

RESEARCH

Open Access



Total and different types of olive oil consumption, gut microbiota, and cognitive function changes in older adults

Jiaqi Ni^{1,2,3}, Stephanie K. Nishi^{1,2,3,4,5}, Nancy Babio^{1,2,3}, Clara Belzer⁶, Jesús Vioque^{7,8}, Dolores Corella^{3,9}, Javier Hernando-Redondo^{3,10}, Josep Vidal^{11,12}, Isabel Moreno-Indias^{3,13}, Laura Compañ-Gabucio^{7,8}, Oscar Coltell^{3,14}, Montse Fitó^{3,10}, Estefanía Toledo^{3,15}, Dong D. Wang^{16,17,18}, Francisco J. Tinahones^{3,13} and Jordi Salas-Salvadó^{1,2,3*}

Abstract

Background Over the past decade, emerging evidence has shed light on the role of the gut microbiota in the interface between diet and brain health. Olive oil, particularly virgin olive oil, a key component and major fat source in the Mediterranean diet, has exhibited widespread healthful benefits, including improvements in gut microbiota and cognitive health. Despite insights from preclinical studies into the relationship between virgin olive oil consumption, gut microbiota, and cognitive function, human research in this area remains limited. Therefore, our study aims to investigate the interplay between total olive oil consumption and its subtypes, gut microbiota, and changes in cognitive function in older adults who were cognitively healthy at baseline but at high risk of cognitive decline.

Methods In this prospective cohort study, we assessed a total of 656 participants aged 55 to 75y (mean age 65.0 ± 4.9y, 47.9% women) with overweight/obesity and metabolic syndrome who provided stool samples and completed a validated semi-quantitative food frequency questionnaire at baseline and a comprehensive battery of neuropsychological tests at baseline and at a 2-y follow-up.

Results Results from the multivariable linear regression models showed that higher consumption of virgin olive oil was associated with improved cognitive function over a 2-y follow-up, and a more diverse gut microbiota overall structure at baseline. Conversely, increased consumption of common olive oil is linked to lower alpha diversity of the microbial communities, and accelerated cognitive decline. Mediation analysis suggests that gut microbiota and particularly the *Adlercreutzia*, may serve as a mediator taxon in the association between virgin olive oil consumption and positive changes in general cognitive function.

Conclusions Higher consumption of virgin olive oil was associated with cognitive preservation, possibly mediated by favorable alterations in gut microbiota composition. Our study provides novel insights into the complex interplay between different types of olive oil consumption, gut microbiota, and changes in cognitive function. These findings underscore the potential of microbiota-targeted dietary strategies to promote cognitive health in aging populations, though further high-quality and clinical cohort studies are required.

Keywords Virgin olive oil, Common olive oil, Cognitive function, Gut microbiota, Mediation, Gut-brain axis

*Correspondence:

Jordi Salas-Salvadó

jordi.salas@urv.cat

Full list of author information is available at the end of the article



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Background

Cognitive decline poses a significant public health challenge in a globally aging population, with an increasing incidence of neurodegenerative diseases (NDs) such as Alzheimer's disease (AD) and other forms of dementia, and a substantial impact on societal healthcare costs [1]. Among various modifiable lifestyle factors, dietary patterns with a particular focus on the Mediterranean diet (MedDiet), have emerged as critical preventive strategies to mitigate age-related cognitive decline and neurodegeneration [2–4].

Virgin olive oil (VOO), a key component of the MedDiet, is distinguished for its high content of mono-unsaturated fatty acids and phenolic compounds, playing a crucial role in disease prevention [5–7]. Specifically, VOO is obtained directly from olives using only mechanical means and contains multiple bioactive components, such as polyphenols (e.g. hydroxytyrosol (HTyr) and oleuropein (OLE)), phytosterols and tocopherols [6]. These bioactive components, known for their antioxidant and anti-inflammatory properties, may confer neuroprotective benefits beyond the lipid profile [8, 9]. In contrast, common olive oil (COO) comprises a higher proportion of refined olive oil or olive–pomace oil and a minimal amount of VOO [10], resulting in a reduced content of phytochemicals despite sharing a similar lipid profile with VOO [7]. While recent findings have shown the cognitive benefits of olive oil consumption, many studies have not distinguished between olive oil types [5]. Given that olive oil types differ in both organoleptic characteristics and nutritional properties [7, 10], the diverse effects of different olive oil types on cognitive function remain to be comprehensively explored.

Emerging evidence from human and animal studies demonstrates the potential benefits of olive oil consumption—particularly VOO—on gut microbiota and intestinal health [6]. Given the critical role of gut microbiota in regulating cognitive function and modifying neurodegenerative disease risk through the “gut-brain” axis [11–13], investigating the mechanism underlying olive oil's effect on gut microbiota as a target of nutritional strategies for improving brain health and function is of particular interest [6, 14, 15]. Olive oil, especially VOO for its higher concentrations of phenolic compounds, may exert its cognitive benefits through modulation of the gut microbiota, influencing various immunological, neuronal, and metabolic pathways involved in the gut-brain communication network [6, 8, 9, 16]. Although preclinical studies have begun to elucidate the interplay between VOO, gut microbiota, and cognitive health [6], there has been limited human research investigating this intricate relationship thus far.

Therefore, to address the current gaps in knowledge, we analyzed the interplay between total olive oil (TOO) consumption—defined as the sum of VOO and COO—and its subtypes, gut microbiota, and changes in cognitive function in a subpopulation from the PREDIMED (PREvenición con Dieta MEDiterránea)-Plus study (Fig. 1). Our study had four main objectives. First, to prospectively analyze the association between the amount of TOO and its subtypes (VOO and COO) consumed, and changes in cognitive function after 2 years of follow-up in a population at high risk of cognitive decline. Second, to analyze cross-sectional associations between different amount and types of olive oil consumption and gut microbiota diversity and composition. Third, to prospectively analyze whether these olive oil-microbial associations are connected with changes in cognitive function during follow-up. Finally, to determine whether the gut microbiota mediates the association between TOO, VOO or COO consumption, and cognitive function.

Methods

Study design and participants

The present prospective cohort study was conducted within the framework of the PREDIMED-Plus primary prevention trial. PREDIMED-Plus is an ongoing, multicenter, parallel-group, randomized controlled trial (RCT). The aim of the trial is to evaluate the effects of lifestyle interventions on a combined cardiovascular primary endpoint, as well as other secondary and intermediate outcomes, including neurodegenerative disorders and cognitive function. The intervention group receives an energy-reduced MedDiet, physical activity recommendations and behavioral modifications, while the control group receives usual care and *adlibitum* MedDiet recommendations. A total of 6874 eligible participants, men and women aged 55–75 years with overweight or obesity and metabolic syndrome [17], without cardiovascular or severe neurodegenerative disease, schizophrenia, bipolar disease, eating disorders, depression with hospitalization, or any other condition that may interfere with adherence to the study protocol at baseline, were randomly allocated to either the intervention group or the control group in a ratio of 1:1. All participants provided written informed consent. A detailed trial protocol is available at <https://www.predimedplus.com/en/> and elsewhere [18]. The trial, approved by the research ethics committees of all participating institutions, was registered at the International Standard Randomized Controlled Trial Number Registry (ISRCTN89898870).

In the present study, a random subsample of 656 participants was included in the downstream analysis (Supplementary Fig. S1). Participants were excluded based on the following criteria: (1) absence of baseline stool

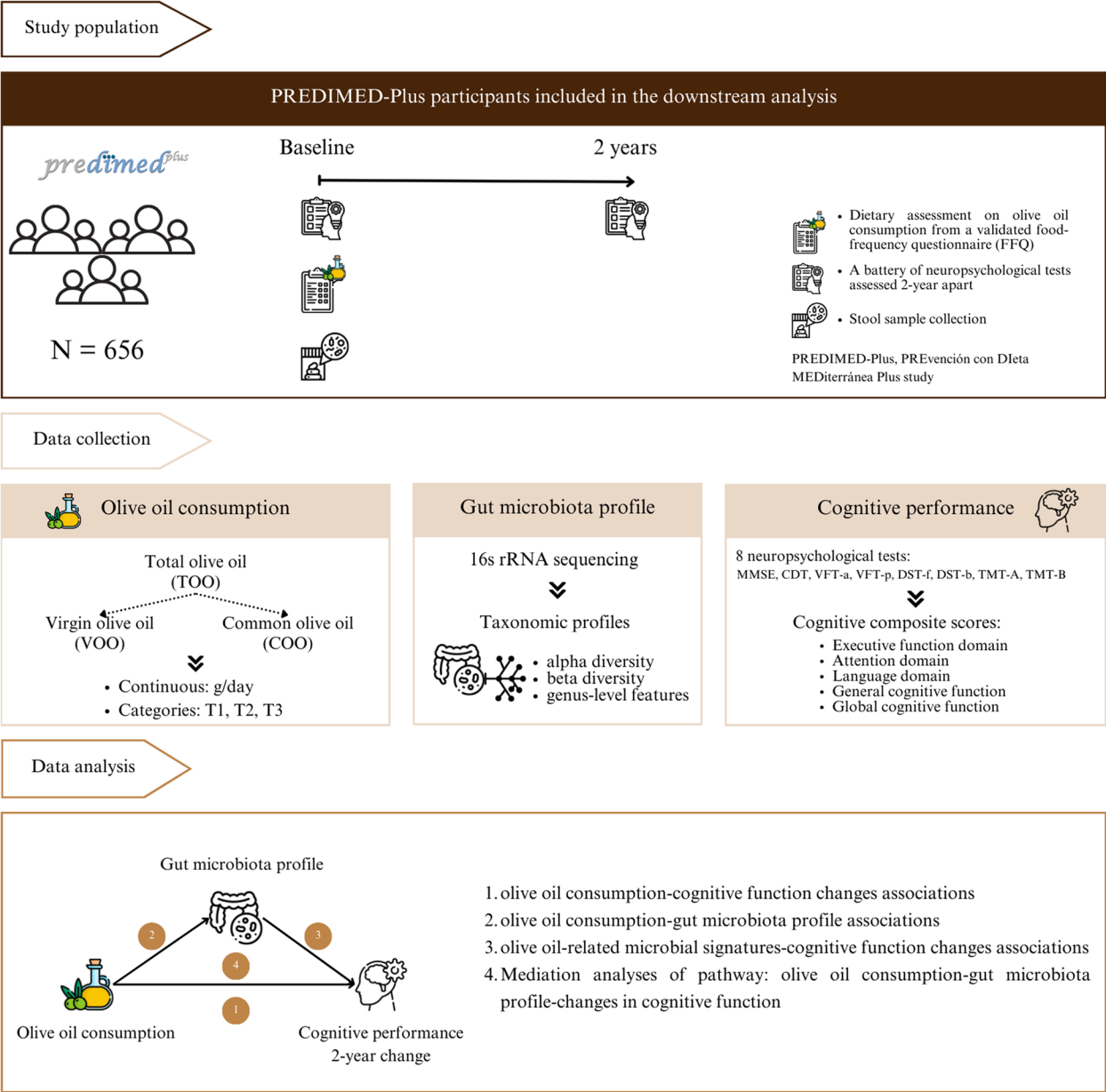


Fig. 1 Overview of the study design. Abbreviations: CDT, Clock Drawing Test; COO, common olive oil; DST-b, Digit Span test-backward; DST-f, Digit Span test-forward; FFQ, food frequency questionnaire; MMSE, Mini-Mental State Examination; PREDIMED-Plus, PREvención con Dieta MEDiterránea-Plus; TMT-A, Trail Making Test Part A; TMT-B, Trail Making Test Part B; TOO, total olive oil; VFT-a, Verbal Fluency tasks semantical; VFT-p, Verbal Fluency tasks phonological; VOO, virgin olive oil

samples or antibiotic use within 30 days before stool sample collection; (2) missing baseline food frequency questionnaire (FFQ) or incomplete neuropsychological test assessments at baseline and after 2 years of follow-up; (3) reported baseline energy intakes outside predefined limits (≥ 800 to ≤ 4000 kcal/day for men, ≥ 500 to ≤ 3500 kcal/day for women) [19]; or (4) TOO consumption exceeding 100 g/d.

