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Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population

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Abstract

This study aimed to explore the potential associations of adherence to the Mediterranean diet with gut microbiota characteristics and gastrointestinal symptomatology in an adult population. Other long-term dietary habits (e.g. consumption of snacks and junk food or stimulant intake) were also evaluated in terms of the gut microbiota profile. Participants (n 120) underwent anthropometric, dietary, physical activity and lifestyle evaluation. Adherence to the Mediterranean diet was assessed using a Mediterranean diet score, the MedDietScore, and subjects were classified into three tertiles according to individual adherence scoring. Gut microbiota composition was determined using quantitative PCR and plate-count techniques, and faecal SCFA were analysed using GC. Gastrointestinal symptoms were also evaluated. Participants with a high adherence to the Mediterranean diet had lower *Escherichia coli* counts ($P=0{\cdot}022$), a higher bifidobacteria:*E. coli* ratio ($P=0{\cdot}025$), increased levels and prevalence of *Candida albicans* ($P=0{\cdot}039$ and $P=0{\cdot}050$, respectively), greater molar ratio of acetate ($P=0{\cdot}009$), higher defaecation frequency ($P=0{\cdot}028$) and a more pronounced gastrointestinal symptomatology compared with those reporting low adherence. A lower molar ratio of valerate was also observed in the case of high adherence to the Mediterranean diet compared with the other two tertiles ($P_{\text{for trend}}=0{\cdot}005$). Positive correlations of MedDietScore with gastrointestinal symptoms, faecal moisture, total bacteria, bifidobacteria:*E. coli* ratio, relative share of Bacteroides, *C. albicans* and total SCFA, as well as negative associations with cultivable *E. coli* levels and valerate were indicated. Fast food consumption was characterised by suppressed representation of lactobacilli and butyrate-producing bacteria. In conclusion, our findings support a link between adherence to the Mediterranean diet and gut microbiota characteristics.

Key words: Gut microbiota: Mediterranean diet: Junk foods: Yeasts: SCFA

The gut microbiota is a diverse and dynamic microbial ecosystem, which supports important gastrointestinal and systemic metabolic functions of the host[1,2]. Nowadays, the gut microbiota is increasingly being accepted as a novel environmental factor associated with the occurrence and progress of various pathological conditions, including obesity and related metabolic comorbidities as well as cancer and inflammatory and degenerative diseases[1–3]. Though several gut micro-organisms (e.g. *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Methanobrevibacter smithii*) have emerged as microbial indicators of metabolic health and inflammation[4–6], more research is necessary to elucidate the potential role of intestinal microbiota in health and disease, taking into consideration the potential effect of lifestyle patterns, including diet[2]. Long-term dietary habits have a considerable effect on the human gut microbiota, and epidemiological data have already indicated connections between diet constituents or dietary patterns and gut microbiota profile and functionality[7].

The Mediterranean diet is a healthy dietary pattern, inspired by the food patterns of populations living around the Mediterranean Sea in the early 1960s[8,9]. Its characteristic features are high consumption of fruits, vegetables, legumes, unrefined cereals and nuts, moderate consumption of fish, poultry and dairy products (principally cheese and yogurt), low consumption of red meat products, use of olive oil as the main edible-fat source and regular but moderate wine consumption[9]. Greater adherence to the Mediterranean diet has been linked to a significant reduction in overall mortality and morbidity, inspiring a beneficial dietary approach in the management of CVD, type 2 diabetes, obesity, inflammatory diseases, degenerative diseases and cancer[3,8,10]. In addition to the proposed protective mechanisms of the Mediterranean diet against major chronic diseases (e.g. anti-inflammatory, antioxidant, satiogenic and fat-oxidation effects)[11], this dietary pattern has recently generated interest regarding the manipulation of gut microbiota characteristics in the battle against inflammatory and metabolic disorders[3,10–19].

Abbreviation: qPCR, quantitative PCR.

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The aim of this cross-sectional study was to elucidate the potential associations of adherence to the Mediterranean diet with gut microbiota characteristics and gastrointestinal symptomatology in an adult population. We particularly focused on proposed microbial indicators of inflammation and metabolic health and on suggested diet-responsive groups of bacteria (e.g. *Prevotella*, *Roseburia-Eubacterium rectale*, *Clostridium coccoides* group)[4–7]. Further, we have determined additional members of faecal microbiota, such as *Candida* spp., with rather unexplored contributions. Other long-term dietary habits (e.g. consumption of snacks and junk food or stimulant intake) were also evaluated in terms of the gut microbiota profile.

Methods

Study population

A total of 120 participants were recruited from Athens, Greece, during the period 2011–2015. Volunteer recruitment was carried out through word of mouth and local press announcements. Eligible participants were men and women, aged 18–65 years, without a history of gastrointestinal disease, autoimmune disease, coronary disease, liver and/or kidney malfunction, epileptic seizures, without current inflammation, present pregnancy, recent weight loss, extreme dietary behaviours, no consumption of antibiotics 2 months before the study and/or intake of non-steroid anti-inflammatory agents, antioxidant and *n*-3 supplements, probiotic and/or prebiotic supplements 2 weeks before the study.

Ethical standards

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Bioethics Committee of Harokopio University. Written informed consent was obtained from all subjects before their inclusion in the study.

Demographic and clinical information

Subjects completed a series of questionnaires in relation to sociodemographic parameters (including age, sex, marital status and education level), smoking habits, medical history, psychological parameters[20] and sleeping patterns. Blood pressure (mmHg) and heart rate of fasted volunteers were measured in the sitting position.

