Adherence to Mediterranean diet and 10-year incidence (2012-2014) of diabetes; the mediating effect of inflammatory and oxidative stress biomarkers: results from ATTICA cohort study.

Mediterranean diet and diabetes

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Abstract

Background: the purpose of this work was to investigate the potential mediating effect of oxidative stress, inflammation and coagulation on Mediterranean diet- diabetes link.

Methods: in 2001-02, a random sample of 1514 men (18-87 years old) and 1528 women (18-89 years old) was selected to participate in the ATTICA study, where Athens is a major metropolis. A validated questionnaire was used to assess lifestyle and dietary factors. Adherence to Mediterranean diet was recorded using *MedDietScore*. Among others, oxidative stress and inflammatory biomarkers were recorded. During 2011-2012, the 10-year follow-up was performed. Diabetes incidence was defined according to American Diabetes Association criteria.

Results: 191 incident cases of diabetes were documented, yielding to an incidence of 12.9% (13.4% in men and 12.4% in women). Medium and high adherence were found to decrease diabetes risk by 49% (95%CI: 0.30, 0.88), and 62% (95%CI: 0.16, 0.88) respectively, compared to low adherence. A logarithmic trend between Mediterranean diet and diabetes incidence was also revealed (p for trend=0.042). Individuals with abnormal waist circumference (>94 for men, >80 for women) were benefited the most. Wholegrain cereals, fruits and legumes had the greatest predictive ability. The anti-diabetic effect of Mediterranean diet was mediated by TNF-α, homocysteine and TAC.

Conclusions: the reported results underline the role of Mediterranean diet as a promising dietary tool for the primary prevention of diabetes, by attenuating inflammation and fostering TAC; thus, this dietary pattern may have a therapeutic potential for a plethora of cardio-metabolic disorders, resulting from inflammation and/or oxidative stress.

Keywords: Cohort study, Mediterranean diet, Risk, Type 2 diabetes, Oxidative stress, Inflammation

INTRODUCTION

The worldwide diabetes epidemic seems to be unstoppable, affecting approximately 8% of the adult population; and the global health expenditure for diabetes treatment, i.e., 548 billion dollars in 2013, continuously escalates with catastrophic consequences for vulnerable economies and people with diabetes [1]. Lack of treatment makes primary prevention a cornerstone of the global response to diabetes. Although many dietary strategies are available for diabetes secondary prevention [2], no definite consensus regarding the best diet against diabetes onset has been achieved.

Mediterranean diet is a nutritional model inspired by the traditional dietary habits of people living in the Mediterranean basin [3]. It is primarily characterized by high consumption of olive oil, legumes, whole grain cereals, fruits and vegetables and moderate wine drinking, and, secondarily, moderate consumption of fish, dairy products and low consumption of poultry, meat and its products, highly processed foods, refined grains and sugars [4]. Recently, Mediterranean diet, which has long been celebrated for its cardiovascular health benefits [5], was systematically reviewed in relation to diabetes incidence. Koloverou et al., reported that higher adherence to Mediterranean diet is associated with 23% reduced risk of diabetes development (combined RR for upper versus lowest available centile: 0.77; 95%CI: 0.66, 0.89) [6]. However, it cannot be ignored that statistical heterogeneity was detected, while it still remains unclear whether this protection is exhibited when adherence to Mediterranean diet is medium, not high.

The underlying pathway of this potential protection requires even more investigation. As a combination of many different foods, Mediterranean diet could exert its anti-diabetic effect variously. For example, since insulin production and secretion gets defective, when pancreas is chronically exposed to high oxidative stress levels [7], Mediterranean diet, which has been associated to elevated total antioxidant capacity (TAC) levels and low oxidized

LDL-cholesterol [8], could avert this deficiency. In addition, adherence to Mediterranean diet has been found to attenuate inflammation and coagulation, in healthy adults [9], a process also implicated in diabetes patho-physiological mechanism [10]. Finally, the tested relationship could be mediated by Mediterranean diet's positive impact on abdominal fat and obesity [11]; however, this still remains unclear, considering a recent study, reporting that Mediterranean diet did not protect from abdominal obesity in the long term [12], as well as the study of Sacks et al, who found that reduction of calories is enough to achieve clinically meaningful weight loss, regardless of diet content [13].