Dietary and covariate assessment

Dietary intake was assessed annually by trained dietitians using a validated semi-quantitative FFQ [20]. Nutrients and energy intake were estimated using Spanish food composition tables [21, 22]. Within the FFQ, 3 items focused on different types of olive oil consumption: (1) VOO intake, including extra virgin olive oil (acidity $\leq 0.8\%$) and virgin olive oil (acidity $\leq 2\%$); (2) refined

olive oil intake, which referred to oils obtained through refining process (acidity $\leq 0.3\%$); and (3) olive–pomace oil intake, comprising oils obtained from treating the byproduct after extracting olive oil and those directly obtained from olives acidity $\leq 0.3\%$) [10, 23]. For analysis, COO consumption was defined as the combination of “refined olive oil intake” and “olive–pomace oil intake”, while TOO consumption was calculated as the sum of all 3 items. VOO is minimally processed and retains a high content of polyphenols, tocopherols, and other bioactive compounds. In contrast, COO undergoes industrial processing that removes most of these minor components, resulting in a lower concentration of bioactive compounds but a similar fatty acid profile to VOO [10]. The collected information was converted into grams per day. The baseline consumption of TOO, VOO, and COO were adjusted for total energy intake using the residual method [24], and these adjusted values were considered as the exposures in this study. Subsequently, participants were grouped into tertiles based on their energy-adjusted consumption of TOO, VOO, or COO.

Moreover, dietitians administered a validated 14-item Mediterranean Diet Adherence Screener (MEDAS) [25] designed to assess adherence to the traditional MedDiet, to all participants. Two questions within the MEDAS are related to olive oil intake: the use of olive oil as the primary cooking oil and dressing fat (1 point for an affirmative response) and the daily consumption of four or more tablespoons of olive oil (1 point for an affirmative response). To control for the overall dietary quality, a modified 12-point MedDiet score derived from the MEDAS after removing the olive oil related items was used as a covariate in the statistical models.

Sociodemographic and lifestyle information were collected through interviewer-administered questionnaires conducted by trained personnel at baseline. The data encompassed information such as age, sex, education level (categorized as primary school or lower, high school, or college), civil status (categorized as single, divorced, separated, married, or widower), smoking status (categorized as current smoker, former smoker, or never smoked), physical activity (measured in METs-min/day). Physical activity was estimated using the REGICOR questionnaire, a validated Spanish short version of the Minnesota Leisure Time Physical Activity Questionnaire [26]. Personal medical history (e.g., prevalence of type 2 diabetes, hypertension, and hypercholesterolemia) and medication use were either self-reported or collected from the participants' medical records. Anthropometric measurements, including weight and height, were conducted by trained personnel using calibrated scales and wall-mounted stadiometers, respectively. Measurements were taken with participants wearing light clothing and

without shoes or accessories. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured using an anthropometric tape midway between the lowest rib and the iliac crest. Depressive symptomatology was evaluated by the Beck Depression Inventory (BDI-II), and a cut-off point of ≥ 14 was established to identify individuals at risk for depressive status [27, 28].

Cognitive function assessment

Cognitive function was evaluated by trained staff at baseline and at 2 years of follow-up using an extensive neuropsychological test battery, including the Mini-Mental State Examination (MMSE): examining orientation, memory, attention, and language; Clock Drawing Test (CDT): visuospatial, visuo-construction, verbal and numerical knowledge, memory, and executive function; Verbal Fluency Test (VFT)-Animals: verbal abilities, and VFT-Letter “p”: verbal abilities and executive function; Digit Span Test (DST)-Forward of the Wechsler Adult Intelligence Scale-III (WAIS-III): attention capacities and short-term memory, and DST-Backward: executive function and working memory; Trail Making Test (TMT) part A: attention and processing speed, and TMT part B: executive function. Detailed information about the neuropsychological test battery was described in the Supplementary Methods, Additional file 1.

For each cognitive test, a cohort-specific z-score was calculated for each participant at baseline and follow-up, based on the means and standard deviations (SDs) of the test scores at baseline. These standardized scores were then combined to form composite scores for neurocognitive domains, i.e., executive function, attention, and language [29]. Additionally, given that MMSE and CDT assess multiple cognitive domains, they were incorporated into the general cognitive function composite as a cognitive screening-summary score. Furthermore, the global cognitive function composite score was computed by adding or subtracting the z-scores of all eight tests, depending on whether a higher score indicates better or worse cognitive performance, respectively.

The specific formulas for composite scores were as follows:

- Executive function = $(z\text{VFT-a} + z\text{VFT-p} + (-z\text{TMT-B}) + z\text{DST-b})/4$
- Attention = $((-z\text{TMT-A}) + z\text{DST-f})/2$
- Language = $(z\text{VFT-a} + z\text{VFT-p})/2$
- General cognitive function = $(z\text{MMSE} + z\text{CDT})/2$.
- Global cognitive function = $(z\text{MMSE} + z\text{CDT} + z\text{VFT-a} + z\text{VFT-p} + (-z\text{TMT-A}) + (-z\text{TMT-B}) + z\text{DST-f} + z\text{DST-b})/8$

These composite scores, calculated at each visit, were further re-standardized to z-scores using the means and SDs of composite scores at baseline, where higher scores indicated better cognitive performance. In the present study, changes in standardized composite scores between baseline and 2 years of follow-up were considered as the main outcome.

Taxonomic profiling of gut microbiota

Details on stool sample collection, microbial DNA extraction, and 16S ribosomal RNA (rRNA) amplicon sequencing were described in the Supplementary Methods, Additional file 1. Briefly, stool samples were collected by participants at baseline and were stored at -80°C until extracting microbial DNA. DNA concentration and purity were assessed before 16S rRNA amplicon sequencing. The V4 region of 16S rRNA gene was amplified in triplicate PCR reactions and sequenced using the Illumina Novaseq platform. Quality control for the sequencing data included artificial mock communities with known composition as positive controls and negative control samples to account for potential contaminant sequences. Raw sequence data were processed using the NG-Tax pipeline, paired-end reads were demultiplexed and filtered to be summarized to amplicon sequence variants (ASVs). Taxonomic profiles of each ASV were generated using the USEARCH algorithm and the Silva database (v138.1).

Statistical analyses

Baseline characteristics of the study population were compared across energy-adjusted TOO consumption tertiles. The results are presented as numbers (percentages) for categorical variables, and means $\pm$ SD for continuous variables.

The regression analyses comprised 4 sets of statistical approaches: (1) prospective associations between olive oil consumption and changes in cognitive function over a 2-year follow-up period; (2) cross-sectional associations between olive oil consumption and gut microbiota diversity (alpha and beta diversity) and composition (differential taxa) at baseline; (3) prospective associations between olive oil-related microbial signatures and changes in cognitive function; and (4) causal mediation analyses of the pathway: olive oil consumption — gut microbiota diversity and composition — changes in cognitive function. Specifically,

- 1) Prospective association between olive oil consumption and cognitive change. Multivariable linear regression models were used to assess longitudinal associations between olive oil consumption at baseline and changes in cognitive function over a 2-year

follow-up period. Total and different types of olive oil consumption, i.e., TOO, VOO, and COO consumption, were evaluated as continuous (per 10 g/d increment) and categorized into tertiles, with the first tertile being the reference. Results from three models were presented: a basic model (adjusted for respective cognitive composite scores at baseline, age, and sex), a socio-demographically adjusted model (further adjusted for PREDIMED-Plus groups of randomization, geographical area, educational level, civil status) and a fully adjusted model (further adjusted for BMI, physical activity, smoking status, alcohol consumption and adding the quadratic term, depressive symptomatology, prevalence of diabetes, hypertension, and hypercholesterolemia, and MedDiet adherence). To assess the linear trend across tertiles of TOO, VOO, COO consumption, the median value of each consumption tertile was treated as a scored trend variable and modelled continuously.

- 2) Cross-sectional association between olive oil consumption and gut microbiota. Alpha and beta diversity were assessed to evaluate overall patterns in gut microbiome variation, while differential abundance analysis was conducted to identify differentially abundant taxa in the comparison of various types and amount of olive oil consumption.

Chao1, Simpson, Shannon, and Inverse Simpson indices [30–32] were calculated from ASV-level absolute abundance counts to measure alpha-diversity, specifically species richness, evenness, and diversity (a combination of richness and evenness) within individual samples. Differences in alpha diversity indices across tertiles of olive oil consumption were evaluated using the Kruskal-Wallis non-parametric test, with post-hoc pairwise comparisons conducted using the Wilcoxon rank sum test. Beta diversity, measured with Aitchison distance metric (Euclidean distance derived from centered log-ratio (clr) transformed abundance counts) [33], was used to quantify the dissimilarity between samples. Permutational multivariate analysis of variance (PERMANOVA) [34] with 999 permutations was performed to test for significant beta diversity clustering across tertiles of olive oil consumption, using the *adonis2* function with the argument “by” set to “margin” in the R package *vegan* (version 2.6-4). Additionally, principal component analysis (PCA) was employed to visually inspect the unsupervised ordination of the two first principal components (PC1 and PC2) of Aitchison distances. These principal components were selected for use as variables representing beta diversity of microbial community composition in the subsequent analysis. For per-feature analysis, a

taxonomic filtering process was initially conducted. Microbial features at the genus level detected at a minimum relative abundance of 0.1% in at least 10% of samples were included. Subsequently, the relative abundance of these features was clr-transformed before conducting downstream analyses. Differential abundance analysis was performed using the R package MaAsLin2 (version 1.16.0) [35] with multivariable linear regression models, adjusting for age, sex, geographical area, educational level, civil status, BMI, physical activity, smoking status, alcohol consumption and adding its quadratic term, depressive symptomatology, prevalence of diabetes, hypertension, and hypercholesterolemia, and MedDiet adherence. All high-dimensional tests were corrected for multiple comparison by controlling the false discovery rate using the Benjamini–Hochberg method with a target rate of 0.25 for *q* values. Additionally, linear regression models with the same adjustment were fitted to test potential dose-response relationships of alpha diversity (Chao1, Simpson, Shannon, and Inverse Simpson indices) and beta diversity (PC1 and PC2) with olive oil consumption at baseline.

