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Trimethylamine *N*-oxide, Mediterranean diet, and nutrition in healthy, normal-weight adults: also a matter of sex?

Luigi Barrea M.D., Ph.D. [a,*], Giuseppe Annunziata M.D., Ph.D. [b], Giovanna Muscogiuri M.D Ph.D. [a], Daniela Laudisio M.D. [a], Carolina Di Somma M.D., Ph.D. [c], Maria Maisto Ph.D. [b], Gian Carlo Tenore Ph.D. [b], Annamaria Colao M.D., Ph.D. [a], Silvia Savastano M.D., Ph.D. [a]

[a] *Department of Clinical Medicine and Surgery, Unit of Endocrinology, Federico II University Medical School of Naples, Naples, Italy*
[b] *Department of Pharmacy, University of Naples "Federico II", Naples, Italy*
[c] *Institute for Hospitalization and Healthcare SDN, Naples, Italy*

A R T I C L E   I N F O

A B S T R A C T

Objectives: Sex exerts an important influence on food preferences. The Mediterranean diet (MD) is based on the common dietary characteristics and lifestyle behaviors of the Mediterranean countries. Trimethylamine *N*-oxide (TMAO), a marker of gut dysbiosis linked to the development of atherosclerosis and cardiovascular risk, is mainly dependent on dietary pattern and gut microbiota metabolism. The aim of this study was to evaluate the association between levels of TMAO and adherence to the MD as a function of sex.
Methods: We enrolled 144 healthy adults, of which 67 were men. Participants were 31.55 ± 6.19 y of age and had an average body mass index of 22.84 ± 1.51 kg/m^2. TMAO levels were detected using high-performance liquid chromatography-mass spectrometry analysis. PREDIMED (PREvención con DIeta MEDiterránea [Prevention with Mediterranean Diet]) questionnaire was used to assess the adherence to the MD. Dietary data were collected by a 7-d food records. Receiver operator characteristic curve analysis was performed to determine the predictive values for PREDIMED score in detecting high TMAO values.
Results: Compared with women, the men presented higher levels of TMAO ($P < 0.001$), lower adherence to the MD ($P = 0.017$) and higher energy intake. The men consumed a greater quantity of animal proteins, carbohydrates, and ω-3 polyunsaturated fatty acids, and less plant proteins and ω-6 polyunsaturated fatty acids than the women. At the receiver operator characteristic analyses, the lowest levels of TMAO were well predicted by a score of adherence of ≤10 in men and ≤9 in women ($P < 0.001$).
Conclusions: A clear sex difference was observed in the apparently novel association between levels of TMAO and MD in healthy adults. Although dietary intervention trials on large series population are mandatory, sex-specific cutpoints of adherence to MD might help identify individuals at high risk for high levels of TMAO who would benefit from personalized dietary interventions.

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Introduction

It is well known that sex and other nonmodifiable and modifiable factors, such as age, body weight, or educational status, influences food preferences and consumption amounts [1]. In particular, women's dietary profiles are characterized by a higher intakes of carbohydrates, including fruits and vegetables, whereas men consume more animal proteins, including meat, eggs, and dairy products, and less fruit [2–4] and that women are more concerned with weight control, natural foods, and ethical issues [5].

Diets rich in animal protein, such as Western diets, could include nonprotein factors that might contribute to the pathogenesis of several diseases. There is a growing body of evidence suggesting an atherogenic role for a group of lipid-like small molecules, mostly found in meat and dairy products, called carnitine, phosphatidylcholine, and its metabolite, choline [6,7]. After their ingestion, phosphatidylcholine and choline are metabolized by gut microorganisms into trimethylamine (TMA) [8]. TMA is absorbed and enzymatically oxidized by flavin-containing monooxygenases (FMO3) in the liver to trimethylamine *N*-oxide (TMAO), a small organic compound also naturally present in fish and seafood,

which may promote atherosclerosis [9,10], also independently of choline itself or its other metabolites [11–13]. As much as 95% of total body TMAO is excreted unchanged by the kidney, through both glomerular filtration and tubular secretion and even modestly impaired renal function might increase circulating TMAO concentrations and alter the intestinal flora, thus inducing increasingly variability in TMAO levels [12]. Because to its gut-derived origin, circulating levels of TMAO have been proposed as a surrogate marker of a proatherogenic microbe community [14]. In particular, elevated levels of TMAO have recently emerged as an independent predictor of chronic kidney disease, atherosclerosis, cardiovascular disease (CVD), and incident risk for major adverse cardiovascular events (myocardial infarction, stroke, and death) [15]. In addition, elevations in TMAO also are associated with type 2 diabetes mellitus, in both animal models of diabetes (i.e., db/db mice) and humans [16], and with a number of different types of cancer, including colorectal and prostate cancers [14,17].

Although there is no uniform definition of the Mediterranean style, the traditional Mediterranean diet (MD) is based on the common dietary characteristics and lifestyle behaviors of Mediterranean countries and, to date, is the most studied diet at the level of evidence-based medicine [18,19]. The food pyramid reflecting Mediterranean dietary traditions is characterized by a high intake of plant foods (fruits, particularly fresh fruits; vegetables; whole grains, including cereals, breads, rice, or pasta; and nuts containing antioxidants) a moderate intake of dairy products (principally cheese and yogurt); fish, particularly fatty fish rich in polyunsaturated fatty acids (PUFAs); a low intake of red meat, processed meats, and sweets; no more than four eggs consumed weekly; both total olive oil and the virgin variety of olive oil as the principal source of fat; and wine consumed in low to moderate amounts, normally with meals [20]. Thus, the MD dietary patterns tended to be moderate in total fat (32–35% of total calories), relatively low in saturated fat (9–10% of total calories), and high in fiber (27–37g/d) and ω-3 PUFAs [19]. Higher adherence to the MD has been reported to have a beneficial effect on cardiovascular risk in both observational cohort studies and clinical trials [21,22]. Intervention trials aiming to investigate the effect of the MD on prevention of CVD reported that circulating levels of TMAO could be a useful marker that may be associated with CVDs [23]. In addition, low levels of urinary TMAO were present after a MD supplemented with extra-virgin olive oil [24]. However, neither the possible association between circulating levels of TMAO and the adherence to the MD nor its association with single dietary nutrients have been explored until now, particularly with how TMAO is associated with sex-related food preferences.

Considering the potential effects of sex on food preferences, the aim of this cross-sectional observational study was to investigate the association between circulating levels of TMAO with the adherence to the MD in function of sex in a cohort of healthy, normal-weight young adults.

Participants and methods

Design and setting

This was a cross-sectional observational study carried out at the Department of Clinical Medicine and Surgery, Unit of Endocrinology, University Federico II, Naples (Italy), from January 2016 to December 2017. The work was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and it was approved by the Ethical Committee of the University of Naples "Federico II" Medical School. The purpose of the protocol was explained to all the study participants, and written informed consent was obtained. This trial was registered at ClinicalTrials.gov (unique identifier: NCT03060811).

Population study

Recruitment strategies included 302 healthy men and women enrolled among hospital volunteers and employees from the same geographical area. Healthy 18-

to 50-y-old individuals (N = 144) with a body mass index (BMI) of 18 to 25 kg/m^2 were included. All participants were white and none had a clinical condition that potentially influences fluid balance, that is, impaired renal function (normal values: estimated glomerular filtration rate ≥90 mL/min/1.73 m^2 calculated by chronic kidney disease epidemiology collaboration equation) or endocrine or myocardial diseases (ascertained by participant questionnaire). All women were non-pregnant and non-lactating and were evaluated in the follicular phase of the menstrual cycle. A full medical history, including drug use, was collected.