Anthropometry

Body weight and height were measured on a levelled platform scale (SECA GmbH) and a wall-mounted stadiometer (SECA GmbH) to the nearest 0·1 kg and 0·5 cm, respectively. BMI was calculated by dividing the weight (kg) by the height (m^2). Waist circumference was measured in the middle between the twelfth rib and the iliac crest, and hip circumference was measured at the widest lateral extension of the hips to the nearest 0·1 cm using a measuring tape. Waist:hip ratio was also calculated. Triceps skinfold was measured at the midpoint between the olecranon process and the acromion process using a Lange Skinfold Caliper (Beta Technology Incorporated). Anthropometric evaluation also included body analysis assessment using bioelectrical impedance (BC-418 Segmental Body Composition Analyzer; Tanita).

Dietary assessment

Dietary intake was evaluated using a validated semi-quantitative FFQ[21]. The FFQ was completed by each participant with the aid of an experienced investigator. Collected data were then analysed in terms of energy and nutrient intakes using the Nutritionist Pro software (version 4.1.0; Axxya Systems). In all, thirteen main food groups (dairy products, starchy products, eggs, meat, fish, legumes, vegetables, fruit, fats and oils, sweets, alcoholic beverages, snacks and junk food, stimulants) were also created, and servings per day/4184 kJ (1000 kcal) of energy intake (EI) for each food group was calculated. The level of adherence to the Mediterranean diet was assessed using an eleven-item composite index, the MedDietScore[22]. For food items presumed to be close to the Mediterranean dietary pattern (i.e. those that are suggested to be consumed on a daily basis or in >4 servings/week: non-refined cereals, fruit, vegetables, potatoes, legumes, olive oil and fish), a score of 0 was assigned when a participant reported no consumption, a score of 1 for reported consumption of 1–4 times/month, a score of 2 for 5–8 times/month, a score of 3 for 9–12 times/month, a score of 4 for 13–18 times/month and a score of 5 for >18 times/month. On the contrary, for the consumption of foods presumed to be away from this dietary pattern (i.e. those suggested to not be consumed on a daily or weekly basis: meat and meat products, poultry and high-fat dairy products), the opposite scores were assigned (i.e. a score of 0 when a participant reported almost daily consumption of the food to a score of 5 for rare or no consumption). For alcohol consumption, a non-monotonic scoring was adopted based on daily intake of 15–30 g ethanol as suggested by the Mediterranean dietary pattern (i.e. a score of 5 was assigned for consumption of <3 glasses/d; 0 for none or consumption of >7 glasses/d; and scores of 4, 3, 2 and 1 for the consumption of 3, 4–5, 6 and 7 glasses/d, respectively). A total score was calculated on the basis of these eleven components (score range: 0–55); higher score values indicate a greater adherence to the Mediterranean diet, whereas lower score values indicate adherence to a Westernised diet. On the basis of calculated values of MedDietScore in our study, the tertiles of this score were applied for classification of individuals into three different groups for further analysis[22,23].

For the assessment of low energy reporting, the ratio of the EI:BMR was determined for each subject. BMR was estimated using the Schofield equations as adopted by the WHO[24]. Participants with EI:BMR ≤1·13 were classified as 'low energy reporters' (LER), on the basis of the methodology developed by Goldberg *et al.*[25].

Physical activity assessment

Physical activity levels were assessed using the International Physical Activity Questionnaire Short Form questionnaire validated for the Greek population[26]. Duration of sedentary activity (sitting or resting) expressed as 'h/week' was also recorded.

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Evaluation of gastrointestinal symptoms

The intensity of gastrointestinal symptoms (i.e. abdominal pain, bloating, flatulence, borborygmi) was recorded on a weekly basis and measured daily on a scale of 0–4, where '0' represented absence of symptoms and '4' severe symptoms. Gastrointestinal symptoms were evaluated as a 7-d symptom score and the total weekly symptom score was calculated as the sum of the four symptom scores[27]. Stool frequency and consistency of evacuations using the Bristol Stool Scale were also recorded[28]. This stool-form scale could also serve as a useful guide to intestinal transit time[28]. Participants were further asked about gastrointestinal pain and the mean number of daily evacuations for the 4-week period preceding the study[29].

Stool collection

Participants were given a faecal collection kit with a sterile stool tube (Oxoid AnaeroGen™; Thermo Scientific Inc.) and a preweighed plastic container to return their faecal sample in during the next few days. Stool samples were processed under anaerobic conditions for plate-count techniques within 2 h after defaecation, or homogenised and stored immediately at −80°C for future molecular analysis.

Gut microbiota analysis

Enumeration of gut microbiota was performed using both plate-count techniques (online Supplementary Table S1) and real-time quantitative PCR (qPCR). Colony counts were expressed as a $\log_{10}$ of the colony-forming units/g wet faeces. The relative share (%) of each microbial group was calculated[30] and detection frequencies were also estimated. For molecular analysis, genomic DNA was extracted according to Salonen *et al.*[31] using QIAamp® DNA Mini Kit (QIAGEN GmbH). Quantitative real-time PCR based on SYBR Green I detection chemistry was used to characterise the gut microbiota using species-, genus- and group-specific primers targeting 16S rRNA genes of different bacterial groups or the nuc gene in the case of *Staphylococcus aureus* (online Supplementary Table S2) and the KAPA SYBR® Fast Master Mix (2×) Universal Kit (Kapa Biosystems Inc.). PCR amplification and detection were performed in a LightCycler® 2.0 Real-Time PCR System (Roche Diagnostics GmbH). Microbial quantification was based on standard curves of genomic DNA from reference strains with the LightCycler® software version 4.1 (Roche Diagnostics GmbH). Data are expressed as $\log_{10}$ copies of 16S rRNA gene/g wet faeces or $\log_{10}$ copies of nuc gene/g wet faeces in the case of *S. aureus*.

Faecal SCFA and pH determination

Faecal SCFA concentrations were determined using capillary GC, as previously described[32], after 1:3 dilution of frozen faecal samples (1·5 g, −80°C) using 0·9 % saline. Faecal pH of fresh samples and stool moisture were also determined[33].