- 3) Prospective association of the identified gut microbial signatures with cognitive changes. Linear regression models were performed to assess the associations between identified olive oil consumption-related microbial signatures and changes in cognitive function after a 2-year follow-up. These models were adjusted for respective cognitive composite scores at baseline, and the aforementioned covariates. All gut microbial signatures, i.e., alpha diversity indices, beta diversity (PC1 and PC2), and clr-transformed relative abundance of identified olive oil-related taxa, were converted to z-scores before analysis when used as predictors in the models.
- 4) Mediation analysis. To assess the mediatory effect of the gut microbiota, two methods were employed. First, causal mediation analysis functions from the R package mediation (version 4.5.0) [36] were performed using the quasi-Bayesian Monte Carlo simulation approaches with 1000 iterations. In this analysis, olive oil consumption served as the exposure variable, alpha diversity indices, PC1, and PC2 accounted for the mediation by overall microbial composition, and cognitive changes were the outcome variable. Second, acknowledging the compositionality and sparsity inherent of the microbiome abundance data, nonparametric bootstrap approaches with 1000 iterations were applied, using the same exposure/outcome variables [37]. This approach aimed to address the identification of olive oil-related taxa as potential mediators. All continu-

ous mediators were converted to z scores before analysis, and genus-level relative abundances were clr-transformed before z-conversion. In addition, the potential interaction between exposure and mediator to the outcome was tested using the test.TMint function from the R package mediation (version 4.5.0) [36, 38, 39]. Supplementary Fig. S4 illustrates the path diagram of the causal mediation analysis models assessed. The average causal mediation effect (ACME) denoted the indirect associations of olive oil consumption with cognitive changes through the mediators. The average direct effect (ADE) represented the associations of the baseline olive oil consumption with cognitive changes without the involvement of a mediator. The proportion of mediation was calculated via dividing the ACME by the total effect (TE), which encompasses all direct and indirect effects of the exposure on the outcome.

To test the robustness of the results of the first three sets of statistical approaches, several sensitivity analyses were conducted: 1) we removed participants with baseline MMSE score < 24, and ran analyses within the remaining sample (*N*=649); 2) to account for potential multiple testing in the analysis of associations between identified gut microbial signatures and cognitive change, we controlled the false discovery rate using the Benjamini–Hochberg method with a target rate of 0.25 for *q* values; and 3) we further adjusted in all models with an additional covariate representing the consumption of other types of oil, as well as conducting analyses restricted to participants who were exclusive VOO consumers (*N*=502) by excluding COO users.

All analyses were two-sided and performed in R version 4.3.1. A *p*-value < 0.05 was deemed statistically significant.

Results

Baseline characteristics of the study population

A total of 656 participants (mean age 65.0 ± 4.9 y, 47.9% women) were included. Table 1 outlines baseline characteristics of the study population across tertiles of energy-adjusted TOO consumption. Mean raw consumption of TOO was approximately 30 ± 11 , 50 ± 1 , and 58 ± 10 g/day in lowest, medium, and highest tertiles, respectively. Participants in the medium tertile presented with lower prevalence of type 2 diabetes, hypercholesterolemia and depression, and better cognitive performance at baseline. No substantial differences in the consumption of other dietary components were observed across tertiles of TOO intake. Additionally, participants in the highest tertile of COO consumption were more likely to have lower

Table 1 Baseline characteristics of the study population according to tertiles of energy-adjusted total olive oil consumption^a

	Overall (n = 656)	T1 (n = 219)	T2 (n = 219)	T3 (n = 218)
Total olive oil, g/d	46 (15)	30 (11)	50 (1)	58 (10)
Virgin olive oil, g/d	40 (21)	21 (16)	46 (12)	53 (17)
Common olive oil, g/d	6 (13)	9 (12)	4 (12)	5 (14)
Women, n (%)	314 (48)	105 (48)	87 (40)	122 (56)
Age, y	65.0 (4.9)	65.0 (4.6)	64.9 (5.2)	65.2 (4.9)
Education level, n (%)				
Primary school or less	355 (54)	133 (61)	109 (50)	113 (52)
High school	183 (28)	52 (24)	71 (32)	60 (28)
College	118 (18)	34 (16)	39 (18)	45 (21)
Civil status, n (%)				
Single, divorced or separated	87 (13)	25 (11)	28 (13)	34 (16)
Married	504 (77)	163 (74)	178 (81)	163 (75)
Widower	65 (10)	31 (14)	13 (6)	21 (10)
Geographically area, n (%)				
Catalonia	403 (61)	97 (44)	170 (78)	136 (62)
Valencia	253 (39)	122 (56)	49 (22)	82 (38)
Intervention group, n (%)	312 (48)	108 (49)	107 (49)	97 (45)
BMI, kg/m ²	32.7 (3.5)	32.8 (3.4)	32.5 (3.5)	33.0 (3.6)
Waist circumference, cm	108 (10)	107 (10)	107 (10)	108 (10)
Smoking status, n (%)				
Never smoker	312 (48)	103 (47)	95 (43)	114 (52)
Former smoker	255 (39)	78 (36)	100 (46)	77 (35)
Smoker	89 (14)	38 (17)	24 (11)	27 (12)
Physical activity, METs-min/day	372 (350)	367 (326)	370 (361)	378 (364)
Modified MedDiet score, 12-point MEDAS	6.9 (1.6)	6.7 (1.7)	6.9 (1.7)	7.0 (1.6)
Type 2 diabetes, n (%)	174 (27)	80 (37)	35 (16)	59 (27)
Hypertension, n (%)	532 (81)	179 (82)	182 (83)	171 (78)
Hypercholesterolemia, n (%)	434 (66)	158 (72)	134 (61)	142 (65)
Depressive symptomatology, n (%)	127 (19)	50 (23)	27 (12)	50 (23)
Global cognitive function, z-score	0.0 (1.0)	-0.1 (1.1)	0.1 (1.0)	-0.0 (1.0)
General cognitive function, z-score	0.0 (1.0)	-0.1 (1.1)	0.1 (0.9)	0.0 (1.0)
Executive function, z-score	0.0 (1.0)	-0.1 (1.0)	0.1 (1.0)	-0.1 (1.0)
Attention, z-score	0.0 (1.0)	-0.1 (1.1)	0.1 (1.0)	-0.0 (0.9)
Language, z-score	0.0 (1.0)	-0.1 (1.0)	0.1 (1.0)	-0.1 (1.0)
Energy intake, kcal/d	2,474 (502)	2,502 (607)	2,640 (236)	2,280 (519)
Fatty acids intake, % of total energy				
Monounsaturated	22 (4)	18 (3)	22 (3)	25 (3)
Polyunsaturated	7 (2)	7 (2)	7 (1)	7 (1)
Saturated	10 (2)	10 (2)	10 (2)	10 (2)
Fibre, g/d	27 (8)	28 (8)	27 (7)	26 (9)
Alcohol, g/d	11 (14)	12 (16)	13 (14)	7 (10)
Vegetables, g/d	349 (140)	378 (142)	337 (140)	330 (134)
Fruits, g/d	368 (203)	373 (204)	351 (169)	380 (231)
Legumes, g/d	20 (10)	19 (10)	20 (10)	20 (12)
Cereals, g/d	160 (69)	169 (78)	174 (58)	137 (65)
Dairy products, g/d	307 (177)	332 (176)	327 (188)	263 (158)
Meat, g/d	161 (55)	174 (59)	168 (50)	139 (50)
Fish, g/d	107 (46)	109 (51)	109 (41)	103 (47)
Nuts, g/d	17 (16)	19 (18)	16 (14)	15 (16)
Confectionery products, g/d	25 (27)	31 (32)	27 (25)	17 (19)

Data are presented as n (%) and mean (SD) for categorical and continuous variables, respectively

Abbreviations: BMI body mass index, MedDiet Mediterranean diet, MEDAS Mediterranean Diet Adherence Screener, METs metabolic equivalents, SD standard deviation, T tertile

^a Adjustment for total energy intake with the residual method. Dietary variables presented in accordance with tertiles of energy-adjusted total olive oil consumption were raw values before energy adjustment

educational levels and a higher prevalence of smoking (Supplementary Table S1, Additional file 1).

Associations between olive oil consumption and changes in cognitive function

Results from the multivariable regression models revealed that (Table 2), compared to participants in the lowest tertile of TOO, those in the higher tertiles had increases in global cognitive function, general cognitive function, and attention after a 2-year follow-up, with a significant dose–response relationship (all p -trend < 0.05). Additionally, a 10 g increment consumption of TOO per day was positively associated with changes in global cognitive function (β 0.044 z-score; 95% CI 0.013, 0.075; p = 0.006), general cognitive function (0.051; 0.009, 0.093; p = 0.018), executive function (0.034; 0.000, 0.067; p = 0.047), and attention (0.046; 0.008, 0.084; p = 0.017). Similar results were observed for VOO, where a 10 g increment in VOO consumption per day was positively associated with changes in global cognitive function, general cognitive function, executive function, and language. Positive dose–response associations were also observed in global, general cognitive function, executive function, and attention changes when comparing tertiles (all p -trend < 0.05). Conversely, in terms of COO, a 10 g increment in COO consumption per day was associated with a decrease in executive function and a more pronounced decline in global and general cognitive function, executive function, and language (all p -trend < 0.05). These findings remained consistent after removal of participants with baseline MMSE score < 24, further adjusting the model for consumption of other type of oils, or restricting the analysis to exclusive VOO consumers (data not shown).

Associations between olive oil consumption and gut microbiota

Significant differences, assessed by Kruskal–Wallis non-parametric test, in alpha-diversity indices were observed across tertiles of VOO and COO consumption (Fig. 2a). Multivariable linear regression models showed that higher consumption of VOO was positively associated with higher scores of Chao1 (p -trend = 0.016) and Inverse Simpson indices (p -trend = 0.041) (Supplementary Table S2, Additional file 1). Conversely, higher consumption of COO was significantly associated with lower alpha diversity (p -trend = 0.019, 0.043, 0.008, < 0.001 for Chao1, Simpson, Shannon, and Inverse Simpson indices, respectively), even after fully adjusting for confounding variables. No statistically significant difference was found in alpha diversity across TOO consumption tertiles (Fig. 2a).