Criteria for exclusion from the study included:

- Postmenopausal status (defined as ≥50 y of age and amenorrhoea for ≥3 y or amenorrhoea for ≥1 but <3 y and plasma follicle-stimulating hormone concentrations elevated to the postmenopausal range; 12 women).
- Current, continued use of hormonal contraceptives (24 women).
- Use of antibiotics or probiotics within 2 mo of recruitment (26 individuals).
- Specific nutritional regimens, including vegan or vegetarian diets (4 individuals).
- Vitamin/mineral or antioxidant supplementation (21 individuals).
- Occasional or current of use of drugs that could influence fluid balance, including non-steroidal anti-inflammatory drugs (21 individuals), diuretics (18 individuals), or laxative use (6 individuals).
- Alcohol abuse according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-V diagnostic criteria (7 individuals).

Finally, 19 individuals left the study after refusing to participate. Figure 1 depicts the flow chart of study participants.

Lifestyle habits

Lifestyle habits, including smoking and physical activity level, were investigated using a standard questionnaire. Participants smoking at least one cigarette per day were considered current smokers. Former smokers were the those who stopped smoking ≥1 y before the interview. Remaining participants were defined as non-current smokers. Physical activity levels were expressed according to whether the participant habitually engaged at least 30 min/d of aerobic exercise (yes/no).

Anthropometric measurements

Measurements were performed between 08:00 h to 12:00 h. All were measured after an overnight fast. The measurements were made by one operator (a nutritionist experienced in providing nutritional assessment and body composition). All anthropometric measurements were taken with participants wearing only light clothes and without shoes. For each participant, weight and height were measured to calculate BMI (weight [kg] divided by height squared [m^2], kg/m^2). Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Seca 711; Seca, Hamburg, Germany). Body weight was determined to the nearest 0.1 kg using a calibrated balance beam scale (Seca 711; Seca). BMI was classified according to the criteria of the World Health Organization, with normal weight being 18.5 to 24.9 kg/m^2. Waist circumference (WC) was measured to the nearest 0.1 cm with a non-stretchable measuring tape, at the end of several consecutive natural breaths, at the level parallel to the floor, midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the midaxillary line [25].

Adherence to the Mediterranean diet

As already widely reported in the literature [26–29], adherence to the MD was evaluated using a 14-item questionnaire previously validated for the assessment of PREDIMED (Prevención con Dieta Mediterránea [Prevention with Mediterranean Diet]) [30]. A qualified nutritionist administered the questionnaire during a face-to-face interview with all the participants. Briefly, each item was assigned a score of 1 or 0. PREDIMED categories were calculated as follows: 0– 5, lowest adherence; 6 to 9, average adherence; ≥10, highest adherence [30].

Dietary assessment

As widely reported in the literature [31–33], data were obtained during a face-to-face interview between the patient and a qualified nutritionist. In detail, the dietary interview allowed the quantification of foods and drinks by using a photographic food atlas (~1000 photographs) of known portion sizes to ensure accurate completion of the diary records [34]. On day 1 of the diary recording, nutritionists who were trained to standardize protocols provided participants with instructions on how to complete the diary at the health check and asked participants to recall their food intake from the previous day. Participants prospectively completed the remaining 6 d and returned the diaries to the nutritionist. Data were processed using a commercial software (Terapia Alimentare
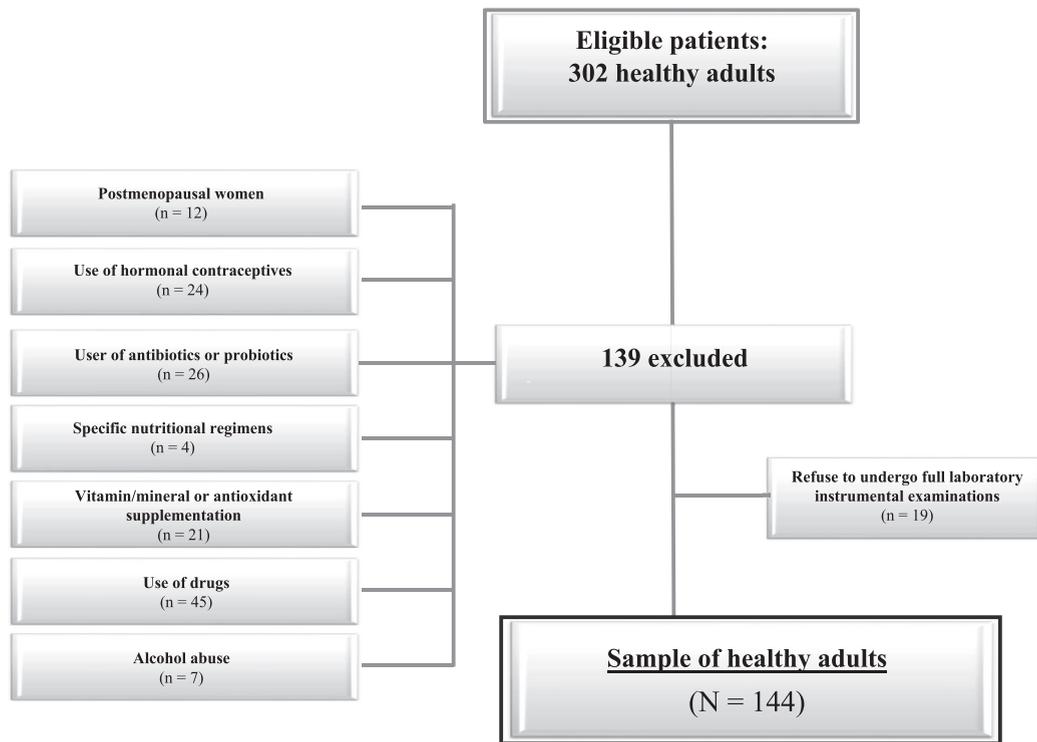
Fig. 1. Flow chart of study design. In both sexes, >50% of participants had a high adherence to the Mediterranean diet and there were no significant sex differences in the low adherence ($\chi^2 = 0.00$; $P = 0.959$), average adherence ($\chi^2 = 0.47$; $P = 0.499$), or high adherence ($\chi^2 = 0.22$; $P = 0.640$) groups.

Dietosystem DS-Medica, http://www.dsmedica.info). Considering quantities and qualities of foods consumed, the software is able to calculate not only the total energy intake, expressed in kcal, but also the quantities of macronutrients (protein; total, complex, and simple carbohydrates; total fat, saturated fatty acids [SFAs], monounsaturated fatty acids [MUFA], ω-6 and ω-3 PUFAs, and the ratio of ω-6 to ω-3 PUFAs; cholesterol; and fiber).

Assay methods

Samples were collected between 0800 h and 1000 h, after an overnight fast ≥8 h and stored at −80 °C until being processed. All biochemical analyses including fasting plasma glucose, total cholesterol, fasting plasma triacylglycerides, alanine transaminase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (γ-GT) were performed with a Roche Modular Analytics System in the Central Biochemistry Laboratory of our institution. Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by a direct method (homogeneous enzymatic assay for the direct quantitative determination of LDL-C and HDL-C). Fasting insulin levels were measured by a solid-phase chemiluminescent enzyme immunoassay using commercially available kits (Immunolite Diagnostic Products Co, Los Angeles, CA, SUA). The intra-assay coefficients of variations (CV) was <5.5%. Homeostasis model assessment of insulin resistance (HoMA-IR) was calculated according to Matthews et al., with a HOMA-IR value of >2.5 used as cutoff of IR [35].