Statistical analysis

Normality of the distribution of variables was tested using the Kolmogorov–Smirnov test. Comparisons of normally distributed variables between study groups were performed using one-way ANOVA and univariate ANCOVA, whereas for skewed variables the Kruskal–Wallis H test and rank ANCOVA were used, as appropriate; Bonferroni's correction rule for the inflation of type I error was applied. The χ^2 was applied for checking dependency between categorical variables; whereas Pearson's r or Spearman's ρ correlation coefficients were used to evaluate linear relationships among continuous variables. Multiple linear regression analysis (presented as β-coefficients with their standard errors and P values) and logistic regression analysis were performed to evaluate MedDietScore or specific food groups (independent variable) in relation to faecal, gut microbiota and gastrointestinal characteristics (dependent outcomes) of the participants. The statistical analysis was performed using SPSS® Statistical software version 21 (IBM Hellas). The sample size of the present study was defined *a priori* in order to evaluate 0·5 standardised differences between Mediterranean diet adherers and non-adherers in various bacteria and SCFA studied in the present protocol. In particular, to achieve 80 % statistical power at a 5 % significance level of two-sided hypotheses, fifty-eight participants were considered adequate to evaluate the aforementioned differences.

Results

A total of 116 subjects (sixty-one male and fifty-five female; mean age 42 years) completed the study. Dropout ($n = 4$) was because of failure of faecal sampling. LER represented 13·8 % of the sample (sixteen subjects; nine obese, six overweight, one normal weight) and they were excluded from the analyses below. On the basis of values of MedDietScore, subjects in our study (n 100) were classified into three tertiles of adherence to the Mediterranean diet according to individual MedDietScore (low tertile (score 19·0–30·0, n 31), medium tertile (score 31·0–33·0, n 29) and high tertile (score 34·0–41·0, n 40)). Descriptive characteristics of the study participants according the tertile of adherence to the Mediterranean diet are available in Table 1.

Diet, physical activity and sleeping patterns

Participants with the highest adherence to the Mediterranean diet reported higher consumption of starchy products, vegetables, fruits, fish and eggs, but lower consumption of meat, snacks and stimulants (e.g. coffee/tea, sodas) compared with those in the low tertile (online Supplementary Table S3). No significant difference was detected among MedDietScore categories in levels of physical activity, total physical activity score, sedentary lifestyle and sleeping duration with or without adjustment for sex, age and BMI (online Supplementary Table S4).

Gastrointestinal symptoms and evacuation characteristics

Subjects in the high-adherence tertile reported greater total number of evacuations during the 7-d period compared with the low-adherence tertile ($P = 0·028$) (Table 2). Though no significant differences were detected among tertiles in terms of Bristol stool scale values, there was a trend for lower scale rating in the participants of medium tertile compared with those

Table 1. Subjects' basic characteristics
(Mean values and standard deviations; medians and quartiles 1–3 (Q1–Q3))

	Total (*n* 100)		Tertiles of MedDietScore						
			Low (*n* 31)		Medium (*n* 29)		High (*n* 40)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	$P_{for\ trend}$
Sociodemographic parameters									
Age (years)	41·27	13·33	40·42	13·12	40·14	13·16	42·75	13·33	0·657
Sex (male/female)	48/52		19/12[a]		15/14[a,b]		14/26[b]		0·080
Years of education	17·05	4·77	17·18	5·00	17·16	5·02	16·87	4·52	0·953
Marital status (no. of singles)	51		16		14		15		0·453
Current smoking (no. of smokers)	31		14		7		10		0·121
Medical and psychological status									
SBP (mmHg)	118·86	16·24	118·15	18·25	121·97	10·67	117·15	17·93	0·462
DBP (mmHg)	76·32	10·44	76·60	10·38	76·90	8·28	75·67	11·98	0·878
Heart rate (beats/min)	73·66	10·04	76·48	9·12	72·43	10·72	72·32	10·01	0·167
Self-evaluated health status score (score 0·0–100·0)									0·846
Median	85·0		80·0		85·0		85·0		
Q1–Q3	80·0–90·0		78·8–90·0		75·0–90·0		80·0–90·0		
ZDRS score (score 20·0–80·0)	33·41	6·87	33·90	7·10	33·39	6·21	33·05	7·26	0·879
STAI score (score 20·0–80·0)	34·76	9·07	35·17	9·57	36·11	9·02	33·50	8·78	0·489
Anthropometric measurements									
Weight (kg)	77·48	15·31	78·44	17·05	80·48	12·38	74·56	15·64	0·262
Height (m)	1·68	0·09	1·69	0·10	1·70	0·08	1·66	0·09	0·134
BMI (kg/m^2)	27·29	4·48	27·46	4·80	27·68	3·66	26·88	4·83	0·743
WC (cm)	90·08	13·46	91·34	14·01	92·48	10·58	87·49	14·59	0·273
HC (cm)	107·99	8·20	108·10	8·40	109·93	6·31	106·61	9·06	0·270
WHR	0·83	0·09	0·84	0·09	0·84	0·08	0·82	0·10	0·425
Triceps skinfold (mm)	24·02	6·46	23·59	6·61	24·69	7·34	23·90	5·80	0·805
Body fat (%)	29·11	8·78	27·42	8·92	29·46	9·61	30·16	8·06	0·418
FFM (kg)									0·081
Median	51·70		59·90[a,b]		57·40[a]		47·50[b]		
Q1–Q3	43·80–64·20		43·60–66·50		45·80–67·60		43·00–61·40		

SBP, systolic blood pressure; DBP, diastolic blood pressure; ZDRS, Zung Depression Rating Scale; STAI, State-Trait Anxiety Inventory; WC, waist circumference; HC, hip circumference; WHR, waist:hip ratio; FFM, fat-free mass.
[a,b] Mean or median values within a row with unlike superscript letters were significantly different ($P<0·05$).