PERMANOVA tests demonstrated significant differences in fully adjusted models across tertiles of TOO

(p = 0.008), VOO (p = 0.011), and COO (p = 0.001), indicating that participants in different tertiles presented overall compositional differences in their gut microbiota, independent of other microbiota-influencing covariates (Fig. 2b and Supplementary Table S3, Additional file 1). PCA plots revealed modest clustering across tertiles of TOO, VOO, COO consumption, where PC1 and PC2 accounted for approximately 70.3% and 4.7% to the total variation, respectively (Fig. 2b). Although olive oil consumption was not a major driver of overall gut microbial community composition variation, explaining 0.3% by TOO, 0.4% by VOO and 0.3% by COO, VOO accounted for the second largest proportion of variation in taxonomy among the olive oil types, covariables and baseline cognitive performance considered in this analysis (Supplementary Fig. 2, Additional file 1).

A total of 19 genera were identified to be related to the olive oil consumption (q < 0.25) in the differential abundance analysis (Fig. 3a and Supplementary Table S4, Additional file 1). Among these, 3 taxa (*Bacteroides*, *Phascolarctobacterium*, *Acidaminococcus*) showed positive and 6 taxa (*Eubacterium_hallii_group*, *Clostridium_sensu_stricto_1*, *Collinsella*, *Dorea*, *Senegalimassilia*, *Adlercreutzia*) showed negative associations with TOO consumption. For VOO, 4 taxa (*Bacteroides*, *Phascolarctobacterium*, *Acidaminococcus*, CAG.56) showed positive and 12 taxa (*Blautia*, *Akkermansia*, *Streptococcus*, *Eubacterium_hallii_group*, *uncultured_5*, *Clostridium_sensu_stricto_1*, *Romboutsia*, *Dorea*, *Collinsella*, *uncultured_8*, *Mogibacterium*, *Adlercreutzia*) showed negative associations. In the case of COO, 4 taxa (*Streptococcus*, *Christensenellaceae_R.7_group*, *Eubacterium_hallii_group*, *Adlercreutzia*) were positively and 1 taxon (*Faecalibacterium*) was negatively associated. *Adlercreutzia*, *Eubacterium_hallii_group*, and *Streptococcus* were found to be commonly associated with all three exposures, showing negative associations with TOO and/or VOO while being positively associated with COO consumption.

Identified gut microbial signatures and changes in cognitive function

After adjusting for potential confounders, no significant association between alpha-diversity indices and changes in cognitive function were observed (Supplementary Fig. 3, Additional file 1). However, PC1 and PC2, which were derived from the Aitchison dissimilarity metric and represent beta-diversity, summarizing overall microbial community composition, were significantly associated with changes in executive function and language, respectively (p < 0.05) (Supplementary Fig. 3, Additional file 1). Furthermore, 6 identified gut microbial features at the genus level, which were related to olive oil consumption,

Table 2 Associations between baseline energy-adjusted olive oil consumption and changes in cognitive performance after 2-y follow-up^a

	Continuous (per 10 g/d increment)		T1 (low)	T2 (medium)	T3 (high)	
Total olive oil^b	β (95% CI)	p	29 [25, 34] g/d Reference	49 [48, 50] g/d β (95% CI)	55 [53, 66] g/d β (95% CI)	p-trend^c
Global cognitive function						
Basic model	0.055 [0.024, 0.086]	< 0.001	reference	0.242 [0.135, 0.348]	0.181 [0.075, 0.287]	< 0.001
Socio-demographically adjusted model	0.043 [0.012, 0.073]	0.006	reference	0.180 [0.072, 0.289]	0.132 [0.026, 0.237]	0.003
Fully adjusted model	0.044 [0.013, 0.075]	0.006	reference	0.182 [0.070, 0.294]	0.138 [0.030, 0.246]	0.003
General cognitive function						
Basic model	0.065 [0.024, 0.107]	0.002	reference	0.243 [0.100, 0.386]	0.238 [0.095, 0.380]	< 0.001
Socio-demographically adjusted model	0.055 [0.013, 0.096]	0.010	reference	0.210 [0.063, 0.358]	0.205 [0.061, 0.349]	0.002
Fully adjusted model	0.051 [0.009, 0.093]	0.018	reference	0.187 [0.035, 0.339]	0.194 [0.047, 0.341]	0.005
Executive function						
Basic model	0.045 [0.012, 0.079]	0.007	reference	0.194 [0.080, 0.308]	0.137 [0.023, 0.251]	0.003
Socio-demographically adjusted model	0.032 [-0.001, 0.064]	0.057	reference	0.131 [0.014, 0.247]	0.082 [-0.031, 0.195]	0.071
Fully adjusted model	0.034 [0.000, 0.067]	0.047	reference	0.134 [0.014, 0.254]	0.089 [-0.027, 0.205]	0.063
Attention						
Basic model	0.057 [0.020, 0.095]	0.003	reference	0.289 [0.161, 0.418]	0.166 [0.037, 0.294]	< 0.001
Socio-demographically adjusted model	0.043 [0.006, 0.080]	0.023	reference	0.208 [0.077, 0.339]	0.111 [-0.016, 0.239]	0.021
Fully adjusted model	0.046 [0.008, 0.084]	0.017	reference	0.220 [0.083, 0.356]	0.124 [-0.008, 0.255]	0.016
Language						
Basic model	0.043 [0.004, 0.081]	0.029	reference	0.146 [0.014, 0.279]	0.115 [-0.017, 0.248]	0.038
Socio-demographically adjusted model	0.031 [-0.007, 0.070]	0.110	reference	0.124 [-0.013, 0.261]	0.076 [-0.057, 0.210]	0.149
Fully adjusted model	0.031 [-0.008, 0.070]	0.115	reference	0.116 [-0.025, 0.257]	0.083 [-0.053, 0.219]	0.150
Virgin olive oil^b	β (95% CI)	p	15 [4, 26] g/d Reference	48 [46, 49] g/d β (95% CI)	54 [53, 65] g/d β (95% CI)	p-trend^c
Global cognitive function						
Basic model	0.046 [0.024, 0.067]	< 0.001	reference	0.240 [0.132, 0.348]	0.204 [0.098, 0.311]	< 0.001
Socio-demographically adjusted model	0.035 [0.014, 0.056]	0.001	reference	0.177 [0.068, 0.286]	0.167 [0.062, 0.272]	< 0.001
Fully adjusted model	0.036 [0.014, 0.057]	0.001	reference	0.180 [0.069, 0.292]	0.174 [0.068, 0.280]	< 0.001
General cognitive function						
Basic model	0.051 [0.022, 0.080]	< 0.001	reference	0.226 [0.081, 0.370]	0.214 [0.072, 0.357]	< 0.001
Socio-demographically adjusted model	0.043 [0.014, 0.072]	0.004	reference	0.184 [0.035, 0.334]	0.189 [0.045, 0.332]	0.004
Fully adjusted model	0.041 [0.011, 0.071]	0.007	reference	0.179 [0.027, 0.330]	0.189 [0.044, 0.333]	0.005
Executive function						
Basic model	0.047 [0.024, 0.070]	< 0.001	reference	0.217 [0.102, 0.332]	0.193 [0.079, 0.306]	< 0.001
Socio-demographically adjusted model	0.035 [0.013, 0.058]	0.002	reference	0.152 [0.034, 0.269]	0.149 [0.036, 0.261]	0.004
Fully adjusted model	0.036 [0.013, 0.060]	0.002	reference	0.152 [0.033, 0.271]	0.156 [0.043, 0.269]	0.003
Attention						
Basic model	0.036 [0.010, 0.062]	0.008	reference	0.266 [0.136, 0.396]	0.172 [0.043, 0.301]	< 0.001
Socio-demographically adjusted model	0.023 [-0.003, 0.049]	0.083	reference	0.187 [0.055, 0.319]	0.130 [0.003, 0.258]	0.011
Fully adjusted model	0.025 [-0.001, 0.052]	0.062	reference	0.200 [0.065, 0.336]	0.139 [0.010, 0.269]	0.007

Table 2 (continued)

	Continuous (per 10 g/d increment)		T1 (low)	T2 (medium)	T3 (high)	
Language						
Basic model	0.040 [0.014, 0.067]	0.003	reference	0.173 [0.039, 0.307]	0.143 [0.011, 0.276]	0.009
Socio-demographically adjusted model	0.031 [0.004, 0.058]	0.026	reference	0.133 [-0.005, 0.271]	0.107 [-0.026, 0.239]	0.057
Fully adjusted model	0.030 [0.003, 0.057]	0.031	reference	0.117 [-0.023, 0.258]	0.110 [-0.024, 0.244]	0.066
Common olive oil^b						
	β (95% CI)	p	-0.08 [-0.1, -0.04] g/d Reference	0.05 [0.01, 0.08] g/d β (95% CI)	24.8 [0.2, 25.0] g/d β (95% CI)	p-trend^c
Global cognitive function						
Basic model	-0.047 [-0.081, -0.012]	0.008	reference	0.000 [-0.108, 0.109]	-0.220 [-0.329, -0.110]	< 0.001
Socio-demographically adjusted model	-0.034 [-0.067, 0.000]	0.048	reference	0.001 [-0.105, 0.107]	-0.172 [-0.279, -0.064]	< 0.001
Fully adjusted model	-0.033 [-0.067, 0.001]	0.055	reference	0.009 [-0.100, 0.118]	-0.166 [-0.276, -0.056]	< 0.001
General cognitive function						
Basic model	-0.048 [-0.094, -0.002]	0.040	reference	-0.042 [-0.187, 0.103]	-0.242 [-0.389, -0.095]	< 0.001
Socio-demographically adjusted model	-0.038 [-0.084, 0.008]	0.102	reference	-0.027 [-0.171, 0.118]	-0.202 [-0.349, -0.055]	0.003
Fully adjusted model	-0.037 [-0.083, 0.009]	0.114	reference	-0.031 [-0.180, 0.117]	-0.208 [-0.358, -0.058]	0.003
Executive function						
Basic model	-0.063 [-0.099, -0.026]	< 0.001	reference	0.009 [-0.107, 0.125]	-0.219 [-0.336, -0.103]	< 0.001
Socio-demographically adjusted model	-0.049 [-0.084, -0.013]	0.008	reference	0.013 [-0.101, 0.126]	-0.169 [-0.284, -0.054]	< 0.001
Fully adjusted model	-0.047 [-0.083, -0.011]	0.010	reference	0.026 [-0.090, 0.143]	-0.153 [-0.270, -0.035]	0.001
Attention						
Basic model	-0.020 [-0.061, 0.022]	0.349	reference	0.002 [-0.130, 0.135]	-0.137 [-0.271, -0.003]	0.018
Socio-demographically adjusted model	-0.004 [-0.045, 0.036]	0.831	reference	0.005 [-0.124, 0.135]	-0.074 [-0.206, 0.057]	0.178
Fully adjusted model	-0.006 [-0.047, 0.035]	0.771	reference	0.014 [-0.120, 0.148]	-0.073 [-0.209, 0.062]	0.170
Language						
Basic model	-0.049 [-0.091, -0.007]	0.023	reference	-0.019 [-0.155, 0.116]	-0.170 [-0.306, -0.034]	0.007
Socio-demographically adjusted model	-0.037 [-0.079, 0.005]	0.082	reference	-0.003 [-0.138, 0.131]	-0.135 [-0.271, 0.001]	0.024
Fully adjusted model	-0.035 [-0.077, 0.007]	0.107	reference	0.015 [-0.123, 0.153]	-0.112 [-0.251, 0.027]	0.047