Determination of circulating levels of TMAO

Circulating levels of TMAO were measured in serum samples that were stored at −80 °C. Stability studies revealed that TMAO is stable under these storage conditions for several years [36]. For the quantification of circulating levels of TMAO from samples, a first sample preparation was performed. More specifically, 160 μL methanol was added to an 80-μL serum. Samples were centrifuged at 14 000 g for 10 min (4°C). The supernatant of each sample was collected and then transferred to a sample vial for high performance liquid chromatography-mass spectrometry (HPLC/MS) analysis. The HPLC system Jasco Extrema LC-4000 system (Jasco Inc., Easton, MD, USA) was coupled to a single quadrupole mass spectrometer (Advion ExpressIon^L CMS, Advion Inc., Ithaca, NY, USA) equipped with an electrospray ionization (ESI) source, operating in positive ion mode. The chromatographic separation was performed with a Luna HILIC column (150 × 3 mm, 5 μm particles) in combination with a guard column (HILIC), both supplied by Phenomenex (Torrance, CA, USA). The oven temperature was 60°C. Mobile phase A was 0.15% formic acid and 10 mM ammonium acetate in water; mobile phase B was 100% methanol

(LC/MS grade). According to Beale and Airs [37], an isocratical run was performed at a flow rate of 0.35 mL/min for 6 min, in the ratio 80:20 (A:B), with an injection volume of 10 μL. The capillary temperature was set at 300°C; capillary voltage +150 V; source voltage offset +25 V; source voltage span +70 V; gas source temperature 350°C; and electrospray ionization voltage 3500 V. Data were acquired in SIM mode. TMAO was detected at m/z 76. Mass spectra were recorded from m/z = 50 to 500, in positive ionization mode. TMAO was identified and quantified by analytical standard (Sigma-Aldrich St. Louis, MO, USA) calibration curve ($r^2 = 0.9994$). Each analysis was performed in triplicate. According to Beale and Airs [37], the precision of the method, determined by calculating the intra- and interday coefficients of variation for TMAO, was 6%.

Statistical analysis

We estimated the minimum sample size required to demonstrate the sex differences in circulating levels of TMAO in healthy adults in attaining the primary outcome (association between circulating levels of TMAO and adherence of MD). The minimum sample size was calculated based on the following assumptions: α = 0.05, β = 0.05, and a power of 95% observed meant the study population for circulating levels of TMAO in men and women (2.70 ± 1.99 versus 4.87 ± 2.01, respectively) was 22 men and 22 women. To further increase the power of the study, 144 patients (67 men and 77 women) were be enrolled to complete the study.

To correct for skewed distributions, all variables were logarithmically transformed and back-transformed for presentation in tables and figures. Results are expressed as mean ± standard deviation (SD). The χ^2 test was used to determine the significance of differences in frequency distribution. Differences between the two groups were analyzed by Student's unpaired *t* test. The correlations between study variables were performed using Pearson's *r* correlation coefficients. Proportional odds ratio (OR) models, 95% confidence interval (CI), and R^2, were performed to assess the association among quantitative variables (single items of PREDIMED questionnaire) with TMAO levels. Receiver operator characteristic (ROC) curve analysis was performed to determine sensitivity and specificity, area under the curve (AUC) and CI, and cutoff values for PREDIMED score in detecting TMAO above the median values in both participants. Test AUC for ROC analysis was also performed. We wanted to show that AUC resulting in 0.957 for a particular test was significant from the null hypothesis value 0.5 (meaning no discriminating power), so we entered 0.957 for AUC ROC and 0.5 for null hypothesis values. For the α level, we selected 0.05 type I error, and for the β level, we selected 0.20 type II error. In these analyses, we entered only those variables that had a $P < 0.05$ in the univariate analysis (partial correlation). To avoid multicollinearity, variables

with a variance inflation factor (VIP) >10 were excluded. Values ≤5% were considered statistically significant. Data were stored and analyzed using the MedCalc package (version 12.3.0 1993- 2012 MedCalc Software bvba - MedCalc Software, Mariakerke, Belgium).

Results

The study population consisted of 144 healthy adults (67 men and 77 women). Table 1 shows age, lifestyle habits, anthropometric measurements, metabolic profile, adherence to the MD, and circulating levels of TMAO either in total population, or grouped according to sex. As shown in Table 1, the men exhibited statistical differences in anthropometric measurements and metabolic parameters compared with women. The men also demonstrated higher circulating levels of TMAO ($P < 0.001$) and lower adherence to the MD ($P = 0.017$) than women. No sex differences were observed in smoking and physical activity ($P = 0.664$ and $P = 0.982$, respectively). Circulating levels of TMAO were higher among smokers (4.05 ± 2.25 versus 3.08 ± 2.18 μM, $P = 0.010$) and sedentary individuals (4.22 ± 2.44 versus 3.23 ± 1.99 μM, $P = 0.008$). We further analyzed the number of individuals in each category of adherence to the MD according to sex. There were no significant sex differences in the low ($\chi^2 = 0.00$, $P = 0.959$), average ($\chi^2 = 0.47$, $P = 0.499$), or high adherence groups ($\chi^2 = 0.22$, $P = 0.640$; Fig. 2). Analyzing the response frequency of dietary components included in the PREDIMED questionnaire, we found that the men exhibited statistically significant differences in some items of PREDIMED questionnaire than the women. In particular, more women consumed red meat <1/d, whereas men reported to consume more fish than women (Table 2). Data on the Mediterranean food questionnaire were further analyzed by using the 7-d food records. As shown in the Table 3, the men had significantly higher energy

intake and consumed more animal protein and less plant protein; higher total, complex, and simple carbohydrates; less ω-6 PUFA; and more ω-3 PUFA than the women.

ROC analysis was performed to determine the cutoff values of adherence to the MD, predictive of circulating levels of TMAO above the median value, according to sex (4.56 and 2.21 in men and women, respectively; Fig. 3A, B). An adherence score of ≤10 for men ($P < 0.001$; AUC 0.759; standard error [SE] 0.059; 95% CI, 0.639−0.855) and ≤9 in women ($P < 0.001$; AUC 0.787; SE 0.052; 95% CI, 0.679−0.872) could serve as a threshold for significantly increased risk for high circulating levels of TMAO.

Correlation studies

The correlations between circulating levels of TMAO, anthropometric measurements, metabolic profile, and adherence to the MD are summarized in Table 4. Circulating levels of TMAO showed a significant positive correlation in both sexes with almost all metabolic parameters and PREDIMED score ($P < 0.001$), whereas positive associations with BMI ($P < 0.008$) and WC ($P = 0.004$) were evident in men only. Two bivariate proportional OR models were performed to assess the association of circulating levels of TMAO with smoking, physical activity, and adherence to the MD. In particular, in the first model, smoking and being sedentary were associated positively with circulating levels of TMAO (OR, 1.23; $P = 0.016$; 95% CI, 1.038−1.447; $R^2 = 0.042$ and OR, 0.82; $P = 0.010$; 95% CI, 0.703−0.953, $R^2 = 0.048$, respectively). The second model reported the results of the bivariate proportional OR model, performed to assess the association of circulating levels of TMAO with single food items of PREDIMED questionnaire and PREDIMED categories (Table 5). In both sexes, the lowest circulating levels of TMAO were significantly associated

Table 1
Lifestyle habits, anthropometric characteristics, metabolic profile, and adherence to the MD in healthy adults grouped on the basis of sex