Table 2. Gastrointestinal symptoms and evacuation characteristics
(Medians and quartiles 1–3 (Q1–Q3))

	Tertiles of MedDietScore						
	Low (*n* 31)		Medium (*n* 29)		High (*n* 40)		
	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	$P_{for\ trend}$
During the last 4 weeks							
Evacuations (no./d)*							0·388
<1	6		5		9		
1	19		22		19		
2	4		1		9		
3	1		1		2		
≥4	0		0		1		
Abdominal or epigastric pain (score 0·00–10·00)*	0·00	0·00–0·63	0·00	0·00–0·76	0·00	0·00–1·09	0·930†
7-d symptom questionnaire‡							
Evacuations (no./week)	7·00[a]	5·75–9·00	7·50[a,b]	6·25–9·00	9·00[b]	6·00–11·00	0·078†
Diarrhoeic evacuations (no./week)	0·00	0·00–0·00	0·00	0·00–0·00	0·00	0·00–1·00	0·678†
Bristol scale (range 1·0–7·0)	4·0	3·0–4·0	3·0	3·0–4·0	4·0	3·0–4·0	0·224†
Faecal colour (range 1·0–4·0)	3·00	2·75–3·00	3·00	2·00–3·00	3·00	2·00–3·00	0·497†
Faecal smell (range 1·0–4·0)	2·00	1·00–2·00	2·00	1·25–2·75	2·00	1·00–3·00	0·305†
Abdominal pain (score 0·0–28·0)	0·00[a]	0·00–2·00	0·50[a,b]	0·00–2·75	2·00[b]	0·00–4·00	0·087†
Bloating (score 0·0–28·0)	0·00[a]	0·00–3·25	0·50[a,b]	0·00–5·00	4·00[b]	0·00–6·25	0·066†
Flatulence (score 0·0–28·0)	1·00	0·00–7·50	5·00	1·00–8·75	6·00	1·00–11·25	0·379†
Borborygmi (score 0·0–28·0)	0·00	0·00–2·00	0·00	0·00–3·75	1·00	0·00–5·00	0·302†
Sum of symptoms (score 0·0–112·0)	8·50	1·75–15·25	11·00	6·00–16·75	14·50	6·75–26·00	0·145†

[a,b] Median values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For evacuations (no./d) and abdominal or epigastric pain: *N* 99 (low tertile: *n* 30, medium tertile: *n* 29, high tertile: *n* 40).
† Sex-, age- and BMI-adjusted *P* values.
‡ For 7-d symptom questionnaire: *n* 96 (low tertile: *n* 30, medium tertile: *n* 28, high tertile: *n* 38).

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in the high tertile ($P=0{\cdot}085$), implying a difference in gut transit time between these groups. In terms of gastrointestinal symptoms, scores of pain ($P=0{\cdot}029$) and bloating were higher ($P=0{\cdot}028$) and the sum of symptoms tended to be higher ($P=0{\cdot}052$) in the high compared with the low tertile. The majority of subjects (89 %) experienced mild total gastrointestinal symptomatology, with flatulence being the most commonly reported symptom.

Faecal SCFA, stool pH and moisture content

Faecal moisture (%) tended to be higher in the high compared with the low tertile ($P=0{\cdot}056$) (Table 3). Total levels of SCFA differed significantly among adherence groups, with detection of lower levels in the case of medium compared with high ($P=0{\cdot}013$) and low tertiles ($P=0{\cdot}078$), whereas no significant difference was detected between adherence groups in terms of stool pH (Table 3). High adherence to the Mediterranean diet was characterised by a significantly greater molar ratio of acetate compared with the low tertile ($P=0{\cdot}009$) and medium tertile ($P=0{\cdot}075$), whereas valerate was detected in a lower ratio in the high tertile compared with both medium ($P=0{\cdot}002$) and low tertiles ($P=0{\cdot}014$).

Gut microbiota analysis

Enumeration of gut microbiota by plate-count techniques and detection frequency of each microbial group are presented in Table 4. Data concerning bacteria, yeasts and fungi are available for ninety-two cases, whereas data for *Candida* genus and species are available for the entire cohort (n 100). Relative shares of each microbial group were also calculated on the basis of total cultured bacteria or eukaryotes (data not shown).

Gut microbiota profiling analysis revealed significantly lower counts of *Escherichia coli* ($P=0{\cdot}022$) in the high- compared with the low-adherence group after adjustment for sex, age and BMI (Table 4). This fact further explained the notable difference in bifidobacteria:*E. coli* ratio between these groups ($P=0{\cdot}025$), though no significant differentiation could be detected between groups in terms of bifidobacteria levels (Table 4). Counts of *Candida albicans* were higher in the high tertile compared with the low- ($P=0{\cdot}039$) and medium- ($P=0{\cdot}093$) MedDietScore groups and a higher detection frequency was observed in the high tertile compared with the low-adherence group (42·5 *v.* 22·6 %, $P=0{\cdot}050$), though no signs or symptoms of candidiasis were reported among subjects (Table 4).

No significant differences were observed between tertiles with respect to qPCR analysis after sex, age and BMI adjustment (Table 5).

