	Reference	0.33 (0.14-0.68)	0.11 (0.03-0.50)
Model 3	0.98 (0.92-1.04)	Reference	0.34 (0.14-0.83)	0.12 (0.03-0.54)
Model 4	0.99 (0.93-1.05)	Reference	0.31 (0.13-0.83)	0.13 (0.03-0.63)

Legend: Results of multiple logistic regression models are presented as odds ratios and the corresponding 95% confidence interval. Model 1: Adjusted for age and sex. Model 2: Adjusted for variables as in Model 1 plus family history of diabetes, and cardiovascular disease risk factors (smoking, hypertension, and hypercholesterolemia). Model 3: Adjusted for variables as in Model 2 plus educational status (years of school) and physical activity status. Model 4: Adjusted for variables as in Model 3 plus waist circumference category (women >80 cm, men >94 cm).

Table 4. Results from multiple logistic regression models applied to evaluate the adherence to the Mediterranean diet and other characteristics of participants with impaired fasting glucose in relation to the 10-year incidence of cardiovascular disease (n = 343)

Model	**OR for MedDietScore (per 1 unit)**	**Level of adherence to the Mediterranean diet**		
		MedDietScore <25 (n = 151)	MedDietScore 26-35 (n = 120)	MedDietScore >35 (n = 72)
Model 1	0.98 (0.93-1.04)	Reference	0.55 (0.27-1.15)	0.28 (0.07-1.12)
Model 2	0.97 (0.91-1.03)	Reference	0.59 (0.27-1.28)	0.28 (0.07-1.15)
Model 3	0.97 (0.91-1.03)	Reference	0.61 (0.28-1.34)	0.32 (0.08-1.32)
Model 4	0.97 (0.91-1.03)	Reference	0.56 (0.24-1.32)	0.28 (0.06-1.27)
Model 5	0.96 (0.90-1.02)	Reference	0.39 (0.16-0.96)	0.18 (0.04-0.84)

Legend: Results of multiple logistic regression models are presented as odds ratios and the corresponding 95% confidence interval. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, and cardiovascular disease risk factors (smoking, hypertension, and hypercholesterolemia). Model 3: Adjusted for variables as in Model 2 plus educational status (years of school) and physical activity status. Model 4: Adjusted for variables as in Model 3 plus waist circumference category (men >94 cm, women >80 cm). Model 5: Adjusted for variables as in Model 3 plus waist circumference (in cm).

- N = 43 cases (29%) among participants not following the Mediterranean diet.
- N = 15 cases (13%) among participants with medium adherence to the Mediterranean diet.
- N = 3 cases (4%) in the high adherence group (p < 0.001).

Thus, the incidence of CVD in patients with IFG and medium/high adherence to the Mediterranean diet was similar to that observed in subjects with normal glucose levels.

The protective effect of high adherence to the Mediterranean diet on the 10-year CVD incidence was also controlled for potential confounders. The crude age- and sex-adjusted model showed a non-significant inverse association for both medium (OR: 0.55, 95% CI: 0.27-1.15) and high (OR: 0.28, 95% CI: 0.07-1.12, p = 0.07) adherence compared to low adherence to the Mediterranean diet (**Table 4**, model 1). However, in a model that included cardiovascular risk factors (smoking, hypercholesterolemia, and hypertension), education, physical activity, and waist circumference (in cm), medium (OR: 0.39, 95% CI: 0.16-0.96) and high adherence (OR: 0.18, 95% CI: 0.04-0.84) were both significantly protective in relation to the 10-year incidence of CVD compared with low adherence (**Table 4**, model 5).

Participants with IFG aged <30 years (n = 30) tended to follow a pattern close to the Mediterranean diet (70% high adherence) in contrast to those aged ≥30 years (n = 313, 47.2% low adherence). In the subgroup of participants aged ≥30 years, the age- and sex-adjusted crude model showed a significant protective effect of high adherence to the Mediterranean diet in contrast to low adherence (**Table 5**, model 1). This finding remained significant even after controlling for cardiovascular risk factors (i.e., hypertension, hypercholesterolemia, and smoking status) (**Table 5**, model 2), and for educational level, physical activity status, and waist circumference (**Table 5**, models 3 and 5).