Parameters	Total (N =144)	Men[*] (n = 67)	Women (n = 77)	P-value[†]
Lifestyle habits				
Age (y)	31.55 ± 6.19	31.96 ± 6.22	31.19 ± 6.17	0.439
Smoker, % (n)	65.3 (94)	62.7 (42)	67.5 (52)	$\chi^2 = 0.19$; $P = 0.664$
Physically active, % (n)	51.4 (74)	52.2 (35)	50.6 (39)	$\chi^2 = 0.00$; $P = 0.982$
Anthropometric measurements				
BMI (kg/m^2)	22.84 ± 1.51	23.35 ± 1.30	22.40 ± 1.55	**<0.001**
WC (cm)	85.04 ± 10.22	90.47 ± 8.34	80.32 ± 9.36	**<0.001**
Metabolic profile				
Circulating levels of TMAO (μM)	3.71 ± 2.27	4.87 ± 2.01	2.70 ± 1.99	**<0.001**
Fasting glucose (mg/dL)	80.72 ± 11.50	83.48 ± 10.56	78.32 ± 11.81	**0.005**
Insulin (μU/mL)	2.50 ± 1.76	2.29 ± 0.95	2.67 ± 2.26	0.868
HoMA-IR	0.51 ± 0.35	0.49 ± 0.25	0.52 ± 0.42	0.574
Total cholesterol (mg/dL)	143.32 ± 23.70	145.12 ± 18.99	141.75 ± 27.17	0.227
HDL cholesterol (mg/dL)	60.09 ± 8.19	58.13 ± 6.81	61.81 ± 8.92	**0.010**
LDL cholesterol (mg/dL)	65.30 ± 25.18	69.24 ± 20.65	61.87 ± 28.22	**0.015**
Triacylgycerides (mg/dL)	89.63 ± 26.95	88.73 ± 23.21	90.42 ± 29.97	0.988
ALT (U/L)	21.91 ± 6.41	21.85 ± 5.80	21.96 ± 6.95	0.817
AST (U/L)	19.97 ± 6.37	19.82 ± 5.05	20.09 ± 7.36	0.671
γ-GT (U/L)	24.54 ± 7.31	24.49 ± 6.57	24.58 ± 7.93	0.810
Adherence to the MD				
PREDIMED score	9.08 ± 2.29	8.60 ± 2.30	9.51 ± 2.27	**0.017**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GT, γ-glutamyl transferase; HDL, high-density lipoprotein; HoMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MD, Mediterranean diet; PREDIMED, PREvención con DIetaMEDiterránea; TMAO, Trimethylamine N-oxide; WC, waist circumference.

All variables were logarithmically transformed and back-transformed for presentation in the table. PREDIMED score includes 14 items. Results are expressed as mean ± SD. χ^2 test was used to test the significance of differences between the two groups. Differences between groups were analyzed by unpaired Student's t test.

[*]Men exhibited statistically significant differences in anthropometric measurements, circulating levels of TMAO, fasting glucose, HDL, and LDL cholesterol compared with women.

[†]P-value indicates comparison between those with men versus women. A P-value in **bold** denotes a significant difference ($P < 0.05$).
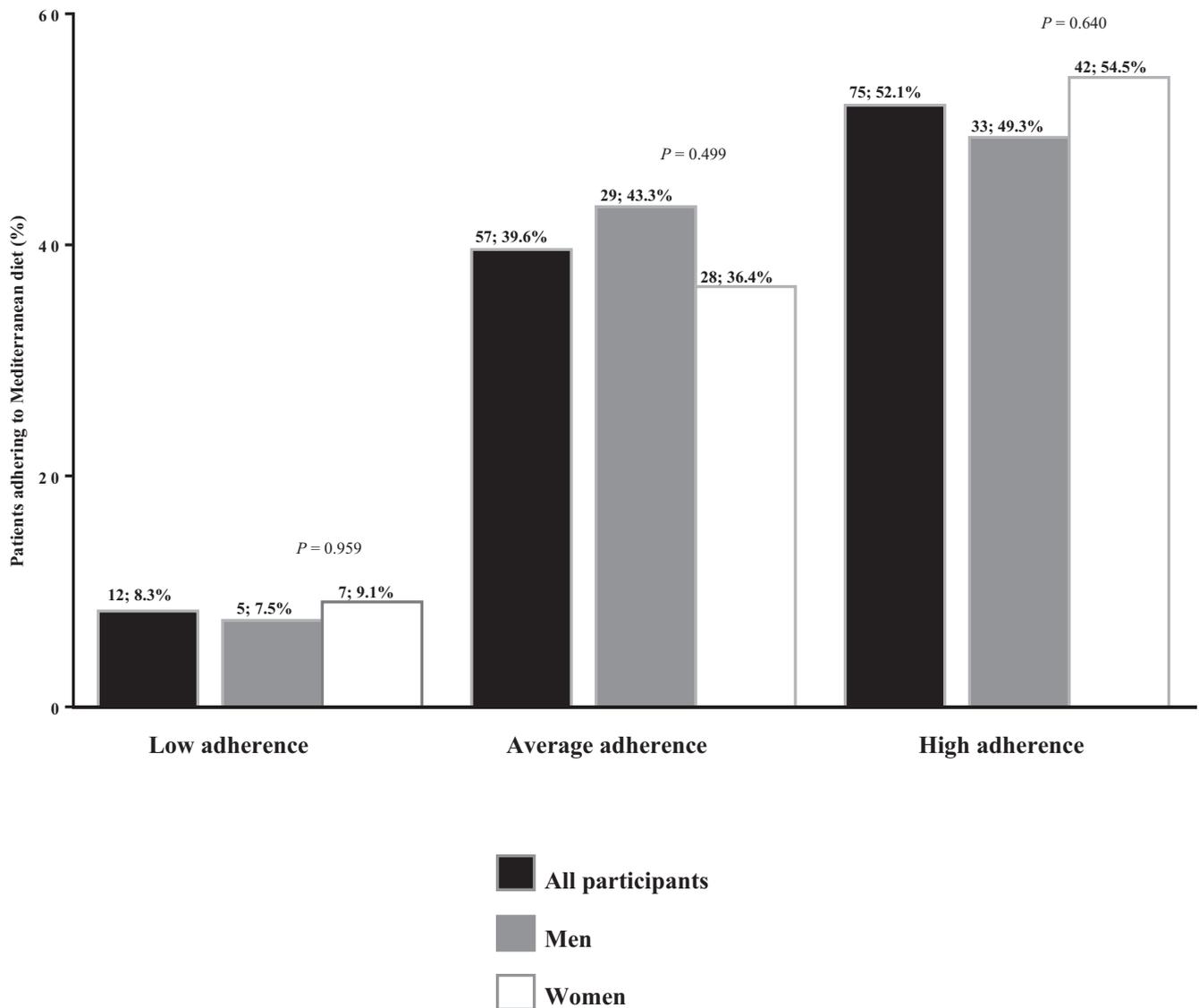
Fig. 2. Number of participants in each category of the adherence to the Mediterranean diet according to sex.

with highest OR of consumption the Mediterranean food items, in particular vegetables, fruits, and legumes, whereas nuts and sofrito were only recorded in women. On the contrary, the highest circulating levels of TMAO were significantly associated with the highest OR of consumption of red meat, butter, and fish, whereas commercial sweets were only recorded in women. Consequently, the lowest circulating levels of TMAO were significantly associated with highest score of adherence to the MD.

Table 6 reports the correlations among circulating levels of TMAO, total energy, and daily macronutrients intake as evaluated using the 7-d food records. In both sexes, circulating levels of TMAO showed highly positive correlations with total energy intake; consumption of animal protein, total and complex carbohydrates, fats, SFAs, MUFAs, and PUFAs; and a negative correlation with ω-3 PUFAs. Considering the possible effects of confounding variables included in this study, we found that the correlation between circulating levels of TMAO and PREDIMED score remained significant after adjusting for BMI, smoking, physical activity, and total energy intake, in both men (Fig. 4A) and women (Fig. 4B).