Basic model adjusted for respective cognitive composite scores at baseline, age (years), and sex

Socio-demographically adjusted model further adjusted for PREDIMED-Plus groups of randomization, geographical area (Catalonia or Valencia), educational level (primary or lower, high school, or college), civil status (single, divorced, separated, married, or widower)

Fully adjusted model further adjusted for BMI (kg/m²), physical activity (METs-min/day), smoking status (never, former, or current smoker), alcohol consumption in g/day and adding the quadratic term, depressive symptomatology (yes or no), prevalence of diabetes (yes or no), hypertension (yes or no), and hypercholesterolemia (yes or no), and Mediterranean diet adherence (modified 12-point MEDAS score)

Abbreviations: BMI, body mass index; CI, confidence interval; IQR, interquartile range; MEDAS, Mediterranean diet adherence screener; METs, metabolic equivalents; PREDIMED-Plus, PREvención con Dieta MEDiterránea-Plus

^a Values are β-coefficients [95% CI] estimated from linear regression models. In the continuous model, β-coefficients represent changes in cognitive composite z-scores associated with 10 g increment in olive oil consumption per day. In the categorical model, β-coefficients represent changes in cognitive composite z-scores in each tertile of olive oil consumption versus reference tertile (T1). *p* < 0.05 was deemed statistically significant

^b Energy-adjusted olive oil consumption per each tertile was presented as median [IQR]. Total, virgin, and common olive oil consumption were adjusted for total energy intake using the residual method. Negative values in the lowest tertile of common olive oil consumption indicate an observed intake below the predicted mean for the given total energy intake

^c Linear trend was calculated by assigning the median values of each tertile of olive oil and treating these values across tertiles as a continuous variable in the linear regression models

showed significant associations with changes in cognitive function (*p* < 0.05) (Supplementary Fig. 3, Additional file 1). Two taxa remained relevant even after controlling for the false discovery rate (*q* < 0.25) (Fig. 3b and

Supplementary Table S5, Additional file 1). Specifically, higher consumption of TOO and VOO were associated with decreased abundance of *Adlercreutzia*, while this taxon was negatively associated with changes in general

cognitive function. *Akkermansia*, inversely associated with VOO consumption, showed a negative association with changes in attention (Fig. 3).

Gut microbiota mediates the associations between olive oil consumption and changes in cognitive function

The mediation analysis suggested that gut microbiota partially mediated the association between olive oil consumption and changes in cognitive function (Table 3). Specifically, *Adlercreutzia* emerged as a significant mediator taxon between VOO consumption and changes in general cognitive function (ACME: β 0.008; 95% CI 0.002, 0.017; $p=0.016$), contributing 20% of the total effect. In the analysis of the overall gut microbial community composition as a mediator, the total effect of TOO on executive function change was β 0.033 (95% CI 0.001, 0.064; $p=0.050$), and the indirect effect through PC1 was β -0.003 (95% CI -0.008, -0.000; $p=0.044$). For the VOO, the indirect effect through PC1 on changes in executive function and language was β -0.003 (95% CI -0.007, -0.000; $p=0.024$) and β -0.003 (95% CI -0.008, -0.000; $p=0.046$). There was no interaction between the exposures and the assessed mediators (data not shown).

Discussion

The present study marks a significant advancement in the understanding of the interplay between the consumption of total and different olive oil types, gut microbiota diversity and composition, and changes in cognitive function in older adults at high risk of cognitive decline. We observed a positive association between TOO and/or VOO consumption and favorable cognitive changes across multiple cognitive domains over a 2-year follow-up, contrasting with the greater cognitive decline associated with higher COO consumption. Our findings also underscore the potential role of olive oil consumption

and its different types in shaping gut microbiota diversity and composition. Mediation analysis suggests that gut microbiota and particularly *Adlercreutzia*, may serve as a mediator taxon in the association between VOO consumption and changes in general cognitive function.

Previous clinical trials have reported similar findings regarding the positive association between VOO consumption and cognitive function [5, 9]. Sub-studies within the PREDIMED randomized trial consistently demonstrated that adherence to MedDiet supplemented with extra virgin olive oil (EVOO) resulted in improved cognitive performance and a lower incidence of mild cognitive impairment compared to a low-fat diet [40–42]. Similarly, small RCTs conducted in diverse Mediterranean regions [43, 44], have suggested that EVOO may confer added protection to the MedDiet against age-related cognitive decline. Although observational studies have yielded inconsistent results [5], the overall evidence suggests that TOO consumption, especially VOO, has a positive relationship on cognitive function. Differential effects of distinct olive oil types on cognitive function have recently been reported in individuals with mild cognitive impairment [45]. In that RCT, EVOO consumption significantly reduced blood–brain barrier permeability and enhanced brain functional connectivity compared to refined olive oil [45]. In line with their findings, in our study, higher consumption of COO was associated with accelerated cognitive decline, providing additional insights into the differential associations of olive oil types on cognitive health.

Beyond the positive association with changes in cognitive function, our study also demonstrated that higher consumption of TOO and/or VOO was linked to the presence of a beneficial gut microbiota composition, characterized by greater alpha diversity, consistent with previous preclinical and clinical studies [6, 46]. This was accompanied by higher abundance of *Acidaminococcus*,

(See figure on next page.)

Fig. 2 Associations of different amount and types of olive oil consumption with overall gut microbiota composition. **a**, Violin plots with included boxplots representing the differences in alpha diversity indices, i.e., Chao1, Simpson, Shannon, and Inverse Simpson, across tertiles of baseline energy-adjusted TOO, VOO, and COO consumption. Differences across tertiles were examined through Kruskal–Wallis non-parametric test. Post-hoc pairwise comparison was performed with Wilcoxon rank sum test. **b**, Ordinations of beta diversity principal components 1 and 2 for tertiles of baseline energy-adjusted TOO, VOO, and COO consumption. The ordination plots are based on the first 2 principal components derived from Aitchison distances. The ellipsoids depict the spread of 95% of the respective groups according to Student t-distribution. The amount of variance explained by each respective principal component is marked on their axis labels. Boxplots alongside the x and y axes represents PC1 and PC2 per tertile, respectively. Results of PERMANOVA tests across tertiles of baseline energy-adjusted TOO, VOO, and COO consumption are provided. The model was adjusted for age (years), sex, geographical area (Catalonia or Valencia), educational level (primary or lower, high school, or college), civil status (single, divorced, separated, married, or widower), BMI (kg/m^2), physical activity ($\text{METs}\cdot\text{min}/\text{day}$), smoking status (never, former, or current smoker), alcohol consumption in g/day and adding the quadratic term, depressive symptomatology (yes or no), prevalence of diabetes (yes or no), hypertension (yes or no), and hypercholesterolemia (yes or no), and Mediterranean diet adherence (modified 12-point MEDAS score). $p < 0.05$ (*) was deemed statistically significant. Abbreviations: BMI, body mass index; COO, common olive oil; MEDAS, Mediterranean diet adherence screener; METs, metabolic equivalents; PC, principal component; PERMANOVA, permutational multivariate analysis of variance; TOO, total olive oil; VOO, virgin olive oil

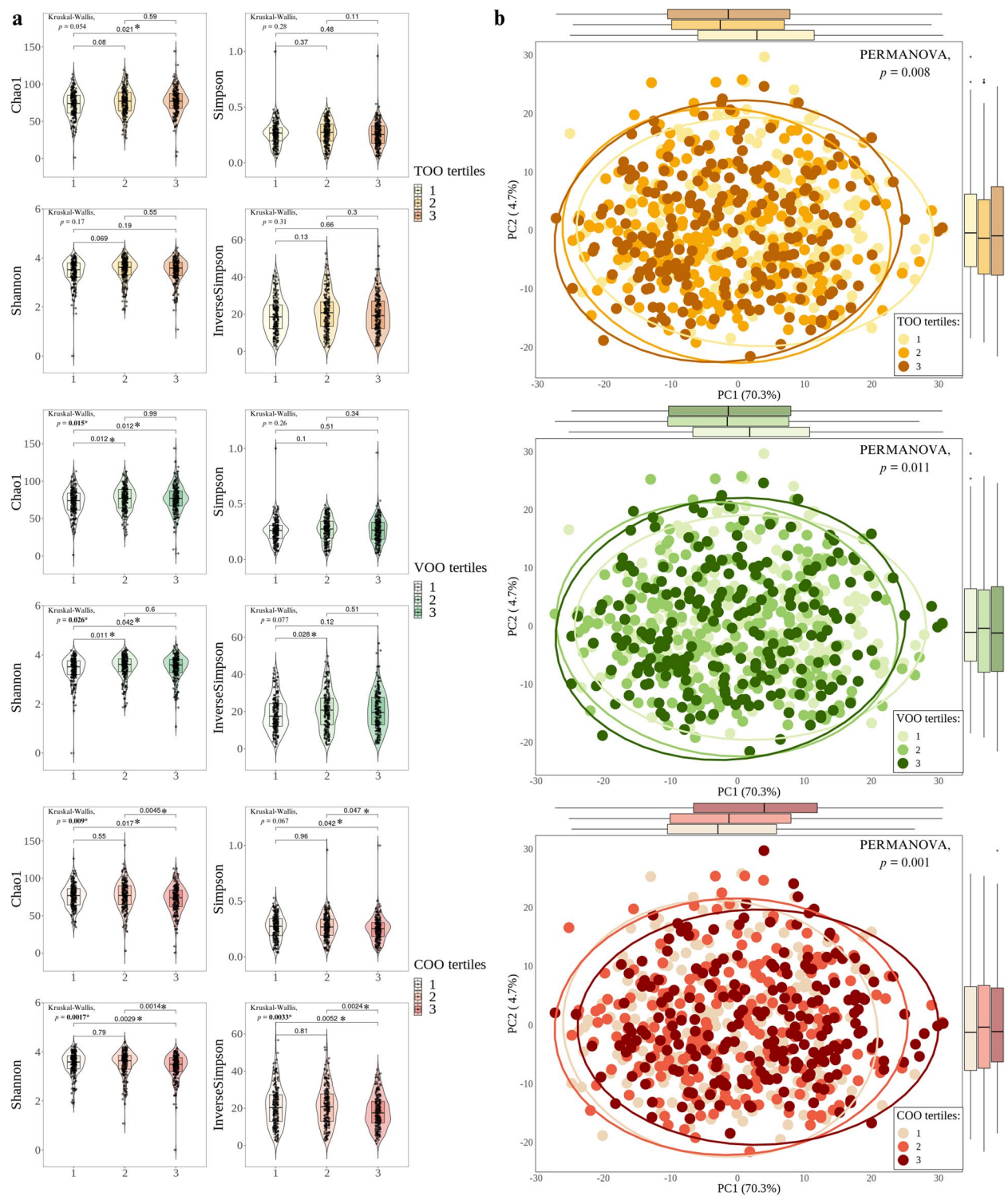


Fig. 2 (See legend on previous page.)