To determine which components of the Mediterranean diet contributed the most to the observed

Table 5. Results from multiple logistic regression models applied to evaluate the adherence to the Mediterranean Diet as well as other characteristics of participants with impaired fasting glucose aged ≥30 years in relation to 10-year incidence of cardiovascular disease (n = 313)

Model	**OR for MedDietScore (per 1 unit)**	**Level of adherence to the Mediterranean diet**		
		MedDietScore <25 (n = 148)	MedDietScore 26-35 (n = 114)	MedDietScore >35 (n = 51)
Model 1	0.98 (0.93-1.04)	Reference	0.54 (0.26-1.13)	0.19 (0.04-0.95)
Model 2	0.97 (0.91-1.03)	Reference	0.58 (0.27-1.27)	0.19 (0.04-0.95)
Model 3	0.97 (0.91-1.03)	Reference	0.60 (0.27-1.34)	0.21 (0.04-1.09)
Model 4	0.97 (0.91-1.03)	Reference	0.57 (0.24-1.36)	0.20 (0.04-1.08)
Model 5	0.96 (0.89-1.02)	Reference	0.37 (0.15-0.93)	0.12 (0.02-0.68)

Legend: Results of multiple logistic regression models are presented as odds ratios and the corresponding 95% confidence interval. Model 1: Adjusted for age and sex. Model 2: Adjusted for variables as in Model 1 plus cardiovascular disease risk factors (smoking, hypertension, and hypercholesterolemia). Model 3: Adjusted for variables as in Model 2 plus educational status (years of school) and physical activity status. Model 4: Adjusted for variables as in Model 3 plus waist circumference category (women >80 cm, men >94 cm). Model 5: Adjusted for variables as in Model 3 plus waist circumference (in cm).

Table 6. Multiple logistic regression assessing the role of prediabetes and Mediterranean diet adherence level in competing and composite events vs. healthy status at the end of the follow-up period (n = 1,817)

Event	OR for Diabetes (95% CI)	OR for CVD (95% CI)	OR for diabetes and CVD (95% CI)
Prediabetes at baseline (yes vs. no)	3.43 (2.17-5.44)	1.08 (0.63-1.85)	3.06 (1.49-6.31)
MedDietScore tertiles			
1st tertile	Reference	Reference	Reference
2nd tertile	0.60 (0.35-1.02)	0.67 (0.40-1.12)	0.16 (0.06-0.43)
3rd tertile	0.29 (0.13-0.65)	0.43 (0.19-0.96)	0.05 (0.01-0.40)

Legend: Model adjusted for age, sex, smoking, physical activity, and history of hypertension and hyperlipidemia. *Abbreviations*: CI - confidence interval, CVD - cardiovascular disease, OR - odds ratio.

inverse association between high adherence and CVD, all Mediterranean diet components were added one by one to model 5, and -2Loglikelihood values were compared, with the lowest values indicating better predictive ability. The three components with the lowest values were MUFAs, vegetables, and full-fat dairy products with -2Loglikelihood values equal to 89.58, 91.13, and 91.26, respectively.

3.6 Competing risks (diabetes or/and CVD outcome)

To explore the role of prediabetes in the future development of diabetes mellitus or/and CVD endpoints, independently of the level of adherence to the Mediterranean diet, multiple logistic regression models were performed for the combined outcome (healthy, diabetes, CVD, diabetes and CVD). Confounding bias was reduced by adjusting for age, gender, smoking status, physical activity status, and presence of hypertension and hyperlipidemia. The presence of prediabetes at baseline was associated with a significant increase in developing diabetes and the composite outcome risk, but not with CVD risk alone. The higher level of adherence to the Mediterranean diet (3rd tertile vs. 1st tertile) was associated with significant reduction for all outcomes, whereas moderate level of adherence (2nd tertile vs. 1st tertile) led to significant reduction only for the composite (diabetes and CVD outcome) event risk (**Table 6**).

4. Discussion

The Mediterranean diet is a healthy dietary pattern associated with improvement of CVD risk factors and other health benefits [12, 13]. The present work extended previous results from the ATTICA study by examining the 10-year diabetes and CVD incidence in subjects with prediabetes (i.e. IFG) in relation to the Mediterranean diet. The study showed a reduction of the 10-year diabetes risk by almost 70% with medium adherence to the Mediterranean pattern, and a greater than 85% reduction with high adherence. Moreover, we observed a decrease in CVD incidence by almost 60% and over 80% with medium and high adherence to the Mediterranean diet, respectively, especially in subjects aged >30 years. These results show for the first time that even subjects with IFG may benefit from a healthy dietary pattern.

Prediabetes is considered a risk factor for the development of diabetes [14]. In the present study, subjects with IFG had an increased, 2.5-fold 10-year incidence of diabetes compared with normoglycemic subjects. Furthermore, multiple regression analyses showed that the presence of prediabetes at baseline was associated with a significant increased risk of developing diabetes, even when adjusted for age, gender, smoking status, physical activity status, and presence of hypertension and hyperlipidemia. The main pathophysiological mechanisms linking IFG with risk of diabetes are increased hepatic insulin resistance and decreased first-phase (0-10 min) insulin secretory response to intravenous glucose. However, many IFG patients also have IGT, exhibiting a defect in both early and late-phase insulin secretion [15].

The increased risk of diabetes was reduced substantially in IFG subjects with medium and high adherence to the Mediterranean diet, resulting in a similar and lower, respectively, diabetes incidence compared with subjects having normal glucose levels at baseline. A recent analysis of the 10-year results of the ATTICA cohort study revealed that the risk of diabetes decreased by 49% and 62%, respectively, with medium and high adherence to the Mediterranean diet in the general non-diabetic population [6]. The present results show that the protective effect of the Mediterranean diet is even stronger in subjects with prediabetes, suggesting that this dietary pattern is protective over the entire spectrum of non-diabetic subjects including subjects with prediabetes.