Discussion

This cross-sectional observational study investigated the circulating levels of TMAO and adherence to the MD in a sample of healthy, normal-weight adults stratified according to sex. A novel association was reported between adherence to the MD, a dietary pattern associated with positive effects on health and well-being [18], and circulating levels of TMAO, a gut microbiome-dependent metabolite that modulates an increased atherogenic risk and incidence of major adverse cardiovascular events and type 2 diabetes [7,38,39]. Of interest, this association, which was independent of common confounding variables including body weight, smoking, physical activity, and total energy intake, presented a clear sexual dimorphism. In particular, although the highest circulating levels of TMAO were associated with the lowest adherence to the MD in both sexes, men showed higher circulating levels of TMAO and lower adherence to the MD than women. Based on ROC curve analysis, the lowest circulating levels of TMAO were well predicted by an adherence score of ≤ 10 in men and ≤ 9 in women. In addition to the adherence to the MD score, the careful investigation of the

Table 2
Response frequency of dietary components included in the PREDIMED questionnaire of healthy adults grouped on the basis of sex

Questions PREDIMED questionnaire	Total (N = 144)		Men* (n = 67)		Women (n = 77)		χ^2	P-value[†]
	n	%	n	%	n	%		
Use of extra virgin olive oil as main culinary lipid	138	95.8	64	95.5	74	96.1	0.06	0.807
Extra virgin olive oil >4 tablespoons	100	69.4	48	71.6	52	67.5	0.17	0.681
Vegetables ≥2 servings/d	95	66.0	43	64.2	52	67.5	0.06	0.805
Fruits ≥3 servings/d	101	70.1	46	68.7	55	71.4	0.00	0.980
Red/processed meats <1/d	94	65.3	35	52.2	59	76.6	8.35	**0.004**
Butter, cream, margarine <1/d	76	52.8	37	55.2	39	50.6	0.15	0.703
Soda drinks <1/d	100	69.4	41	61.2	59	76.6	3.33	0.068
Wine glasses ≥7/wk	89	61.8	40	59.7	49	63.6	0.09	0.754
Legumes ≥3/wk	91	63.2	40	59.7	51	66.2	0.41	0.524
Fish/seafood ≥3/week	88	61.1	48	71.6	40	51.9	5.05	**0.025**
Commercial sweets and confectionery ≤2/wk	96	66.7	44	65.7	52	67.5	0.00	0.953
Tree nuts ≥3/wk	84	58.3	33	49.3	51	66.2	3.58	0.059
Poultry more than red meats	91	63.2	42	62.7	49	63.6	0.00	0.956
Use of sofrito sauce ≥2/wk	65	45.1	15	22.4	50	64.9	24.50	**0.001**

PREDIMED, PREvención con DIetaMEDiterránea.
Extra virgin olive oil was the most consumed food item, followed by fruits.
Results are expressed as percentage of response obtained with PREDIMED questionnaire. No differences were observed in PREDIMED score. The χ^2 test was used to test the significance of differences between the two groups (men and women).
*Men exhibited statistically significant differences in some items of PREDIMED questionnaire compared with women.
[†] P-value indicates comparison between those with men versus women. A P-value in **bold** denotes a significant difference (P < 0.05).

dietary pattern using the 7-d food record provided evidence that the circulating levels of TMAO were positively correlated with a dietary pattern characterized by a prevalent consumption of animal-derived proteins, and negatively correlated with the consumption of ω-3 PUFAs.

There is considerable debate on both the physiological and pathophysiologic actions of TMAO [40]. However, despite being burdened by a significant intraindividual variation owing to gut microbiota composition, liver enzyme activity, and the excretion rate of methylamines, there is a large consensus that circulating levels of TMAO are mainly dependent on dietary patterns [14,41,42]. Nevertheless, sex-aggregated data on the association between circulating levels of TMAO and single essential nutrients that act as precursors to TMAO or dietary patterns are still missing. The present study further extended previous observations because it demonstrated that in normal-weight individuals, lower circulating levels of TMAO not only were actually associated with a higher

adherence to an eating pattern contributing to overall health, such as in the MD [43], but it evidences also how food preferences between the sexes are also associated with sex differences in circulating levels of TMAO, independent of confounding variables.

It is well known that the intake of specific food rich in TMAO precursors such as eggs, beef, and fish increased the blood and urine levels of TMAO [44]. Tang et al. described the generation of the proatherogenic metabolite TMAO after a phosphatidylcholine challenge in 40 healthy adults and demonstrated the obligatory role of intestinal microorganisms in the production of TMAO from phosphatidylcholine in humans by using oral broad-spectrum antibiotics to temporarily suppress the production of TMAO [45]. Afterward, Miller et al. reported a steep dose−response relationship between both plasma and urinary TMAO and egg consumption, which are a major dietary source of choline in the form of phosphatidylcholine, in six healthy volunteers, although with considerable interindividual variations in the TMAO response, likely linked to

Table 3
Total energy and daily macronutrients/micronutrients intake of healthy adults grouped on the basis of sex

Parameters	Total (N =144)	Men* (n = 67)	Women (n = 77)	P-value[†]
Total energy (kcal)	2288.77 ± 264.52	2336.10 ± 246.10	2247.59 ± 274.53	**0.037**
Protein (g of total kcal)	101.74 ± 15.83	102.79 ± 17.03	100.82 ± 14.76	0.529
Animal (g of total kcal)	69.51 ± 11.67	77.92 ± 10.45	66.53 ± 11.91	**0.001**
Plant (g of total kcal)	32.24 ± 11.64	29.87 ± 12.15	34.29 ± 10.84	**0.008**
Carbohydrate (g of total kcal)	307.74 ± 36.26	315.90 ± 32.00	300.63 ± 38.40	**0.007**
Complex (g of total kcal)	205.82 ± 25.69	211.02 ± 23.17	201.29 ± 27.03	**0.016**
Simple (g of total kcal)	101.92 ± 13.13	104.88 ± 11.83	99.34 ± 13.73	**0.008**
Fat (g of total kcal)	72.32 ± 9.83	73.48 ± 9.19	71.30 ± 10.31	0.146
SFA (g of total kcal)	23.66 ± 3.37	23.56 ± 3.58	23.74 ± 3.20	0.687
MUFA (g of total kcal)	33.25 ± 4.97	33.61 ± 5.38	32.94 ± 4.59	0.474
PUFA (g of total kcal)	15.41 ± 2.75	15.71 ± 3.32	15.15 ± 2.13	0.412
ω-6 PUFA (g/d)	4.99 ± 2.79	5.65 ± 3.25	4.41 ± 2.19	**0.032**
ω-3 PUFA (g/d)	10.42 ± 1.84	10.05 ± 1.48	10.74 ± 2.06	**0.023**
ω-6/ω-3 PUFA ratio	0.52 ± 0.37	0.58 ± 0.37	0.46 ± 0.36	**0.019**