Until now only two prospective studies, examining Mediterranean diet-diabetes link, have attempted to identify potential mediators. Among patients with recent myocardial infarction, higher HDL-cholesterol and lower triglycerides levels [14] have been found to mediate the protective effect of Mediterranean diet and among women with prior gestational diabetes lower body mass index (BMI) [15]. To the best of our knowledge, no previous study has assessed the mediating effect of inflammatory, coagulation and oxidative stress biomarkers, especially among apparently healthy individuals. Thus, and under the context of the ATTICA study [20], in the present work the effect of Mediterranean diet on 10-year diabetes incidence in a Greek sample was evaluated, while oxidative stress, inflammation and coagulation status of the participants were examined as plausible mediators.

MATERIALS AND METHODS

Baseline sampling procedure (2001-2002)

The ATTICA study is a large-scale, health and nutrition, prospective survey, which was carried out during 2001-2002, in the province of Attica, where Athens is a major metropolis. People with history of CVD or other atherosclerotic disease, or having chronic viral infections or living in institutions were excluded from participation. Of the initially invited 4056 individuals and after excluding those with CVD (i.e., n=117) or those having chronic viral infections (n=107), 3042 finally agreed to participate (75% participation rate); 1514 of the participants were men (aged 46±13 y; range 18-87 y) and 1528 were women (aged 45±13 y; range: 18–89 y). Trained personnel (i.e., cardiologists, general practitioners, dietitians and nurses) interviewed the participants, using a standard questionnaire.

More details about the aims, design and methods used in the ATTICA Study may be found elsewhere in the literature [16].

Baseline measurements

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. Smokers were defined as those who smoked at least one cigarette per day or had quitted within the previous year; the rest were defined as non-smokers. The International Physical Activity Questionnaire (IPAQ) was used to evaluate the level of physical activity. IPAQ is an index of weekly energy expenditure using frequency (times per week), duration (in minutes per time) and intensity of sports or other habits related to physical activity (in expended calories per time) [17].

The evaluation of the nutritional habits was based on a validated semi-quantitative food-frequency questionnaire, the EPIC-Greek questionnaire that was kindly provided by the Unit of Nutrition of Athens Medical School; participants were asked to report the average intake of several food items and liquids consumed during the previous year [18]. Adherence to Mediterranean diet was evaluated using the *MedDietScore* (range 0-55, higher values greater adherence) [19]. The tertiles of the score were also calculated, yielding three categories, i.e., low, medium and high level of adherence.

Weight (in Kg), height (in m), waist (in cm) and hip (in cm) circumferences, as well as clinical characteristics, were measured using standardized procedures. Arterial blood pressure was measured 3 times by using the right arm. All measurements were made at the end of the physical examination while subjects were in a sitting position for at least 30 min. Patients whose average blood pressure was ≥140/90 mm Hg or those under 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 diabetes mellitus (type 2) was based on the criteria of the American Diabetes Association [20], i.e., fasting blood glucose>125 mg/dL or the use of antidiabetic medication.

Biochemical measurements were carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. Blood samples were collected from the antecubital vein between 8 to 10 a.m., in a sitting position after 12 hours of fasting and avoiding of alcohol. Serum total cholesterol, HDL-cholesterol, triglycerides and glucose concentrations were measured using chromatographic enzymic method in a Technicon automatic analyser RA-1000. LDL cholesterol calculated using the Friedewald formulae. Serum insulin concentrations were assayed by means of radioimmunoassay. Inflammatory markers were assayed using the following techniques: C-reactive protein (CRP) and Serum Amyloid-A (SAA) by particle-enhanced immunonephelometry, interleukin 6 (IL-6) by a high-sensitivity enzyme-linked immunoassay, human tumor necrosis factor -a (TNF-a) by the enzyme-linked immunosorbent assay method for the quantitative determination, homocysteine levels by an automatic analyzer based on the technology of fluorescence polarization immunoassay and fibrinogen by automatic nephelometry. Finally, serum TAC was measured with a colorimetric test and plasma oxidized LDL-cholesterol with an enzyme-linked immunosorbent assay kit.