Associations of the Mediterranean diet with gut microbiota, faecal and gastrointestinal characteristics

Bivariate models revealed several significant correlations between adherence to the Mediterranean diet, as evaluated by MedDietScore, and gastrointestinal, stool and gut microbiota characteristics, which remained significant even after adjustment for sex, age and

Table 3. Faecal total SCFA concentration, molar ratios of SCFA and stool characteristics (Mean values and standard deviations; medians and quartiles 1–3 (Q1–Q3))

	Tertiles of MedDietScore						
	Low (n 31)		Medium (n 29)		High (n 40)		
	Mean	SD	Mean	SD	Mean	SD	$P_{\text{for trend}}$*
Total SCFA (μmol/g wet faeces)	83·07^{a,b}	35·36	67·52^{a}	25·45	83·02^{b}	34·24	0·040
Molar ratios of SCFA							
Acetate (%)	45·41^{a}	4·92	46·62^{a,b}	5·60	49·16^{b}	4·96	0·026
Propionate (%)	19·29	5·22	17·69	3·64	17·39	4·91	0·245
Butyrate (%)	27·74	6·09	27·15	5·82	26·77	5·43	0·928
Branched-chain SCFA (%)†	3·70	1·93	4·28	1·67	3·57	2·34	0·300
Iso-butyrate (%)							0·462
Median	1·31		1·38		1·21		
Q1–Q3	0·76–2·24		0·89–2·58		0·66–2·01		
Iso-valerate (%)	2·27^{a,b}	1·40	2·47^{a}	1·22	1·91^{b}	1·26	0·099
Iso-caproic acid (%)							0·180
Median	0·00		0·00		0·00		
Q1–Q3	0·00–0·00		0·00–0·08		0·00–0·07		
Other SCFA (%)‡	3·49^{a,b}	1·69	4·25^{a}	1·59	3·11^{b}	1·36	0·012
Valerate (%)	2·43^{a}	0·93	2·56^{a}	0·76	1·97^{b}	0·75	0·005
Caproic acid (%)	0·92^{a}	0·81	1·41^{b}	0·85	0·99^{a}	0·78	0·045
Heptanoic acid (%)							0·023
Median	0·08^{a}		0·20^{b}		0·13^{a,b}		
Q1–Q3	0·00–0·20		0·11–0·31		0·00–0·24		
Faecal characteristics							
pH	6·66	0·52	6·96	0·68	6·94	0·65	0·200
Moisture (% of wet weight)	70·65	6·90	71·65	6·25	74·10	6·47	0·131

^{a,b} Mean or median values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* Sex-, age-, and BMI-adjusted P values.
† Sum of iso-butyrate, iso-valerate and iso-caproic acid.
‡ Sum of valerate, caproic acid and heptanoic acid.

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Table 4. Culture-dependent analysis of gut microbiota (bacteria, yeasts, fungi)*
(Mean values and standard deviations; medians and quartiles 1–3 (Q1–Q3); number of positive samples)

	Tertiles of MedDietScore						
	Low (*n* 29)		Medium (*n* 27)		High (*n* 36)		
	Mean	SD	Mean	SD	Mean	SD	$P_{for\ trend}$†
Total aerobes	8·12	1·07	8·14	0·88	8·02	0·79	0·888†
Total coliforms	7·25	1·19	6·98	0·90	6·83	1·05	0·287†
Escherichia coli	7·24[a]	1·20	6·69[a,b]	1·04	6·46[b]	1·38	0·065†
Enterococci	6·08	1·11	5·78	1·73	5·96	1·31	0·727†
Staphylococci	2·79	0·76	3·15	1·17	3·03	0·80	0·295†
Detection of staphylococci	24[a,b]		16[a]		32[b]		0·028
Staphylococcus aureus	2·72	0·85	3·00	1·32	2·51	0·80	0·175†
Detection of *S. aureus*	18[a,b]		12[a]		26[b]		0·114
Total anaerobes	9·81[a]	0·44	9·47[b]	0·54	9·59[a,b]	0·62	0·056†
Clostridia	4·88	1·25	4·69	1·39	4·87	1·05	0·761†
Clostridium perfringens	3·82	1·27	3·91	1·50	4·03	1·35	0·762†
Lactobacillus spp.	5·79	1·34	5·47	1·52	5·73	1·27	0·539†
Bifidobacterium spp.							0·811†
Median	9·07		8·77		8·78		
Q1–Q3	7·39–9·35		7·40–9·38		8·34–9·33		
Bacteroides spp.	8·37	1·23	8·46	1·04	8·43	1·26	0·933†
Yeasts	3·17[a,b]	1·24	2·69[a]	0·84	3·56[b]	0·92	0·016†
Detection of yeasts	22		24		30		0·433
Candida spp.‡	3·15[a,b]	1·30	2·62[a]	0·88	3·39[b]	0·90	0·055†
Detection of *Candida* spp.	18		19		29		0·444
Candida albicans	2·69[a]	0·48	2·99[a,b]	0·82	3·28[b]	0·54	0·074†
Detection of *C. albicans*	7		9		17		0·131
Candida glabrata	2·90	1·31	2·14	0·85	2·89	0·75	0·653†
Detection of *C. glabrata*	7		7		10		0·972
Candida parapsilosis	2·65	1·31	2·04	0·56	3·14	0·96	0·664†
Detection of *C. parapsilosis*	5		3		5		0·795
Candida krusei	2·96	1·01	2·35	0·81	3·06	1·19	0·463†
Detection of *C. krusei*	4		3		9		0·487
Rhodotorula-like yeasts	2·72	1·01	2·18	0·42	2·55	0·73	0·309†
Detection of *Rhodotorula*	8		13		14		0·283
Fungi							0·978†
Median	2·08		1·97		2·31		
Q1–Q3	1·67–2·82		1·87–3·05		1·87–3·05		
Detection of fungi	17		14		18		0·787
Bifidobacteria:*E. coli* ratio							0·077†
Median	1·16[a]		1·30[a,b]		1·24[b]		
Q1–Q3	1·00–1·34		1·08–1·35		1·15–1·49		
Bacteria:yeasts ratio	3·47[a,b]	0·99	3·87[a]	1·17	2·91[b]	0·78	0·006†
Bacteria:*Candida* ratio	3·29[a,b]	0·96	3·89[a]	1·22	3·05[b]	1·02	0·032†
Bacteria:fungi ratio	4·40	1·45	4·40	1·14	4·28	1·49	0·995†

[a,b] Mean or median values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* Values are $\log_{10}$colony-forming units/g wet faeces (counts).
† Sex-, age- and BMI-adjusted *P* values.
‡ For *Candida* spp. and species: *n* 100 (low tertile: *n* 31, medium tertile: *n* 29, high tertile: *n* 40).