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
All variables were logarithmically transformed and back-transformed for presentation in the table. Results are expressed as mean ± SD. Differences between groups were analyzed by unpaired Student's t test.
*Men had a higher total energy intake, a higher intake of energy from animal protein; total, complex, and simple carbohydrates; and ω-6 PUFA than women.
[†] P-value indicates comparison between those with men versus women. A P-value in **bold** denotes a significant difference (P < 0.05).
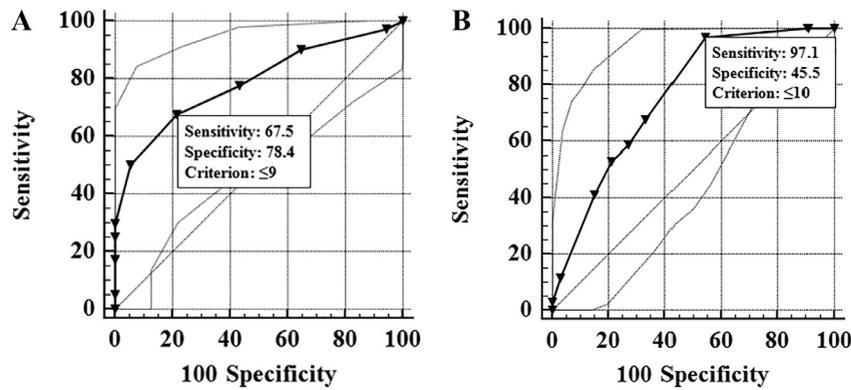
Fig. 3. ROC for predictive values of PREDIMED score in detecting circulating levels of TMAO in men (**A**) and women (**B**). The PREDIMED score ≤10 in (A) men (*P* < 0.001; AUC 0.759, SE 0.059; 95% CI, 0.639–0.855) and ≤9 in (B) women (*P* < 0.001; AUC 0.787, SE 0.052; 95% CI 0.679 to 0.872) could serve as thresholds for significantly increased risk for high circulating levels of TMAO. A *P*-value in **bold** denotes a significant difference (*P* < 0.05). AUC, area under curve; PREDIMED, PREvención con DIeta MEDiterránea; ROC, receiver operating characteristic analysis; TMAO, trimethylamine *N*-oxide.

differences in their intestinal microbiota or hepatic oxidation of trimethylamine mediated by FMO1 and FMO3 [46]. Very recently, Kühn et al. demonstrated that in the highest consumption of food items typical of the MD, such as vegetables, fruits, and nuts, was associated with the lowest circulating levels of TMAO, whereas the consumption of animal-derived protein, such as red meat or fish, the latter important source of protein and vitamins in the MD, was associated with the highest circulating levels of TMAO [41]. This latter finding offered an interesting point for discussion because it is generally accepted that fish and seafood, which contain considerable amounts of TMAO are well-known to exert cardioprotective effects mainly based on the plasma lipid-lowering and anti-inflammatory effects of ω-3 PUFAs [10]. On the other hand, circulating

levels of TMAO have been reported as potential biomarkers of increased risk for major adverse cardiovascular events and death [47]. In humans, plasma levels of TMAO increase rapidly after fish consumption, which yields ∼50 times higher postprandial circulating levels of TMAO than the consumption of eggs (abundant in choline) or beef (abundant in carnitine and choline) among healthy young men, indicating direct uptake of free TMAO [44]. However, diet is a complex combination of foods from various groups and nutrients, and some nutrients are highly correlated, thus it is very challenging, in free-living populations, to separate the effect of a single nutrient or food group from others [48]. In fact, the large amount of egg and meat consumption, both containing the TMA precursors carnitine and choline that are metabolized by anaerobic

Table 4
Correlations among circulating levels of TMAO with age, anthropometric characteristics, metabolic profile, and adherence to the MD in healthy adults grouped on the basis of sex

Parameters	Circulating levels of TMAO (μM)					
	Total (N = 144)		Men (n = 67)		Women (n = 77)	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Age (y)	0.147	0.079	−0.108	0.386	0.208	0.064
Anthropometric measurement						
BMI (kg/m^2)	0.645	<**0.001**	0.686	<**0.001**	0.553	<**0.001**
WC (cm)	0.418	<**0.001**	0.346	**0.004**	0.157	0.172
Metabolic profile						
Fasting glucose (mg/dL)	0.434	<**0.001**	0.479	<**0.001**	0.328	**0.004**
Insulin (μU/mL)	0.295	<**0.001**	0.436	<**0.001**	0.323	**0.004**
HoMA-IR	0.359	<**0.001**	0.475	<**0.001**	0.356	**0.002**
Total cholesterol (mg/dL)	0.385	<**0.001**	0.504	<**0.001**	0.340	**0.002**
HDL cholesterol (mg/dL)	−0.461	<**0.001**	−0.390	**0.001**	−0.434	<**0.001**
LDL cholesterol (mg/dL)	0.490	<**0.001**	0.488	<**0.001**	0.453	<**0.001**
Triacylglycerides (mg/dL)	0.346	<**0.001**	0.460	<**0.001**	0.375	**0.001**
ALT (U/L)	0.294	<**0.001**	0.290	**0.017**	0.352	**0.002**
AST (U/L)	0.361	<**0.001**	0.489	<**0.001**	0.364	**0.001**
γ-GT (U/L)	0.089	0.290	0.175	0.158	0.049	0.670
Adherence to the MD						
PREDIMED score	−0.502	<**0.001**	−0.443	<**0.001**	−0.523	<**0.001**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GT, γ-glutamyl transferase, HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MD, Mediterranean diet; PREDIMED, PREvención con DIetaMEDiterránea; TMAO, Trimethylamine *N*-oxide; WC, waist circumference.
Circulating levels of TMAO were inversely associated in both sexes with BMI, metabolic profile except for γ-GT, and PREDIMED score. All variables were logarithmically transformed and back-transformed for presentation in table. Correlations among variables were performed using Spearman's *r* correlation coefficients. A *P*-value in **bold** denotes a significant difference (*P* < 0.05).

Table 5
Bivariate proportional OR model to assess the association between circulating levels of TMAO and food items included in the PREDIMED questionnaire

Questions	Total (N = 144)				Men (n = 67)				Women (n = 77)			
	OR	P-value	95% CI	R^2	OR	P-value	95% CI	R^2	OR	P-value	95% CI	R^2
Use of extra virgin olive oil as main culinary lipid	0.73	0.607	0.214–2.457	0.002	0.11	0.249	0.003–4.561	0.026	1.18	0.842	0.226–6.209	0.001
Extra virgin olive oil >4 tablespoons	0.64	0.100	0.380–1.089	0.019	0.51	0.276	0.153–1.709	0.019	0.49	0.056	0.240–1.019	0.049
Vegetables ≥2 servings/d	0.41	**0.002**	0.236–0.722	0.073	0.14	**0.008**	0.032–0.590	0.132	0.43	**0.026**	0.206–0.904	0.068
Fruits ≥3 servings/d	0.39	**0.002**	0.217–0.708	0.074	0.27	**0.050**	0.073–1.020	0.064	0.32	**0.005**	0.140–0.712	0.109
Red/processed meats <1/d	0.20	**<0.001**	0.101–0.391	0.186	0.05	**<0.001**	0.011–0.275	0.249	0.37	**0.019**	0.161–0.847	0.076
Butter, cream, margarine <1/d	0.54	**0.012**	0.328–0.873	0.045	0.23	**0.018**	0.067–0.777	0.096	0.45	**0.025**	0.223–0.904	0.069
Soda drinks <1/d	0.62	0.079	0.366–1.057	0.022	0.48	0.194	0.161–1.449	0.027	0.95	0.886	0.446–2.010	0.000
Wine glasses ≥7/wk	0.73	0.201	0.448–1.184	0.012	0.73	0.548	0.261–2.039	0.005	0.70	0.295	0.356–1.369	0.014
Legumes ≥3/wk	0.36	**<0.001**	0.207–0.639	0.095	0.20	**0.015**	0.055–0.713	0.104	0.34	**0.006**	0.157–0.737	0.105
Fish/seafood ≥3/wk	0.59	**<0.001**	2.042–6.302	0.153	2.69	**0.047**	0.899–8.052	0.047	4.32	**0.001**	1.851–10.068	0.179
Commercial sweets and confectionery ≤2/wk	0.55	**0.025**	0.321–0.925	0.037	0.54	0.277	0.175–1.647	0.019	0.43	**0.026**	0.205–0.903	0.068
Tree nuts ≥3/wk	0.32	**<0.001**	0.181–0.559	0.123	0.66	0.416	0.242–1.796	0.010	0.19	**<0.001**	0.077–0.470	0.201
Poultry more than red meats	0.99	0.975	0.616–1.598	0.000	1.75	0.280	0.633–4.858	0.017	0.80	0.503	0.409–1.551	0.006
Use of sofrito sauce ≥2/wk	0.26	**0.003**	0.144–0.456	0.160	0.97	0.963	0.300–3.152	0.000	0.26	**0.001**	0.115–0.594	0.151
PREDIMED categories												
Low adherence	4.32	**0.016**	1.309–14.245	0.052	17.29	0.087	0.661–452.99	0.062	5.22	**0.015**	1.370–19.899	0.089
Average adherence	2.41	**0.001**	1.415–4.128	0.078	3.57	0.035	1.094–11.648	0.075	2.60	**0.012**	1.240–5.481	0.089
High adherence	0.28	**<0.001**	0.159–0.495	0.151	0.15	0.004	0.041–0.551	0.146	0.19	**<0.001**	0.079–0.472	0.210