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

During 2011-2012, the 10-year follow-up was performed. Of the n=3042 participants, *n*=2583 were allocated during the follow-up (85% participation rate). A detailed evaluation of the participants' medical status was performed. Among various endpoints, development of diabetes mellitus was recorded; *n*=210 patients diagnosed with diabetes at baseline and *n*=1347 participants with no data regarding diabetes status at the 10-year follow up were not included in the present analyses, yielding a working sample of *n*=1485 participants without diabetes at baseline. The sample size was adequate to achieve 92% statistical power to evaluate relative risk of 0.70 between the null and the alternative two-sided hypothesis, when the exposure variable (i.e., level of adherence to Mediterranean Diet or *MedDietScore*) was increased by 1-unit and with a significance level (alpha) of 0.05. Further details about the baseline procedures and the 10-year follow-up of the study have been presented elsewhere [16, 21].

Statistical analysis

Incidence of diabetes was calculated as the ratio of new cases (n=191) to the total number (*n*=1485) of participants in the follow-up. Incidence by Mediterranean Diet group was calculated as the ratio of new cases to the number of participants in each group. Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies. Associations between categorical variables were tested using chi-squared test. Comparisons of mean values of normally distributed variables between those who developed diabetes and the rest of the participants were performed using Student's t-test, after ensuring equality of variances using Levene's test. Analysis of variance (ANOVA) was performed to compare the mean values of normally distributed variables by Mediterranean Diet group. Post-hoc analyses using the Bonferroni rule were performed to account for the inflation of the probability of type-I error. For non-normally distributed variables, the

Kruskall-Wallis test was applied, and next the Mann-Whitney test was performed between every two groups, so as to detect significant mean differences. Continuous variables were tested for normality through histograms. The relative risk of developing diabetes during the 10-year period according to the participants' baseline characteristics was estimated through the odds ratio (OR) and the 95% corresponding confidence interval, as derived from logistic regression models. This type of analysis was preferred 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 sub-group analyses were performed. Trend analysis was applied by fitting smoothing lines (linear, logarithmic, quadratic or cubic) on odds ratios derived by age-sex adjusted analysis, by Mediterranean diet category; the corresponding R-squared values indicated which line best fits the observed data. The predictive ability of the components of Mediterranean diet was ranked, calculating the -2log-likelihood of each model (the lower the better). The SPSS version 18 (Statistical Package for Social Sciences, IBM Hellas SA, Greece) software was used for all statistical calculations.

RESULTS

10-year diabetes incidence

During the 10-year follow-up period, 191 diabetes cases were documented; yielding to a crude incidence of 129 per 1000 participants (or 12.9%); of them, 97 (13.4%) were men and 94 (12.4%) were women ($p = 0.79$ for gender difference) [21].

Participants' baseline characteristics by level of adherence to the Mediterranean diet

Demographic and clinical characteristics of the participants by adherence to the Mediterranean diet status are presented in ***Table 1***. As the level of adherence to Mediterranean diet increased, participants were more likely to be women, younger, better educated and to have lower BMI and waist circumference (WC). Hypertension and hypercholesterolemia were also less frequent; Total cholesterol, LDL-cholesterol and triglycerides mean concentrations were lower, whereas HDL-cholesterol higher. With regards to oxidative stress biomarkers, participants, who adopted the Mediterranean diet, had the highest TAC and lowest oxidized-LDL values. Similarly, mean concentrations of fasting glucose and insulin, as well as all inflammatory and coagulation biomarkers were the lowest. Family history of diabetes, smoking status and physical activity status did not differ significantly between the three groups ***(Table 1).***

10-year diabetes incidence and Mediterranean Diet

The 10-year incidence of diabetes was n=83 cases (21%) among participants away from the Mediterranean diet, n=38 (8.0%) among participants with medium adherence and n=8 (5.0%) in the high adherence group (p<0.001) (***Table 1***). Participants who did not develop diabetes within the 10-year follow-up period were equally distributed among the three Mediterranean diet groups (30%, 35% and 35% for low, medium and high adherence), whereas participants who developed diabetes were mostly away from the pattern (55%, 33% and 13% for the three groups respectively). Trend analysis on the ORs derived from age-sex adjusted models per Mediterranean diet group revealed a significant logarithmic shape between diabetes incidence and level of adherence to the Mediterranean diet (R-squared for logarithmic trend = 0.99, p=0.042) (other trends, i.e., linear, exponential, quadratic, were also tested, but found insignificant, p>0.10).