BMI (Table 6). In specific, MedDietScore was associated positively with faecal moisture, total bacteria levels, bifidobacteria:*E. coli* ratio (culture based) and the relative share of *Bacteroides* spp., and negatively with counts of *E. coli* and the molar ratio of valerate after age, sex and BMI adjustment (Table 6). A correlation of MedDietScore with *C. albicans* counts (0·066 (SD 0·033), $P = 0·054$) and total SCFA concentration (1·554 (SD 0·789), $P = 0·052$) was also observed after age, sex and BMI adjustment. In terms of gastrointestinal symptomatology, MedDietScore was positively correlated with bloating, sum of symptoms (Table 6) and score of pain (0·173 (SD 0·088), $P = 0·054$) after age, sex and BMI adjustment.

Though not components of the MedDietScore, consumption of two food groups (snacks and junk food, stimulants) correlated negatively with adherence to the Mediterranean diet in this study and may have significant effects in gut microbiota characteristics. Consumption of snacks and junk food correlated negatively with faecal moisture (−7·541 (SD 2·569), $P = 0·004$), Firmicutes (−0·195 (SD 0·077), $P = 0·013$), the *C. coccoides* group (−0·225 (SD 0·092), $P = 0·016$), the *Clostridium leptum* group (−0·306 (SD 0·112), $P = 0·007$), *F. prausnitzii* (−0·501 (SD 0·144), $P = 0·001$), the *Lactobacillus* group (qPCR; −0·759 (SD 0·333), $P = 0·025$), *Bacteroides* (qPCR; −0·246 (SD 0·113), $P = 0·032$) and the bifidobacteria:*E. coli* ratio (culture based; −0·395 (SD 0·175), $P = 0·027$), and positively with the relative share of coliforms (5·903 (SD 2·524), $P = 0·022$), counts of *E. coli* (culture based; 1·360 (SD 0·551), $P = 0·016$), propionate (5·030 (SD 1·877), $P = 0·009$) and iso-valerate (1·338 (SD 0·508), $P = 0·010$) after

Table 5. Culture-independent analysis of gut microbiota (quantitative PCR)*
(Mean values and standard deviations; medians and quartiles 1–3 (Q1–Q3); percentage of positive samples)

	Tertiles of MedDietScore						
	Low (*n* 31)		Medium (*n* 29)		High (*n* 40)		
	Mean	SD	Mean	SD	Mean	SD	$P_{for\ trend}$
Total bacteria	11·68	0·23	11·75	0·19	11·75	0·23	0·421†
Firmicutes	11·67	0·20	11·77	0·14	11·69	0·22	0·187†
Bacteroidetes	11·09	0·27	11·07	0·19	11·09	0·29	0·832†
Firmicutes:Bacteroidetes ratio	1·05	0·02	1·06	0·02	1·05	0·02	0·121†
Bacteroides spp.	11·00	0·32	10·98	0·22	11·03	0·31	0·596†
Prevotella spp.							0·623†
Median	9·30		9·01		8·83		
Q1–Q3	8·72–10·71		8·77–10·38		8·57–10·58		
% detection of *Prevotella*	90·3		79·3		92·5		0·223
Prevotella:*Bacteroides* ratio							0·547†
Median	0·89		0·82		0·80		
Q1–Q3	0·80–0·98		0·79–0·98		0·78–0·97		
Clostridium coccoides group	10·80	0·26	10·82	0·22	10·77	0·24	0·829†
Roseburia spp.-*Eubacterium rectale*							0·946†
Median	10·42		10·38		10·31		
Q1–Q3	10·10–10·53		10·19–10·54		10·06–10·58		
Clostridial cluster IV (*Clostridium leptum* group)	10·70	0·29	10·81	0·22	10·74	0·32	0·345†
Faecalibacterium prausnitzii							0·745†
Median	10·46		10·54		10·48		
Q1–Q3	10·30–10·64		10·28–10·66		10·29–10·70		
C. coccoides:*C. leptum* ratio	1·01	0·03	1·00	0·02	1·00	0·02	0·292†
Clostridium perfringens group	8·09	0·69	8·11	0·71	8·20	0·73	0·733†
Lactobacillus group	8·05	0·70	7·83	0·92	7·98	0·85	0·549†
Bifidobacterium spp.							0·481†
Median	10·90		10·52		10·55		
Q1–Q3	10·57–10·90		9·94–10·72		10·17–10·77		
Escherichia coli subgroup	8·08	1·41	7·79	1·14	7·90	1·04	0·623†
Bifidobacteria:*E. coli* ratio	1·33	0·25	1·34	0·32	1·34	0·20	0·958†
Staphylococcus aureus	4·81	0·49	4·72	0·87	4·95	0·54	0·534†
% detection of *S. aureus*	58·1		72·4		50·0		0·173
Akkermansia muciniphila							0·607†
Median	8·98		9·09		8·84		
Q1–Q3	6·09–9·52		6·20–9·91		6·43–9·52		
% detection of *A. muciniphila*	87·1		79·3		90·0		0·440
Methanobrevibacter							0·549†
Median	8·48		9·38		9·02		
Q1–Q3	6.44–9.91		6·77–10·17		5·55–9·92		
% detection of methanogens	83·9		75·9		92·5		0·635

* Values are $\log_{10}$ copies of 16 S rRNA gene/g wet faeces (levels) or $\log_{10}$ copies of nuc gene/g wet faeces for *S. aureus*.
† Sex-, age- and BMI-adjusted *P* values.