Bacteroides, and *Phascolarctobacterium* (“TOO/VOO-enriched” taxa), and lower abundance of taxa mainly assigned to *Adlercreutzia*, *Akkermansia*, *Blautia*, *Clostridium sensu stricto 1*, *Collinsella*, *Dorea*, *Eubacterium hallii*

group, *Mogibacterium*, *Romboutsia*, *Senegalimassilia*, *Streptococcus* (“TOO/VOO-depleted” taxa). While the microbiota effects varied by study, most of these “TOO/VOO-depleted” taxa have been previously associated with

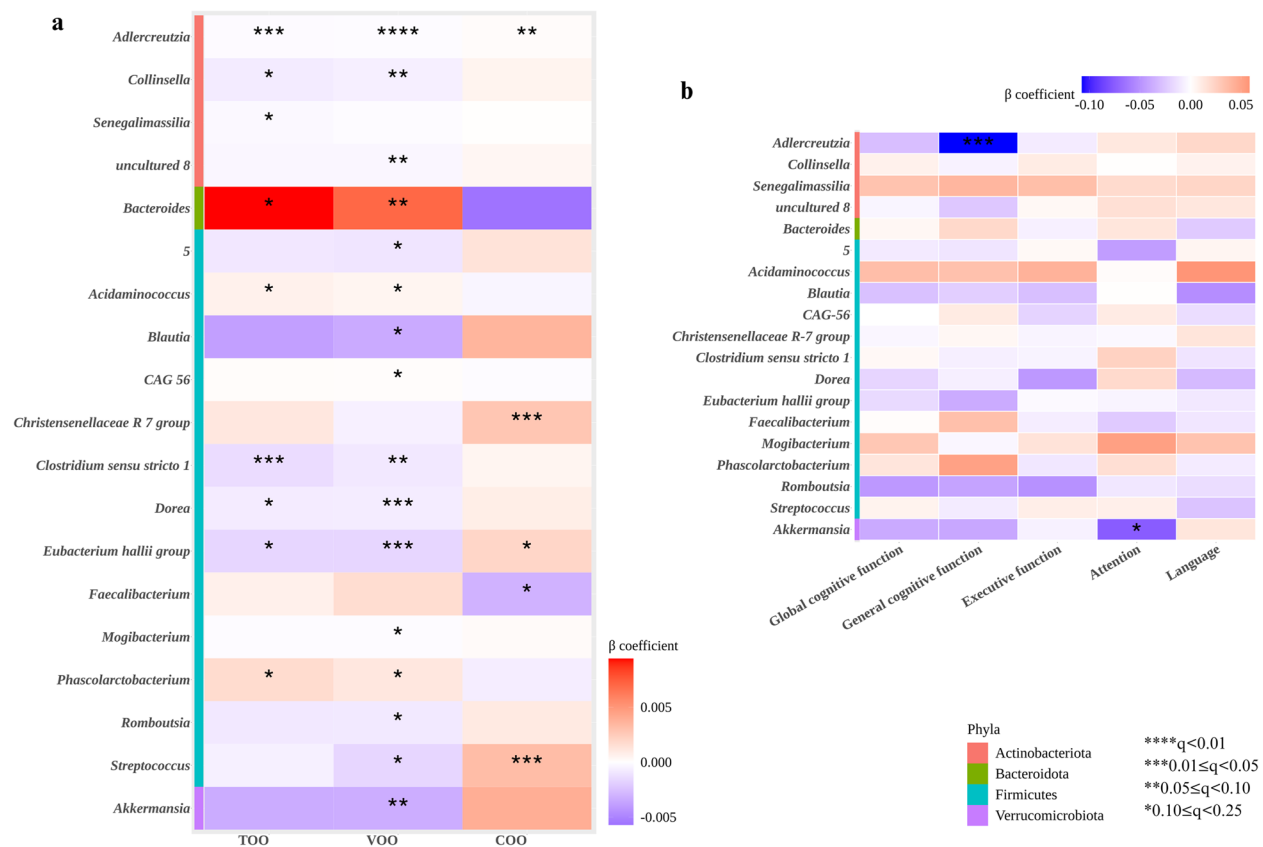


Fig. 3 Interplay between olive oil consumption, genus-level gut microbial features, and 2-y changes in cognitive function. **a**, this heatmap shows the significant associations of olive oil consumption variables (TOO, VOO, COO) with genus-level features abundances ($q < 0.25$). The blue-to-red gradient represents the magnitude and direction of the associations (β -coefficients) estimated by multivariable linear regression models in MaAsLin2 (see the “Methods” section). Microbial features at genus-level with a minimum relative abundance of 0.1% in at least 10% of all samples were included and the relative abundance of these features was clr-transformed before conducting the MaAsLin2 models. Models were adjusted for age (years), sex, geographical area (Catalonia or Valencia), educational level (primary or lower, high school, or college), civil status (single, divorced, separated, married, or widower), BMI (kg/m^2), physical activity ($\text{METs}\cdot\text{min}/\text{day}$), smoking status (never, former, or current smoker), alcohol consumption in g/day and adding the quadratic term, depressive symptomatology (yes or no), prevalence of diabetes (yes or no), hypertension (yes or no), and hypercholesterolemia (yes or no), and Mediterranean diet adherence (modified 12-point MEDAS score). Asterisks denote statistical significance based on the multiple comparison adjustment using Benjamini–Hochberg method with q values at a target false discovery rate of 0.25: * $q < 0.25$, ** $q < 0.10$, *** $q < 0.05$, **** $q < 0.01$. All microbial features with $q < 0.25$ are in Additional file 1: Supplementary Table 4. **b**, this heatmap illustrates the prospective associations between olive oil consumption-related microbial signatures identified in panel **a** and changes in cognitive function after a 2-year follow-up. Genus-level relative abundances of these microbial signatures were clr-transformed and then converted to z scores before analysis. The blue-to-red gradient represents the magnitude and direction of the associations (β -coefficients) estimated by multivariable linear regression models, which were adjusted for respective cognitive composite scores at baseline, PREDIMED-Plus groups of randomization, and aforementioned covariates in panel **a**. Asterisks denote statistical significance levels after controlling the false discovery rate: * $q < 0.25$, ** $q < 0.10$, *** $q < 0.05$. All associations of these individual microbial signatures with 2-year changes in cognitive function are in Additional file 1: Supplementary Table 5. Abbreviations: BMI, body mass index; clr, centered log-ratio; COO, common olive oil; MaAsLin2, Microbiome Multivariable Association with Linear Models; MEDAS, Mediterranean diet adherence screener; METs, metabolic equivalents; PREDIMED-Plus, PREvención con Dieta MEDiterránea-Plus; TOO, total olive oil; VOO, virgin olive oil

diseases like obesity, type 2 diabetes, cardiometabolic disease, metabolic liver disease, and NDs [12, 47]. Conversely, higher COO consumption was associated with lower alpha diversity, and higher abundance of *Adlercreutzia*, *Christensenellaceae R.7 group*, *Eubacterium hallii group* and *Streptococcus*, alongside reduced levels of *Faecalibacterium*, a predominant butyrate-producing

bacterium whose depletion was observed in several diseases, including depression [48]. Remarkably, our study is the first in assessing and demonstrating the potential impacts of different olive oil types on gut microbiota composition in humans, filling a gap in current research. Our findings lend support to the involvement of gut microbiota in modulating cognitive function. Limited

Table 3 Significant results from the causal mediation analysis

Exposure	Mediator	Outcome	Proportion of mediation	ACME [95%CI], <i>p</i> -value	ADE [95%CI], <i>p</i> -value	TE [95%CI], <i>p</i> -value
Overall composition^a						
Total olive oil	PC1	Executive function	-8.9%	-0.003 [-0.008, -0.000], 0.044	0.037 [0.004, 0.067], 0.020	0.033 [0.001, 0.064], 0.050
Virgin olive oil	PC1	Executive function	-8.5%	-0.003 [-0.007, -0.000], 0.024	0.039 [0.017, 0.061], < 0.001	0.035 [0.015, 0.057], 0.004
Virgin olive oil	PC1	Language	-9.8%	-0.003 [-0.008, -0.000], 0.046	0.032 [0.007, 0.059], 0.020	0.029 [0.004, 0.055], 0.026
Olive oil-related feature^b						
Total olive oil	<i>Adlercreutzia</i>	General cognitive function	18.3%	0.009 [0.001, 0.019], 0.014	0.042 [-0.000, 0.086], 0.052	0.051 [0.010, 0.094], 0.014
Virgin olive oil	<i>Adlercreutzia</i>	General cognitive function	20.0%	0.008 [0.002, 0.017], 0.016	0.033 [0.002, 0.063], 0.032	0.041 [0.011, 0.070], 0.014

The ACME denotes the indirect effects of the olive oil consumption on cognitive changes through the mediators. The ADE represents the effects of the baseline olive oil consumption on cognitive changes without the involvement of a mediator. The proportion of mediation was calculated via dividing the ACME by the TE, which encompasses all direct and indirect effects of the exposure on the outcome. All continuous mediators were converted to z scores before analysis, and genus-level relative abundances were clr-transformed before z-conversion

Abbreviations: ACME average causal mediation effect, ADE average direct effect, clr centred log-ratio, PC principal component, TE total effect

^a The mediation analysis was performed using the quasi-Bayesian Monte Carlo simulation method with 1000 iterations

^b The mediation analysis was performed using the nonparametric bootstrap approaches with 1000 iterations to address the compositionality and sparsity nature of the microbiome data

data is available assessing gut microbiota composition and its association with cognitive decline in humans, particularly in individuals without diagnosed neurologic disorders. Previous studies have identified distinct microbiome features associated with different NDs [12]. Notably, *Akkermansia* has consistently exhibited increased abundance in individuals with both Parkinson's disease and AD [12], mirroring our study's findings where we observed an enrichment of *Akkermansia* associated with accelerated cognitive decline. One study involving only 25 AD patients reported a decreased abundance of *Adlercreutzia* [49], contradicting the present results where increased *Adlercreutzia* abundance was linked to cognitive decline. These discrepancies may arise from differences in, among others, factors related to study design and the disease progression stage of the population studied, as in that study, participants had already been diagnosed with the disease compared to our participants, who were free of dementia.

In our study, we have also found that gut microbiota mediates the association between olive oil consumption and changes in cognitive function. While the specific mediation role of *Adlercreutzia* in the association between TOO/VOO consumption and cognitive change is unique in our study, previous research has shed light on the importance of gut microbiota-mediated pathways in modulating cognitive function [6, 8, 50, 51]. The overall gut microbiota composition also has been observed

to mediate the association between the consumption of TOO or VOO and changes in cognitive function. Considering the nascent phase of research in this area, it is essential to exercise caution and avoid overinterpretation of results.