CI, confidence interval; OR, odds ratio; MD, Mediterranean diet; PREDIMED, PREvención con DIetaMEDiterránea; TMAO, Trimethylamine N-oxide.
Circulating levels of TMAO were inversely associated with the highest odds of consumption fruits, extra virgin olive oil, vegetables, fish, and wine and with highest score of adherence of MD. On the contrary, the highest circulating levels of TMAO were positively associated with the lowest odds of consumption of butter. A P-value in **bold** denotes a significant difference (P < 0.05).

gut microbiota, contributes to the putative role for TMAO as a factor linking development of atherosclerosis [10]. Of interest, both in vitro and in vivo studies evidenced that dietary components, such as indoles, found in members of the Cruciferae family of vegetables, inhibited the activity of FMO3, the most important TMAO-producing enzyme in the liver, decreasing the amount of TMAO excreted [49]. In addition, Sayegh et al. reported that active supplementation with capsules containing a combination of fruit and vegetables influenced the circulating levels of TMAO [50]. In line with this finding, we also evidenced a negative association of vegetables and fruits with circulating levels of TMAO. The so-called puzzling paradox that surrounds physiological and pathophysiologic actions

exerted by TMAO could be almost partially due to the fact that traditionally most available studies have looked into association of TMAO with single nutrient or dietary component rather than with a dietary pattern [10]. Indeed, only a limited number of human studies have shown that dietary patterns high in animal proteins, animal fat, and simple carbohydrates, and low in fiber and unrefined carbohydrates are associated with reduced microbiota diversity and increased relative abundance of undesirable bacteria and their toxic metabolites, including the TMAO [51,52]. On the other hand, human studies have determined that vegans and vegetarians produce less TMAO than their omnivorous counterparts after a dietary challenge [6]. In addition to the short-term effects of specific

Table 6
Correlations of circulating levels of TMAO with total energy and daily macronutrient/micronutrient intake

Parameters	Circulating levels of TMAO (μM)					
	Total (N = 144)		Men (n = 67)		Women (n = 77)	
	r	P-value	r	P-value	r	P-value
Total energy (kcal)	0.316	**<0.001**	0.283	**0.020**	0.263	**0.021**
Protein (g of total kcal)	0.195	**0.019**	0.204	0.098	0.198	0.084
Animal (g of total kcal)	0.421	**<0.001**	0.309	**0.011**	0.349	**0.002**
Plant (g of total kcal)	−0.180	**0.031**	−0.046	0.711	−0.112	0.331
Carbohydrate (g of total kcal)	0.334	**<0.001**	0.277	**0.023**	0.259	**0.023**
Complex (g of total kcal)	0.345	**<0.001**	0.300	**0.013**	0.285	**0.012**
Simple (g of total kcal)	0.250	**0.002**	0.162	0.189	0.167	0.146
Fat (g of total kcal)	0.258	**0.002**	0.252	**0.039**	0.221	0.053
SFA (g of total kcal)	0.188	**0.024**	0.249	**0.043**	0.324	**0.004**
MUFA (g of total kcal)	0.214	**0.010**	0.251	**0.040**	0.357	**0.001**
PUFA (g of total kcal)	0.292	**<0.001**	0.298	**0.014**	0.344	**0.002**
ω-6 PUFA (g/d)	0.472	**<0.001**	0.577	**<0.001**	0.385	**0.001**
ω-3 PUFA (g/d)	−0.240	**0.004**	−0.509	**<0.001**	−0.183	**0.035**
ω-6/ω-3 PUFA ratio	0.477	**<0.001**	0.656	**<0.001**	0.343	**0.002**

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TMAO, Trimethylamine N-oxide.
Circulating levels of TMAO were significantly associated with all the macronutrients/micronutrients evaluated in this study, in all individuals. After grouping participants on the basis of sex, some correlation were lost. Correlations among variables were performed Spearman's r correlation coefficients.
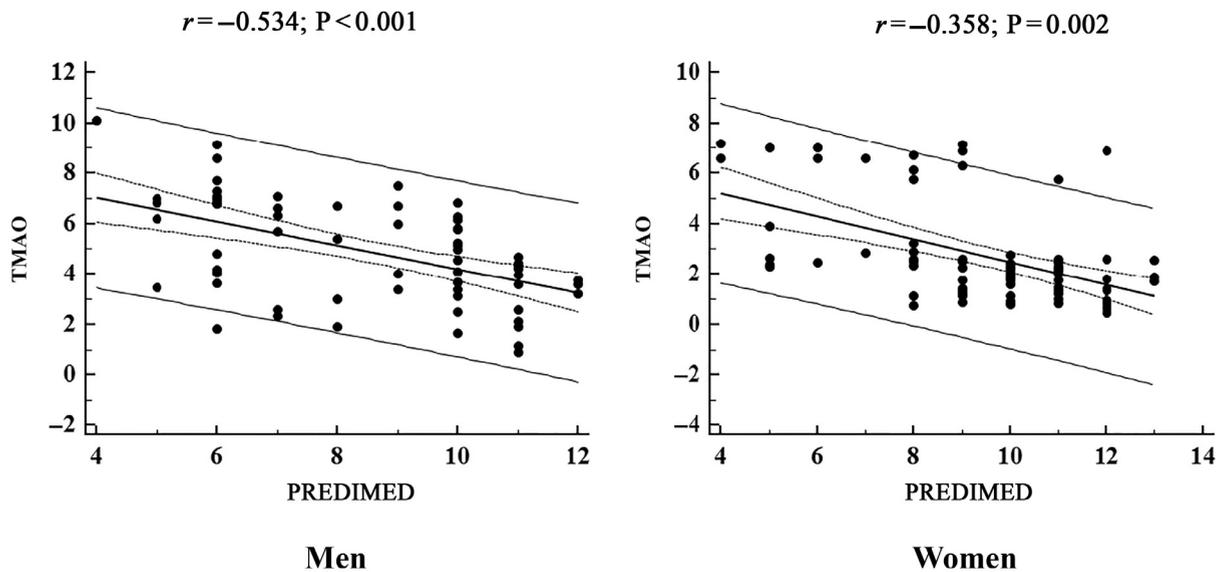
Fig. 4. Correlation between PREDIMED score and circulating levels of TMAO, adjusted for common confounding variables, including body weight, smoking, physical activity, and total energy intake. A significant positive correlation was observed between PREDIMED score and circulating levels of TMAO, independently of body mass index. A *P*-value denotes a significant difference (*P* < 0.05). PREDIMED, PREvención con DIeta MEDiterránea; TMAO, trimethylamine *N*-oxide.