Table 6. Results from simple correlation analysis and multiple linear regression models for adherence to Mediterranean diet (MedDietScore) and gastrointestinal, faecal and gut microbiota characteristics of the participants
(*β*-Coefficients with their standard errors)

		Correlation coefficient	Sex-, age- and BMI-adjusted models		
Dependent outcomes	Independent factor	(*r* and *P* value)	*β*-Coefficient	SE	*P*
% faecal moisture	MedDietScore (per 1 point)	*r* 0·235, *P*=0·019	0·357	0·167	0·035
Total bacteria (qPCR)	MedDietScore (per 1 point)	*r* 0·250, *P*=0·012	0·014	0·006	0·016
Escherichia coli (culture based)	MedDietScore (per 1 point)	*r* −0·317, *P*=0·003	−0·111	0·037	0·004
Bifidobacteria:*E. coli* (culture based)	MedDietScore (per 1 point)	*r* 0·313, *P*=0·004	0·035	0·012	0·003
Relative share of bacteroides	MedDietScore (per 1 point)	*r* 0·210, *P*=0·011	1·310	0·473	0·007
Molar ratio of valerate	MedDietScore (per 1 point)	*r* −0·195, *P*=0·052	−0·042	0·021	0·049
Bloating	MedDietScore (per 1 point)	*r* 0·311, *P*=0·002	0·197	0·095	0·042
Sum of symptoms	MedDietScore (per 1 point)	*r* 0·258, *P*=0·011	0·766	0·315	0·017

qPCR, quantitative PCR.

sex, age and BMI adjustment. Stimulant consumption was negatively associated with stool pH (−0·360 (SD 0·143), *P*=0·013), *S. aureus* (qPCR; −0·573 (SD 0·251), *P*=0·026) and staphylococci counts (culture based; −0·517 (SD 0·239), *P*=0·034) after sex, age and BMI adjustment. Further analysis into the stimulant food group revealed that these effects were

attributable exclusively to coffee or tea consumption and that the consumption of sodas was correlated negatively with faecal levels of *A. muciniphila* (−3·717 (SD 1·641), $P = 0·026$).

Discussion

The present study aimed to explore possible associations of adherence to the Mediterranean diet with the gut microbiota profile and gastrointestinal symptoms in an adult population. Regarding the well-documented geographical and ethnic variation of gut microbial composition in humans[34], similar scientific efforts may contribute to validation of the robustness of the proposed microbial indicators of metabolic health and inflammation. Moreover, the influence of the Mediterranean diet in gut microbial ecology is currently revealed[12–15,18,19], and this scientific field is open to further investigation. Thus, our findings indicate that a high adherence to the Mediterranean diet was characterised by lower *E. coli* counts and a subsequently higher culture-based bifidobacteria:*E. coli* ratio, increased levels and prevalence of *C. albicans*, greater molar ratio of acetate, higher defaecation frequency and a more pronounced gastrointestinal symptomatology compared with the low tertile. An overall lower molar ratio of valerate in the case of high adherence to the Mediterranean diet compared with other score levels was also indicated. Positive correlations of MedDietScore with gastrointestinal symptoms, faecal moisture, total bacteria, bifidobacteria:*E. coli* ratio, relative share of bacteroides, *C. albicans* and total SCFA, and negative associations with cultivable *E. coli* levels and valerate were also indicated. Further associations between gut microbiota characteristics and consumption of snacks and junk food or stimulants were also revealed.

Beneficial effects of the Mediterranean diet in the management of chronic diseases are attributed to the cumulative synergistic and interactive combinations of nutrients[10]. Characteristics of the Mediterranean diet, such as intake of non-refined cereals, vegetables, fruit, olive oil and red wine, are linked to a great repertoire of constituents with potential effects in gut microbiota dynamics[17]. In the present study, positive associations of MedDietScore with total bacteria and bacteroides characteristics could be attributed to the high carbohydrate, fibre, unsaturated lipid and antioxidant content of the Mediterranean diet, as previously reported[13–14,17]. High adherence to the Mediterranean diet was also related to decreased counts of *E. coli*, a representative pathogenic bacterium, and subsequently to an increased ratio of typical beneficial bifidobacteria:*E. coli*, which is considered an important indicator for gut microbiota equilibrium and overall health[35]. Long-term polysaccharide-rich diets have been linked to underrepresentation of Enterobacteriaceae (*Shigella* and *Escherichia*), and dietary antioxidants may inhibit the growth of *E. coli* strains[18,36–38].

Previously reported connections of the Mediterranean diet[12–15,19] or plant-based diets typical in rural, agrarian societies[39] with suggested diet-responsive gut microbiota characteristics (e.g. *Prevotella*, *Prevotella*:*Bacteroides* ratio, *Roseburia-E. rectale*) were not found in the current work. Roager *et al.*[40] also reported stable *Prevotella*:*Bacteroides* ratio in Danish subjects with central obesity and components of the metabolic syndrome after a controlled intervention with the New Nordic Diet, which includes more fruits, vegetables and whole grains, and less added sugar and saturated fat. Factors related to the urban setting of the present research, the variation in fibre, protein and fat content as well as the quality of diets among studies, the differences in definition of adherence level to the Mediterranean diet or even the reported underrepresentation of the *Prevotella* group in Nordic and Southern European countries could be the reason for these discrepancies[12,39,41].

The diversity of the human gut mycobiome remains poorly explored[42] and available data about the Mediterranean diet and gut eukaryotes are rather scarce. In this study, high adherence to the Mediterranean diet was characterised by increased *C. albicans* colonisation patterns. *Candida* and other yeasts are associated with features of diet[43,44], and the connection of high adherence with increased levels of yeasts could be attributed to ingestion of foodstuff that are carriers of yeasts, such as fruits, juices and fermented food products[44,45].