The differing associations of VOO and COO on cognitive function may be attributed to their distinct phenolic compound content. Phenolic compounds, including HTyr, tyrosol (Tyr), and secoiridoids such as OLE, ligstroside, oleacein, and oleocanthal, are predominantly found in VOO [8, 9]. These phenolic compounds have been shown to cross blood–brain barrier (BBB) and may directly enhance cognitive health by mitigating oxidative stress and neuroinflammation, both of which are pathways closely associated with the onset and progression of NDs [9]. Preclinical studies have demonstrated that HTyr and OLE can cross the BBB and accumulate in the brain regions such as hippocampus, where they protect neuronal cells by inhibiting β -amyloid (A β) aggregation, Tau protein fibrillization, apoptotic pathways activation and reducing pro-inflammatory cytokines production, as well as enhancing autophagy and A β deposits clearance [6, 8, 9]. Additionally, they may also indirectly impact cognitive function by shaping the gut microbiota composition through the “gut-brain” axis [6, 8]. Studies have also shown that these two major VOO polyphenols, HTyr and OLE, exhibit beneficial effects on gut microbiota by inhibiting pathogenic bacteria growth, increasing

microbiota richness, reversing gut dysbiosis, and promoting intestinal integrity, thus reducing inflammation and favoring intestinal microbial homeostasis, even in the presence of chronic diseases [6, 8]. Moreover, phenolic compounds impact gut microbiota to produce bioactive metabolites (e.g., short-chain fatty acids), which further enhance cognitive function [6, 8, 16]. These metabolites have been associated with reduced oxidative and neuroinflammatory stress, improved blood–brain barrier permeability, and the modulation of synaptic plasticity [6, 8, 16]. Furthermore, certain gut microbes or microbial metabolites influenced by the consumption of VOO have been shown to stimulate intestinal vagal terminals, promoting the synthesis of neurotrophic factors and neurotransmitters such as γ -aminobutyric acid [6, 16]. All these effects may induce changes on the function and structure of neurons and synapses in the central nervous system, exerting regulatory effects in the brain through the enteric and vagal nervous systems which plays an important part in learning, memory, and mood [16].

The strengths of our study lie in its robust methodology, including a well-characterized cohort with dietary, gut microbiota, and cognitive function data, a prospective design allowing for longitudinal analysis, comprehensive assessment of cognitive function changes across multiple domains, control for potential confounders, and the novel exploration of the mediating role of gut microbiota in the association between olive oil consumption and cognitive health. Furthermore, unlike previous studies that often overlooked the distinctions among different types of olive oil, we conducted separate analyses for VOO and COO, enabling a more detailed examination of their respective associations with cognitive decline and gut microbiota composition. Nevertheless, it is important to recognize certain limitations of the present study. First, due to its observational nature, establishing causality is not feasible and, despite adjusting for numerous potential confounders, residual confounding could not be ruled out. Second, our study population comprised older adults with overweight/obesity and metabolic syndrome from a Mediterranean country, where olive oil, especially VOO, is primarily consumed, potentially limiting the generalizability of our findings to broader populations. Additionally, opposite direction of the associations observed between COO and VOO with changes in cognitive function and gut microbiota composition may be influenced by several factors, warranting cautious interpretation of the results. On the one hand, in our population, the amount of COO consumed is much smaller than that of VOO. On the other hand, participants with higher COO consumption had lower educational levels and were more likely to be smokers. Although we adjusted for these socioeconomic status (SES) related factors, the

absence of information on other SES indicators remains a limitation, leaving the possibility of residual confounding. While our sensitivity analyses did not reveal substantial changes in the results after adjusting for the consumption of other oil types, future research comparing olive oil with other commonly consumed oils in other countries, such as soybean oil or rapeseed oil, would provide additional insights into the associations between dietary oils, gut microbiota, and cognitive function. Furthermore, investigating polyphenol content and its association with cognitive function and microbiota composition in different types of olive oil would be warranted to better understand underlying mechanisms. Third, olive oil consumption was evaluated from a FFQ which may be prone to measurement error and recall bias. Fourth, the sequencing depth of 16S rRNA amplicon sequencing was relatively short, which may introduce some degree of taxonomic misclassification, particularly at lower taxonomic levels. Lastly, we acknowledged that the exposure and the mediator were assessed at the same time point, potentially limiting our ability to establish temporal sequence in the “olive oil-gut microbiota-cognitive function” pathway.

In conclusion, our study provides further insights into the interplay between olive oil consumption, gut microbiota, and changes in cognitive function. Higher consumption of TOO/VOO was associated with improved cognitive function, while increased consumption of COO was linked to accelerated cognitive decline, underscoring the importance of distinguishing between olive oil types in future research and dietary recommendations. Moreover, our findings indicate a mediating role of the gut microbiota in this relationship, suggesting the potential mechanistic pathways involved in the ‘olive oil-gut-brain’ axis. Given the early stage of research in this promising field, additional data, specifically from high-quality and clinical cohort studies are required to provide evidence-based recommendations for developing microbiota-targeted dietary strategies aimed at enhancing brain health and promoting healthy aging.

Abbreviations

ACME	Average causal mediation effect
AD	Alzheimer's disease
ADE	Average direct effect
ASVs	Amplicon sequence variants
BBB	Blood-brain barrier
BDI-II	The Beck Depression Inventory
BMI	Body mass index
CDT	Clock Drawing Test
COO	Common olive oil
DST	Digit Span Test
EVOO	Extra virgin olive oil
FFQ	Food frequency questionnaire
HTyr	Hydroxytyrosol
MaAsLin2	Microbiome Multivariable Association with Linear Models

MEDAS	Mediterranean Diet Adherence Screener
MedDiet	Mediterranean diet
MMSE	Mini-Mental State Examination
METS	Metabolic equivalents
NDs	Neurodegenerative diseases
OLE	Oleuropein
PC	Principal components
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
PREDIMED-Plus	PREvención con Dieta MEDiterránea Plus study
RCT	Randomized controlled trial
SDs	Standard deviations
SES	Socioeconomic status
TE	Total effect
TMT	Trail Making Test
TOO	Total olive oil
VFT	Verbal Fluency Test
VOO	Virgin olive oil
WAIS-III	Wechsler Adult Intelligence Scale-III

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40168-025-02306-4>.

Additional file 1: Supplementary methods. Supplementary Figure 1. Flowchart of the study population. Supplementary Figure 2. Proportion of variation in the microbial communities explained by olive oil consumption, baseline cognitive function and covariates. Supplementary Figure 3. Associations between baseline gut microbial overall composition and 2-y changes in cognitive function. Supplementary Figure 4. Path diagram of the casual mediation analysis models with all potential exposure-mediator-outcome combinations assessed. Supplementary Table S1. Baseline characteristics of the study population according to tertiles of energy-adjusted common olive oil consumption. Supplementary Table S2. Cross-sectional association between baseline energy-adjusted olive oil consumption and overall gut microbial composition. Supplementary Table S3. PERMANOVA test results based on Aitchison distance across tertiles of baseline olive oil consumption. Supplementary Table S4. Results of MaAsLin2 analysis of associations between energy-adjusted olive oil consumption and gut microbial genus-level features ($q < 0.25$). Supplementary Table S5. Prospective associations of identified olive oil consumption-related microbial signatures with changes in cognitive function after a 2-year follow-up.

Additional file 2: Metadata associated with all samples used in the study.

Additional file 3: ASV_table used for this study.

Additional file 4: Taxonomic assignments for ASVs.

Additional file 5: Sequence files link for reviewers.

Acknowledgements

The authors thank all PREDIMED-Plus participants and investigators. CIBEROBN, CIBERESP, and CIBERDEM are initiative of the Instituto de Salud Carlos III (ISCIII), Madrid, Spain. The Hojiblanca (Lucena, Spain) and Patrimonio Comunal Olivarero (Madrid, Spain) food companies donated extra-virgin olive oil. The Almond Board of California (Modesto, CA), American Pistachio Growers (Fresno, CA), and Paramount Farms (Wonderful Company, LLC, Los Angeles, CA) donated nuts for the PREDIMED-Plus pilot study. The authors also thank the PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples.

Authors' contributions

All the principal PREDIMED-Plus investigators contributed to the study concept and design and to data extraction from the PREDIMED-Plus participants. SKN, NB and JSS contributed to the concept and design of the present study. JN wrote the first draft and performed the statistical analyses under the supervision of SKN, NB and JSS. JN and JSS are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility

for the integrity of the data and the accuracy of the data analysis. All authors reviewed the manuscript for important intellectual content and approved the final version to be published.

Funding

This work was supported by the official Spanish Institutions for funding scientific biomedical research, CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) and Instituto de Salud Carlos III (ISCIII), through the Fondo de Investigación para la Salud (FIS), which is co-funded by the European Regional Development Fund (six coordinated FIS projects led by JSS and JVi, including the following projects: PI13/00233, PI13/00728, PI13/00462, PI14/01206, PI14/00696, PI16/00533, PI16/00366, PI16/00501, PI17/01441, PI17/00855, PI19/00017, PI19/00781, PI19/00576, PI20/00557, PI21/0046; the Especial Action Project entitled: Implementación y evaluación de una intervención intensiva sobre la actividad física Cohorte PREDIMED-Plus grant to JSS; the Recercaixa (number 2013ACUP00194) grant to JSS; grants from the Consejería de Salud de la Junta de Andalucía (PI0458/2013, PS0358/2016, PI0137/2018); the PROMETEO/2017/017 and PROMETEO/2021/21 grants from the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital from the Generalitat Valenciana; and by NIH grant R01DK127601. This research was also partially funded by the Eat2beNICE/H2020-SFS-2016–2 EU-H2020 European grant, and the Horizon 2020 PRIME study (Prevention and Remediation of Insulin Multimorbidity in Europe; grant agreement #847879). JN is supported by a predoctoral grant from Ministerio de Ciencia, Innovación y Universidades (FPU 20/00385). SKN is supported by a postdoctoral fellowship from the Canadian Institutes of Health Research (CIHR, MFE-171207). JSS, the senior author of this paper, was partially supported by ICREA under the ICREA Academia program. None of the funding sources took part in the design, collection, analysis, interpretation of the data, writing the report, or in the decision to submit the manuscript for publication.

Data availability

Original R scripts are available in GitHub (<https://github.com/Ni-Jiaqi/Olive-oil-Gut-microbiota-Cognitive-function>). Metadata, the ASVs table, and corresponding taxonomic classifications have all been included as Additional files 2, 3 and 4, respectively, available for reviewers. Reviewer link to sequence files has been included in Additional file 5.