aliments (e.g., fish or eggs) [46] and nutrients (e.g., choline, carnitine, or betaine) [53] on circulating levels of TMAO, a number of studies investigated the effects of long-term dietary habits (e.g., vegan/vegetarian versus omnivore/carnivore) on the generation of TMAO from dietary substrates [6,54]. Of interest, the generation of TMAO from choline or carnitine by the intestinal microbiome was associated with atherosclerosis in omnivores, but not in vegetarians, supporting the protective role of a plant-based diet microbiome, along with an occasional consumption of meat, and suggesting the ratio of polyphenols to TMAO as an indicator of atherogenesis risk [54]. Garcia-Perez et al., by simultaneously measuring a very high number of urinary metabolites, the concentrations of which can be affected by food intake, recently reported that urinary TMAO concentration increased not only after the consumption of fish intake, which is associated with healthy diets that are rich in fish, but also of red meat, which is generally associated with adverse health outcomes owing to the gut bacteria that can synthesize TMAO from choline [43]. In addition, Vázquez-Fresno et al. investigated the long-term effects of the MD on urinary metabolome and found that the urinary excretion of TMAO was lower in a sample population following the MD than in controls on a free diet [24]. However, Pignanelli et al. did not find any significant difference in the plasma levels of the intestinal metabolites associated with an MD score or with intake of dietary precursors among omnivorous vascular patients, although these results could be affected by the low presence of study participants with high adherence to the MD [55]. In this complex scenario, the present study could provide further evidence that the circulating levels of TMAO could be better unravelled through the interplay of different foods with different antioxidant properties, mainly vegetables and fruits, and diet-driven gut microbiota in the context of a dietary pattern rather than a single food item. A further point of discussion is the sex difference observed in circulating levels of TMAO. It is well known that sex, with age and educational status, influences food choice behaviors [56], with women consuming preferentially fruit and vegetables and men consuming more animal protein–rich diets [2]. Nevertheless, sex-specific effects in humans also influence the amount of urinary TMA [9] because estrogens have

reported to decrease the expression of the *FMO3* gene [57], whereas androgens have been found to reduce *FMO3* capacity [58]. In addition, it has been reported that *FMO3* expression is under the negative control of insulin and that fasting glucose levels correlated positively with circulating levels of TMAO in humans [59]. Nevertheless, differences in circulating levels of TMAO in a sample of free-dieting normal-weight men and women living in the same geographical area with high average adherence to the MD have not yet been reported. Despite men consuming more fish than women, their adherence to the MD is lower than women. Indeed, the evaluation of dietary reports in the present sample population indicated that, compared with women, men consumed higher amounts not only of animal-derived proteins, but also of carbohydrates (both complex and simple) and ω-6 PUFA, and have a low intake of ω-3 PUFAs. This particular "male" dietary pattern—rich in animal proteins and low in antioxidant ω-3 PUFAs—could contribute to higher circulating levels of TMAO with the increased atherogenic effect of TMAO, whereas a lower intake of fish, associated with a high adherence to the MD food items, and a higher intake of plant-derived proteins and ω-3 PUFA from nuts, could ensure lower circulating levels of TMAO and lower atherogenic profiles in women.

This study included some limitations and these warrant some considerations. The study's cross-sectional nature did not allow for the identification of any causal association between circulating levels of TMAO and adherence to the MD and did not allow us to clearly determine the prognostic value of the adherence to the MD for predicting the circulating levels of TMAO. Moreover, the suggested cutoff values of the PREDIMED score for identifying the lower circulating levels of TMAO should be viewed with caution until results of studies with larger patient populations are available to perform an appropriate cross-validation. Furthermore, although the gut-derived origin of TMAO is well known, we did not include the gut microbiota analysis. Thus, we could not determine whether the MD could have influenced the circulating levels of TMAO through its effects on gut microbiota composition. However, the study of gut microbiota is burdened by a high intraindividual variability because ≤69 factors associated with gut flora composition and diversity have been identified [60], and there is a large

consensus that circulating levels of TMAO are mainly dependent on dietary pattern [14,41,42]. On these bases, TMAO per se has been used as a surrogate marker of a pro-atherogen microbe community [14]. Nevertheless, further studies are needed to address the influence of the MD by analyzing gut microbiota. A further limitation of the present study was that the plasma levels of TMAO were measured in the fasting state. In addition, we did not include in this study other metabolites in the same pathway, such as choline, betaine, or carnitine, nor did we evaluate the effect of the consumption of TMAO precursors, such as phosphatidylcholine in eggs and carnitine from meat, on levels of TMAO. Metabolites from the choline pathway were associated with an increased risk for major adverse cardiovascular events in elderly Mediterranean individuals at high cardiovascular risk [61]. However, other studies have shown that the associations between plasma TMAO and disease outcomes were independent of choline itself or its other metabolites [11–13], the interindividual variations in TMAO levels mainly attributed to intestinal microbiota differences, and the overall influence of diet on fasting TMAO concentrations, although statistically significant, might be considered rather moderate [62]. A further limitation could derive from the sample size, which was relatively small, and a larger group of individuals would have provided more information. However, for greater statistical accuracy, we calculated the sample size, which was 22 for both groups (men and women), whereas our study consisted of 67 men and 77 women, more than double required by the sample size. In addition, to increase the homogeneity of the sample population, we restricted the study to only healthy, normal-weight men and premenopausal women, living in the same geographical area and with similar eating behaviors. A further strength of this study was the evaluation of TMAO in plasma rather than in urine samples. It is well known that urinary TMAO is largely dependent on fish consumption. Krüger et al. found that fish consumption is correlated with both plasma and urinary levels of TMAO [62]. Contrarily, circulating levels of TMAO are representative not only of fish-derived TMAO, but also of the other dietary sources of TMA metabolites and the gut microbiota that contributes to oxidize TMA. In addition, the nutritional status was evaluated using a 7-d food record by an experienced nutritionist. This method, which is considered the gold standard in validation studies of different types of self-administered food frequency questionnaires, allowed us to better characterize the macronutrients and total energy intake [63].

Conclusion

An apparently novel association was observed between circulating levels of TMAO and the MD in healthy, normal-weight adults, and there was a clear sex difference in this association. We are aware that the determination of the global pattern of food metabolites is more important in indicating dietary patterns than individual biomarkers, such as TMAO, and allows an accurate monitoring of changes in dietary patterns in response to food policy implementation. However, the association between TMAO and MD uncovered a new potential benefit of the MD, as higher adherence by both sexes to the MD was associated with lower circulating levels of TMAO, a marker of gut dysbiosis linked to the development of atherosclerosis and CVDs. These benefits could be of particular importance in the management of patients with renal impairment, especially the elderly. The determination of sex-specific cutpoints for the score of adherence to MD might help identify individuals at high risk for high-circulating levels of TMAO, who could benefit from personalized dietary interventions. Future dietary intervention trials on large populations will be critical to elucidate the beneficial effects of the MD and of macronutrient intake on the circulating levels of TMAO.

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