SCFA are major gut microbial metabolites with a high potential impact on host molecular mechanisms because of their role as substrates and/or signalling molecules[46]. Increased amount of faecal SCFA was also previously observed in high-level adherence to the Mediterranean diet[12–13,19], a fact that could be interpreted by the enhanced microbial-dependent fermentation of indigestible carbohydrates reaching the colon[46]. Host factors such as transit time may also have a pivotal role in the total amount of SCFA excreted in faeces[47], which could merely justify suppressed SCFA levels observed in the medium tertile. Connections of adherence to the Mediterranean diet with acetate and other SCFA levels (e.g. valerate) were also previously reported[12] and reflected differences in consumption of plant- and animal-origin food groups, whereas positive relations with propionate and butyrate[12–13] were not replicated in this study, possibly because of the influence of known factors that affect the human gut-associated metabolome, such as sex and age[12].

A high adherence to the Mediterranean diet was related to higher stool frequency and faecal moisture and characterised by greater, though mild, gastrointestinal symptomatology. Dietary fibre consumption, fermentation and bulking-effect caused directly via water retention are significant contributors in these relationships[48]. Furthermore, the lipid content of olive oil, a core constituent of the Mediterranean diet, could exert a lubricant and stool-softening effect and enhance important stimuli for bowel movements through interactions with bile acids[49].

In the present study, intriguing associations between gut microbiota characteristics and consumption of stimulants or snacks and junk food were found. The inverse relation of stimulant consumption with the prevalence of faecal *S. aureus* could be attributed to more acidic stool pH and to the potential systemic antimicrobial activity of coffee and tea polyphenols against a wide range of pathogenic micro-organisms[50]. The negative association of soda consumption with levels of *A. muciniphila,* a mucin-degrading bacterium with possible beneficial effects against obesity and type 2 diabetes[4,51,52], raises further interest. Higher consumption of snack and junk food products was characterised by increased counts of *E. coli* and suppressed presence of lactobacilli and butyrate-producing

Firmicutes members (e.g. *C. coccoides* group, *C. leptum* group, *F. prausnitzii*), resulting in a potential detrimental inflammatory gut microbiota milieu for the host[1,5,53]. Furthermore, increased faecal levels of the branched SCFA iso-valerate may reflect bacterial catabolism of animal protein[54], whereas elevated amount of propionate in faeces may result from increased dietary intake of propionate salts, common preservatives in the food industry, and may exert versatile effects on host physiology and pathology[55]. Though evidence regarding junk food consumption and gut microbiota profiling in humans is very limited, in animal models it has been documented that following a Westernised 'fast food' style diet or a Western-style high-energy cafeteria diet results in restructuring of the gut microbiota[56,57]. Moreover, anecdotal experimental data have proposed the devastating effects of an exclusively fast food diet on the human gut microbiome diversity, with a 40 % reduction in detectable species within 10 d of consumption[58,59]. Finally, cumulative evidence suggests that following a Western-type, high-fat, refined-carbohydrate-rich diet and frequent consumption of highly processed and preserved foods, which reduce the intake of commensal, food-associated microbes, could disturb the gut microbiota balance and deserves special attention[60,61].

In this study, a detailed record of dietary, exercise, lifestyle and gastrointestinal parameters allowed the in-depth assessment of the population under investigation, whereas analysis adjustments for factors with established effects on gut microbiota composition (e.g. sex, age, BMI) allowed to explore the contribution of possible covariates[6,34]. The exclusion of LER from the analyses could minimise the systematic error of under-reporting. Even though microbiota sequencing was not available in this study, qPCR methodology in combination with cultivation techniques provided a thorough analysis of the gut microbiome and mycobiome. On the other hand, the cross-sectional study design undermines the causality of the reported results and future prospective and intervention studies are essential.

The use of MedDietScore for the assessment of adherence to the Mediterranean diet may also have some limitations. No weighting has been applied to the components of the score, mainly because it is hard to select the best weight because of the lack of sufficient data to support components' weighting (i.e. from meta-analyses on the specific components). Thus, we cannot exclude the possibility that two individuals may have the same score but different dietary intakes. This is an inherent limitation of the composite diet scores presented in the literature. Moreover, we could not use *a priori*-defined cut-off points for the adherence (or non-adherence) to the Mediterranean diet, because the Mediterranean diet as a healthy prototype should be followed in total. The cut-off points used here were the tertiles of the MedDietScore; by dividing the group into three equal-sized sub-groups, optimal statistical power is achieved. This approach has been routinely used in observational studies investigating the role of adherence to the Mediterranean diet in health status[22,23,62–64]. It is true that the cut-off points used in MedDietScore for the description of the characteristics in our sample were data driven. This means that the 'low' cut-off point of the MedDietScore could be different in another population group. However, the classification of MedDietScore was used only for descriptive results, whereas continuous values of the score were used in all multi-adjusted statistical analyses. Thus, the findings of diet–outcome(s) relationships and their significance were not related to the thresholds used for the tertiles of the MedDietScore. Finally, it is interesting to note that in our study, the mean MedDietScore was comparable with values reported in other studies performed outside Greece (e.g. USA, UK), in which adherence to the Mediterranean diet was calculated on the basis of the same diet score as in our research[62–64]. In detail, MedDietScore ranged from 18 to 46 in the study by Tangney *et al.*[64], with a mean value of 26 for the low tertile, 31 for the medium tertile and 37 for the high tertile, whereas in the study by Koyama *et al.*[62], race-specific tertiles for Whites were 12–29 (low), 30–34 (medium) and 35–50 (high tertile). Thus, our data might be applied to other populations beyond Greek individuals and these observations could add considerable strength to the argument about the 'health' aspect of the Mediterranean diet.

In conclusion, our findings support a link between adherence to the Mediterranean diet and the gut microbiota profile, SCFA production and gastrointestinal symptoms. Additional research is necessary to elucidate connections of the Mediterranean food pattern with gut microbiota characteristics, possibly under the prism of other long-term dietary habits (e.g. stimulant and fast food consumption).

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517001593

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