The datasets generated and analyzed during the current study are not available to public due to data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scom-mittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

Declarations

Ethics approval and consent to participate

The PREDIMED-Plus trial was conducted according to the guidelines of the Declaration of Helsinki and received approval from the relevant research ethics committees of all participating institutions. The Ethical Committees involved in overseeing the present study are as follows: CEIC del Hospital Universitari Sant Joan de Reus:13–07-25/7proj2; CEIC Corporativo de Atención Primaria de la Comunitat Valenciana:2011–005398-22; CEIC del Hospital General Universitario de Alicante:CEIC PI2017/02; and CEIC Parc de Salut Mar y IDIAP Jordi Gol:PI13/120.

Consent for publication

Not applicable.

Competing interests

JSS reports serving on the board of and receiving grant support through his institution from the International Nut and Dried Fruit Council, serving on the board of the Instituto Danone Spain and the International Danone institute. None of the other authors declare competing interests.

Author details

¹ Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain.

²Institut d'Investigació Sanitària Pere Virgili (IISPV), Alimentació, Nutrició, Desenvolupament i Salut Mental, Reus, Spain. ³Centro de Investigación Biomédica en Red Fisiopatología de La Obesidad y La Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain. ⁴School of Nutrition, Faculty of Community Services, Toronto Metropolitan University, Toronto, Canada. ⁵Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, Unity Health Toronto, Toronto, Canada. ⁶Laboratory of Microbiology, Wageningen University & Research, Wageningen, the Netherlands. ⁷CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain. ⁸Universidad Miguel Hernández, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL-UMH), Alicante, Spain. ⁹Department of Preventive Medicine, University of Valencia, Valencia, Spain. ¹⁰Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas Municipal d'Investigació Mèdica (IMIM), Barcelona, Spain. ¹¹CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain. ¹²Department of Endocrinology, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain. ¹³Department of Endocrinology and Nutrition, Virgen de La Victoria University Hospital, the Biomedical Research Institute of Malaga and Platform in Nanomedicine (IBIMA-BIONAND Platform), University of Malaga, Malaga, Spain. ¹⁴Department of Computer Languages and Systems, Jaume I University, Castellón, Spain. ¹⁵Department of Preventive Medicine and Public Health, University of Navarra, Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain. ¹⁶Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. ¹⁷Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA. ¹⁸Broad Institute of MIT and Harvard, Cambridge, MA, USA.

Received: 23 April 2024 Accepted: 19 November 2025

Published online: 24 January 2026

References

- Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health*. 2022;7:e105–25.
- Scarmeas N, Anastasiou CA, Yannakoulia M. Nutrition and prevention of cognitive impairment. *Lancet Neurol*. 2018;17:1006–15.
- WHO GUIDELINES. Risk reduction of cognitive decline and dementia: WHO guidelines. 2019.
- Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*. 2020;396:413–46.
- Fazlollahi A, Motlagh Asghari K, Aslan C, Noori M, Nejadghaderi SA, Araj-Khodaie M, et al. The effects of olive oil consumption on cognitive performance: a systematic review. *Front Nutr*. 2023;10:1–13.
- Millman JF, Okamoto S, Teruya T, Uema T, Ikematsu S, Shimabukuro M, et al. Extra-virgin olive oil and the gut-brain axis: influence on gut microbiota, mucosal immunity, and cardiometabolic and cognitive health. *Nutr Rev*. 2021;79:1362–74.
- Marcelino G, Hiane PA, de Freitas KC, Santana LF, Pott A, Donadon JR, et al. Effects of olive oil and its minor components on cardiovascular diseases, inflammation, and gut microbiota. *Nutrients*. 2019;11:1826.
- Micheli L, Bertini L, Bonato A, Villanova N, Caruso C, Caruso M, et al. Role of hydroxytyrosol and oleuropein in the prevention of aging and related disorders: focus on neurodegeneration, skeletal muscle dysfunction and gut microbiota. *Nutrients*. 2023;15:1767.
- Boronat A, Serreli G, Rodríguez-Morató J, Deiana M, de la Torre R. Olive Oil Phenolic Compounds' Activity against Age-Associated Cognitive Decline: Clinical and Experimental Evidence. *Antioxidants*. 2023;12:1472.
- Commission Regulation EEC, Reiter B, Lorbeer E. The characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of the European Communities*. 2001;269:881–8.
- Morais LH, Schreiber HL, Mazmanian SK. The gut microbiota–brain axis in behaviour and brain disorders. *Nat Rev Microbiol*. 2021;19:241–55.
- Fang P, Kazmi SA, Jameson KG, Hsiao EY. The microbiome as a modifier of neurodegenerative disease risk. *Cell Host Microbe*. 2020;28:201–22.
- Cryan JF, O'riordan KJ, Cowan CSM, Sandhu KV, Bastiaansen TFS, Boehme M, et al. The microbiota–gut–brain axis. *Physiol Rev*. 2019;99:1877–2013.
- David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505:559–63.
- Chakrabarti A, Geurts L, Hoyle L, Iozzo P, Kraneveld AD, La Fata G, et al. The microbiota–gut–brain axis: pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. *Cell Mol Life Sci*. 2022;79(2):80.
- Berding K, Vickova K, Marx W, Schellekens H, Stanton C, Clarke G, et al. Diet and the microbiota–gut–brain axis: sowing the seeds of good mental health. *Adv Nutr*. 2021;12:1239–85.
- Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome. *Circulation*. 2009;120:1640–5.
- Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, Basora J, Fitó M, Corella D, et al. Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-Plus trial. *Diabetes Care*. 2019;42:777–88.
- Willett W. *Nutritional Epidemiology*. Oxford: Oxford University Press; 2012.
- Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr*. 2010;103:1808–16.
- Moreiras O, Carbajal A, Cabrera L, Cuadrado C. *Tablas de composición de los alimentos*. 9th. Ediciones Pirámide. Madrid (España). 2005.
- Mataix Verdú J. *Tablas de composición de alimentos*. Instituto de nutrición y tecnología de los alimentos. Granada (España): Editorial Universidad de Granada. 2003.
- European Commission. Commission Regulations (EC) No. 1019/2002 of 13 June 2002 on the marketing standards for olive oil. *Official Journal of the European Communities*. 2002;L155:27–31.
- Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997;65:1220S–1228S.
- Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr*. 2011;141:1140–5.
- Molina L, Sarmiento M, Peñafiel J, Donaire D, García-Aymerich J, Gómez M, et al. Validation of the regicor short physical activity questionnaire for the adult population. *PLoS ONE*. 2017;12:168148.
- Beck A, Steer R, Brown G. BDI-II. Beck depression inventory–II-second edition manual. San Antonio, TX: The Psychological Corporation; 1996.
- Sanz J, Luis A, Carmelo P, Resumen V. CLÍNICA Y SALUD 249 ARTÍCULOS Adaptación española del Inventario para la Depresión de Beck-II (BDI-II): 2. Propiedades psicométricas en población general The spanish adaptation of Beck's Depression Inventory-II (BDI-II): 2. Psychometric properties in the general population. 2003;14:249–80.
- Sachdev PS, Blacker D, Blazer DG, Ganguli M, Jeste DV, Paulsen JS, et al. Classifying neurocognitive disorders: the DSM-5 approach. *Nature Reviews Neurology*. 2014;10:634–42.
- AC. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*. 1987;43:783–91.
- Shannon CE. A mathematical theory of communication. *Bell Syst Tech J*. 1948;27:379–423.
- Simpson EH. Measurement of Diversity. *Nature*. 1949;163:688–688.
- Aitchison J, Barceló-Vidal C, Martín-Fernández JA, Pawłowsky-Glahn V. Logratio analysis and compositional distance. *Math Geol*. 2000;32:271–5.
- Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol*. 2001;26:32–46.
- Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput Biol*. 2021;17:e1009442.
- Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. *J Stat Softw*. 2014;59:1–38.
- Zhang H, Chen J, Li Z, Liu L. Testing for mediation effect with application to human microbiome data. *Stat Biosci*. 2021;13:313.
- Lee H, Cashin AG, Lamb SE, Hopewell S, Vansteelandt S, Vanderweele TJ, et al. A guideline for reporting mediation analyses of randomized trials and observational studies: the AGReMA Statement. *JAMA - Journal of the American Medical Association*. 2021;326:1045–56.

39. Rijnhart JJM, Lamp SJ, Valente MJ, MacKinnon DP, Twisk JWR, Heymans MW. Mediation analysis methods used in observational research: a scoping review and recommendations. *BMC Med Res Methodol*. 2021;21:1–17.
40. Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, De La Torre R, Martínez-González MÁ, et al. Mediterranean diet and age-related cognitive decline: a randomized clinical trial. *JAMA Intern Med*. 2015;175:1094–103.
41. Martínez-Lapiscina EH, Clavero P, Toledo E, Estruch R, Salas-Salvado J, San Julián B, et al. Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. *J Neurol Neurosurg Psychiatry*. 2013;84:1318–25.
42. Martínez-Lapiscina EH, Clavero P, Toledo E, San Julian B, Sanchez-Tainta A, Corella D, et al. Virgin olive oil supplementation and long-term cognition: the Predimed-Navarra randomized, trial. *Journal of Nutrition, Health and Aging*. 2013;17:544–52.
43. Mazza E, Fava A, Ferro Y, Rotundo S, Romeo S, Bosco D, et al. Effect of the replacement of dietary vegetable oils with a low dose of extravirgin olive oil in the Mediterranean Diet on cognitive functions in the elderly. *J Transl Med*. 2018;16:1–10.
44. Tsolaki M, Lazarou E, Kozori M, Petridou N, Tabakis I, Lazarou I, et al. A randomized clinical trial of Greek high phenolic early harvest extra virgin olive oil in mild cognitive impairment: the MICOIL pilot study. *J Alzheimers Dis*. 2020;78:801–17.
45. Kaddoumi A, Denney TS, Deshpande G, Robinson JL, Beyers RJ, Redden DT, et al. Extra-virgin olive oil enhances the blood–brain barrier function in mild cognitive impairment: a randomized controlled trial. *Nutrients*. 2022;14:5102.
46. Meyer K, Lulla A, Debroy K, Shikany JM, Yaffe K, Meirelles O, et al. Association of the gut microbiota with cognitive function in midlife. *JAMA Netw Open*. 2022;5:e2143941–e2143941.
47. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*. 2021;19:55–71.
48. Martín R, Rios-Covian D, Huillet E, Auger S, Khazaal S, Bermúdez-Humarán LG, et al. *Faecalibacterium*: a bacterial genus with promising human health applications. *FEMS Microbiol Rev*. 2023;47:1–18.
49. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*. 2017;7:1–11.
50. Ghosh TS, Rampelli S, Jeffery IB, Santoro A, Neto M, Capri M, et al. Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: the NU-AGE 1-year dietary intervention across five European countries. *Gut*. 2020;69:1218–28.
51. Nagpal R, Neth BJ, Wang S, Craft S, Yadav H. Modified Mediterranean-ketogenic diet modulates gut microbiome and short-chain fatty acids in association with Alzheimer's disease markers in subjects with mild cognitive impairment. *EBioMedicine*. 2019;47:529–42.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.