The protective effect of the Mediterranean diet against the development of diabetes has been consistently demonstrated and various mechanisms have been implicated, including improvement of insulin sensitivity, reduction of endothelial dysfunction and inflammatory markers, and increase in antioxidant markers [5, 16]. The antidiabetic effect of the Mediterranean diet in the non-diabetic population of the ATTICA cohort was mediated by attenuation of inflammation and enhancement of total antioxidant capacity [6].

We also examined the components of the Mediterranean diet to determine separately their protective roles in subjects with prediabetes. The analysis revealed that fruits, poultry, and MUFAs were the most protective components. Fruits may increase antioxidant action and reduce oxidative stress, both of which have been implicated in the impairment of pancreatic beta-cells to produce and secrete insulin [17]. The consumption of more fruits and poultry has also been associated with less low-grade inflammation [18]. Furthermore, MUFAs have been related to increased anti-inflammatory and antidiabetic action through increased glucagon-like peptide 1 (GLP-1) secretion, which may play a role in the delay of diabetes onset [19]. The Mediterranean dietary pattern also contains additional dietary components that may have a protective role, which is related to increased insulin sensitivity and decreased fasting glucagon concentration [20].

Prediabetes has been associated with a clustering of CVD risk factors [21, 22]. In the present study, subjects with IFG had an adverse metabolic profile compared with normoglycemic subjects, but this was counteracted by adherence to the Mediterranean diet, which is consistent with previous reports [23]. As the results of this study showed, medium and high adherence to the Mediterranean diet was associated with a decreased risk of CVD in prediabetic subjects, even after adjustment for known CVD risk factors. The Mediterranean diet is associated with improvements in lipid profile, blood pressure, and other well-known CVD risk factors [24]. It is rich in antioxidant and anti-inflammatory substances, and has been related to the reduction in the expression of several proatherogenic genes involved in vascular inflammation, foam cell formation and thrombosis, and increase of serum markers of atheroma plaque stability [25, 26]. Several components of the Mediterranean diet may play a significant role in CVD prevention. The analysis showed that MUFAs had the best predictive ability of CVD incidence. In this context, olive oil consumption has been associated with reduction of CVD incidence in individuals at high cardiovascular risk, an effect that may be attributed to its antioxidant and anti-inflammatory effects [27].

Prediabetes has been associated with an adverse cardiometabolic profile and, although not consistently, with CVD incidence and mortality [28]. Our study showed that the presence of IFG is associated with the development of diabetes and the combined outcome of diabetes and CVD, but not with CVD alone. This suggests that the association of IFG with CVD is mainly driven by the development of diabetes, a finding that should be taken into account in future studies. Various non-drug and drug-based approaches are effective for the prevention of diabetes in prediabetic subjects, but their effects in relation to CVD incidence are not similarly positive [29]. The present study showed for the first time that a Mediterranean dietary pattern is protective against both diabetes and CVD in subjects with IFG. The analysis showed that medium adherence of prediabetic patients to the Mediterranean diet was protective against the combined outcome of diabetes and CVD, while high adherence was protective against all outcomes. This finding suggests that prediabetic patients can gain full benefits against the pathophysiology of diabetes and vascular complications by following the appropriate dietary patterns as closely as possible.

5. Limitations

This study has some minor limitations that should be taken into account. Firstly, only fasting plasma glucose levels were available. Therefore, it was impossible to determine the number of patients with both IFG and IGT. Since the date of diabetes diagnosis, but not the exact time of diabetes onset, was known in most cases, hazard ratios were estimated through odds ratios, which may have overestimated the true effect. However, odds ratio is generally held to be an accurate estimate (convergence) of the relative risk in the case of low frequency diseases. Furthermore, considering that individuals with CVD were excluded at baseline, and that it was possible to misclassify diabetes status for patients interviewed by phone, an underestimation of diabetes incidence may have been observed rather than an overestimation. Observational studies using semi-quantitative questionnaires may lead to an inaccurate evaluation of dietary habits with possible underreporting and misclassification. Finally, the possibility that some participants may have changed their dietary habits during the long 10-year follow-up period without reporting this promptly should also be considered. Therefore, more research on this issue is needed to confirm the results.

6. Conclusions

The present study is based on previous findings from the ATTICA study showing a protective effect of the Mediterranean diet on the risk of diabetes, and expands those findings by examining its role in CVD prevention in subjects with prediabetes. It confirms the favorable effect of a high and even medium adherence to the Mediterranean dietary pattern against diabetes and CVD incidence in subjects with IFG. The results indicate that prediabetic subjects carry an increased risk of diabetes and CVD, and underline the critical role of dietary prevention of diabetes and CVD in prediabetic subjects.

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