In order to control for potential confounding, multi-adjusted analysis was performed through nested models. In age-sex adjusted model (***Table 2, model 1***), a significant inverse association was observed for participants with both medium and high, compared to low, adherence to Mediterranean diet (RR= 0.57, 95%CI: 0.38, 0.84, and RR= 0.25, 95%CI: 0.13, 0.47 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 2, model 2***), educational status and physical activity status (***Table 2, model 3***), as well as abnormal WC (i.e., WC>94cm for men or >80cm for women) (***Table 2, model 4***). The interaction between *MedDietScore* and WC category was found significant (*p* =0.045) and was kept in the final model. Specifically, in the fully adjusted model, individuals with medium adherence to the Mediterranean diet experienced 49% lower risk for developing diabetes, within the next 10 years (RR=0.51; 95%CI: 0.30, 0.88), whereas for individuals with high adherence, the risk was found decreased by 62% (RR=0.38; 95%CI: 0.16, 0.88), compared to participants with low level of adherence to the pattern.

To identify which components of Mediterranean diet contribute the most to the observed inverse association, all Mediterranean diet components were added to model 4, one by one, and -2Loglikelihood values were compared; with lowest values indicating better predictive ability. The three components with the lowest values were wholegrain cereals, fruits and legumes with -2Loglikelihood values equal to 189.1, 189.5 and 189.9 respectively). Due to the above mentioned significant interaction between WC category and *MedDietScore*, logistic regression analysis was additionally performed after stratification by WC, i.e., *n*=727 participants with increased WC (WC>94cm for men and >80cm for women) versus *n*=549 participants with normal WC. In the fully adjusted model, an inverse association was observed between *MedDietScore* and diabetes development, only among participants with increased abdominal fat (RR=0.44, 95%CI: 0.25, 0.77 for medium adherence to Mediterranean diet, and RR=0.26, 95%CI: 0.10, 0.70 for high adherence). For individuals with normal WC, results were not significant (RR=0.97, 95%CI: 0.29, 3.25 and RR=0.89, 95%CI: 0.16, 4.90 for medium and high adherence respectively); nevertheless, the interpretation of these findings warrants caution due to the small number of events (*n*=32), among participants with WC<90/84.

To investigate the potential mechanism underlying the protective effect of Mediterranean diet for individuals with increased abdominal fat, biomarkers of oxidative stress (i.e., ox-LDL and TAC), inflammation (i.e., IL-6, SAA, TNF-a, CRP and homocysteine) and coagulation (i.e., fibrinogen) were sequentially, and separately, entered to the fully adjusted model. Stratification by WC category was maintained. For men with WC>94cm and women >80cm, statistical analysis revealed the mediating effect of TAC, TNF-α and homocysteine in the examined relationship (all p-values >0.05) (***Table 3***). The mediating effect of these biomarkers was enhanced by the fact that their mean concentrations significantly increased in parallel with the level of adherence to the Mediterranean diet (***Table 1***). Oxidized-LDL, IL-6, CRP, SAA and fibrinogen did not influence the tested association (***Table 3***). Among individuals with normal waist circumference, results remained not significant in all steps (data not shown).

DISCUSSION

Mediterranean diet is a well-known "heart-healthy" pattern, with a potential to extend its health benefits beyond cardiovascular disease. In the present work the 10-year diabetes incidence was studied in relation to Mediterranean diet. Medium adherence to Mediterranean pattern was found to decrease the 10-year diabetes risk by almost 50%, whereas high adherence more than 60%. Trend analysis also revealed a significant logarithmic relationship between diabetes development and adherence to the Mediterranean diet, suggesting that, shifting from low to medium adherence was followed by a greater relative risk reduction, compared to shifting from medium to high. Among *MedDietScore* components, wholegrain cereals, fruits and legumes had the greatest predictive ability on diabetes risk; in other words exerted the greatest protection. Furthermore, abdominal fat was found to have a moderating effect on the tested relationship; the protective effect of the diet was limited, though strengthened, when waist circumference exceeded 94/80cm for men and women respectively. Finally, an effort to identify potential mediators of the Mediterranean diet-diabetes inverse association was attempted. It was revealed that changes in TAC, TNF-α and homocysteine concentrations may underlie diabetes pathogenesis mechanism, suggesting that adherence to the Mediterranean diet may offer its anti-diabetic effect through a decrease in oxidative stress and subclinical inflammation. Despite the limitations of the present observational study, the large, representative sample, the prospective design and follow-up of 10 years, as well as the detailed assessment of lifestyle information, and, therefore, the ability to adjust for several known confounders, may guarantee that the reported findings are of considerable public health importance, as they shed light into the underlying pathway of Mediterranean's diet antidiabetic effect, as well as the group of people that seem to benefit most by the pattern's long term high adherence.

Mediterranean diet has been consistently linked to lower diabetes incidence, a finding confirmed recently by the meta-analysis of Koloverou et al [6]. De Koning et al, have also observed that Mediterranean diet yields its benefit only in the presence of high BMI [22]; similarly to the present study, where increased WC, an even better than BMI predictor of diabetes [23], was found to maximize Mediterranean diet's beneficial effect. Considering the higher levels of oxidative stress and inflammation among individuals with abdominal obesity [24, 25], and the observed mediating effect of TAC, homocysteine and TNF-α on the examined relationship, this finding does make sense, and further confirms the inflammatory and oxidative stress component in diabetes pathogenesis [7, 10].

Mediterranean diet's anti-inflammatory potential is well-known. Whole grains, vegetables, fruit and fish, in all of which Mediterranean diet is abundant, as well as vitamin C, vitamin E and carotenoids are associated with lower circulating concentrations of inflammatory markers [26]. Two long-term randomized clinical trials have proven the effectiveness of Mediterranean diet in lowering inflammatory markers, such as CRP and IL-6, either following weight loss in post-menopausal women [27], or independent of weight loss among patients with metabolic syndrome [28].

In this article, three other biomarkers were identified as plausible mediators of Mediterranean diet – diabetes inverse association, homocysteine, TNF-α and TAC. Mediterranean diet has previously been reported to decrease homocysteine [29], an inflammatory score including TNF-α [30] and to increase TAC [31]. Regarding homocysteine, a recent meta-analysis highlighted that hyperhomocysteinemia, is causally linked to diabetes development [32], which makes the present finding even more robust. Secondly, TNF-α, a pro-inflammatory cytokine, has long been studied in relation to obesity-related insulin resistance and it is found to deteriorate insulin-signaling pathways [33, 34]. Furthermore, TNF-α antagonists improve insulin sensitivity among non-diabetic patients with rheumatoid arthritis [35]. However, a direct association between TNF-α and diabetes incidence has not been established. Finally, TAC has been inversely associated with diabetes biomarkers among healthy participants [36]; nevertheless, similarly with TNF-α, it has not been definitively linked to diabetes incidence.

The proposed effect of Mediterranean diet against diabetes development was postulated to be interplay between the pattern's nutritional components. As it was observed, wholegrain cereals, legumes and fruits consumption were primarily linked to the pattern's anti-diabetic effect. Cereal-oriented dietary fiber have been suggested to act beneficially [37] due to delayed gastric emptying, which slows down glucose absorption and reduces insulin levels, as well as the bran's high magnesium concentration. Decreased intracellular enzymatic activity, due to magnesium deficiency, fosters insulin resistance [38]. Legumes are also excellent magnesium sources. With regards to fruits, their significant antioxidant content may be responsible for their high predictive ability. As mentioned above, dietary antioxidants may suspend oxidative stress accumulation, which inflicts damage on pancreatic b-cell's dynamic for insulin production and secretion [7]. Other dietary components may act protectively. Alcohol consumption in moderation improves insulin sensitivity, reduces basal insulin secretion rate and lowers fasting glucagon concentration [39], as well as plasma Fetuin-A, which in turn inhibits insulin signaling. Moreover, fish and nut derived n-3 FFA, along with olive oil polyphenols shift metabolic balance towards a more anti-inflammatory state [40], which as mentioned above contributes to diabetes onset detainment.

Limitations

This study has some limitations that should be considered. Since the exact time of diabetes onset was not known in all cases, but only the date of diabetes diagnosis, hazard ratios was estimated through odds ratios, which may have over-estimated the true effect. However, for low frequency diseases, odds ratio is suggested to be an accurate estimate (converges) of the relative risk. Furthermore, considering that individuals with CVD were excluded in baseline assessment, as well as the possibility of misclassification of diabetes status for patients interviewed by phone, there may have been an underestimation of diabetes incidence. The difficulty of an accurate evaluation of Mediterranean diet with possible underreporting and misclassification should be acknowledged, as in all observational studies. Finally, the fact that some participants might have changed their dietary habits during the long follow-up of 10 years, without timely information updates should also be acknowledged.

Conclusions

The present study confirmed the favorable effect of Mediterranean dietary pattern against diabetes onset, and identified potential mechanisms. Specifically, the novelty of this work is that Mediterranean diet was found to exert its protection, through attenuation of inflammation process and oxidative stress, especially among individuals with increased waist circumference. Although, focused studies are deemed necessary to draw firm conclusions regarding Mediterranean diet's anti-diabetic pathways, the reported results underline the crucial role of Mediterranean diet, not only as a promising dietary tool for the primary prevention of diabetes, but also a plethora of cardio-metabolic disorders, with an inflammatory or oxidative stress component.

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Table 1. Distribution of baseline lifestyle and clinical characteristics[2] of the ATTICA study's participants, according to the level of adherence to Mediterranean diet (n=1485).

Baseline characteristics	Level of adherence to Mediterranean Diet			
	Low (n= 490)	Medium (n= 518)	High (n=477)	*p*
Diabetes cases, n (%)	83 (21)	38 (12)	8 (5)	<0.001
Age, years	54 ± 12	46 ± 10*	36 ± 10*	<0.001
Male sex, n (%)	371 (76)	289 (56)	66 (14)	<0.001
Education, years of school	11 ± 3.8	12 ± 3.6**	14 ± 2.8**	<0.001
Body mass index, kg/m^2	29 ± 4.2	27 ± 2.8*	22 ± 2.5*	<0.001
Waist circumference, cm	100 ± 12	92 ± 11*	78 ± 10*	<0.001
Family history of diabetes, n (%)	98 (22)	96 (21)	97 (22)	0.91
Hypertensive subjects, n (%)	215 (46)	156 (32)	45 (10)	<0.001
Hypercholesterolemic subjects, n (%)	215 (44)	257 (50)	109 (23)	<0.001
– *Total cholesterol, mg/dL*	202 ± 39	203 ± 42	177 ± 39*	<0.001
– *HDL-cholesterol, mg/dL*	45 ± 10	48 ± 17*	54 ± 14*	<0.001
– *LDL-cholesterol, mg/dL*	131 ± 35	130 ± 36	109 ± 35*	<0.001
– *Triglycerides, mg/dL*	136 ± 80	129 ± 93*	78 ± 37**	<0.001
Current smokers, n (%)	263 (54)	298 (58)	241 (51)	0.091
Physically active, n (%)	212 (43)	202 (39)	211 (44)	0.20
Fasting glucose, mg/dL	92 ± 13	90 ± 12*	86 ± 12**	<0.001
Fasting insulin, μU/mL	14 ± 5.3	13 ± 1.6**	12 ± 1.3**	<0.001
TAC, μmol/L	225 ± 42	229 ± 32	250 ± 45**	<0.001
ox-LDL, mg/dL	66 ± 30	63 ± 28	54 ± 26**	<0.001
IL-6, pg/mL	1.6 ± 0.48	1.5 ± 0.52**	1.3 ± 0.40**	<0.001
TNF-α, pg/mL	8.2 ± 3.9	6.2 ± 3.3**	4.8 ± 4.8**	<0.001
CRP, mg/L	2.3 ± 2.7	1.9 ± 2.3	1.3 ± 1.9**	<0.001
Homocysteine, μmol/L	13 ± 5.1	12 ± 6.0*	11 ± 7.7**	<0.001
SAA, mg/dL	4.7 ± 4.6	4.3 ± 3.8	4.3 ± 5.1*	0.050
Fibrinogen, mg/dL	320 ± 75	311 ± 63	292 ± 65**	<0.001

[a] TAC= Total Antioxidant Capacity, ox-LDL=oxidized LDL-cholesterol, IL-6=Interleykin-6, TNF-a= Tumor Necrosis factor-a, CRP=C-reactive protein, SAA=Serum Amyloid-A.

[b] Data are presented as mean values and standard deviation or absolute and relative frequencies. P-values derived from ANOVA, for the normally distributed variables and Kruskall-Wallis test for the non-normally distributed variables (i.e., triglycerides, years of school, fasting glucose, fasting insulin, TAC, ox-LDL, TNF-a, IL-6, CRP, homocysteine, SAA, fibrinogen), or chi-square test for the categorical variables.

[c] *p<0.05 and **p<0.001 from post-hoc analyses using the Bonferroni rule, using small adherence to the Mediterranean Diet as the reference category, or from the Mann-Whitney test (every two groups) for non-normally distributed variables.

Table 2. Results from multiple logistic regression models (odds ratios and the corresponding confidence intervals) that evaluated participants' adherence to Mediterranean diet as well as other characteristics, in relation to 10-year incidence of diabetes (n=1485)

	Odds Ratio for *MedDietScore* (per 1 unit)	Level of adherence to the Mediterranean Diet		
		Low (n= 490)	Medium (n=518)	High (n=477)
Model 1	0.99; 0.97, 1.02	*Ref*	0.57; 0.38, 0.84	0.25; 0.13, 0.47
Model 2	0.99; 0.96, 1.02	*Ref*	0.53; 0.34, 0.83	0.28; 0.14, 0.57
Model 3	0.99; 0.96, 1.02	*Ref*	0.54; 0.35, 0.85	0.31; 0.15; 0.62
Model 4	1.04; 0.99; 1.09	*Ref*	0.51; 0.30, 0.88	0.38; 0.16, 0.88

Table 3. Results (odds ratio and 95% confidence interval) from multiple logistic regression models, among *n*=727 participants with WC>94/80, that evaluated participants' adherence to Mediterranean diet in relation to 10-year incidence of diabetes, after one-by-one inclusion of various biomarkers in the fully-adjusted model.

Biomarker added in the full model that contained: age, sex, family history of diabetes, history of hypertension, hypercholesterolemia, smoking, education level and physical activity.	Level of adherence to the Mediterranean Diet		
	Low (n= 305)	Medium (n=288)	High (n=134)
Oxidative stress			
TAC, μmol/L	*Ref*	0.08; 0.01, 1.11	0.24; 0.01, 5.98
ox-LDL, mg/dL	*Ref*	0.31; 0.15, 0.64	0.17; 0.05, 0.62
Inflammatory			
IL-6, pg/mL	*Ref*	0.47; 0.26, 0.84	0.28; 0.09, 0.88
TNF-α, pg/mL	*Ref*	0.60; 0.31, 1.16	0.35; 0.12, 1.02
CRP, mg/L	*Ref*	0.47; 0.26, 0.84	0.28; 0.09, 0.88
Homocysteine, μmol/L	*Ref*	0.56; 0.29, 1.10	0.31; 0.09, 1.07
SAA, mg/dL	*Ref*	0.47; 0.25, 0.88	0.22; 0.07, 0.75
Coagulation			
Fibrinogen, mg/dL	*Ref*	0.45; 0.25, 0.83	0.28; 0.10, 0.81