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Exploring the relationship between APOEε4 allele and gut microbiota composition and function in healthy adults

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Abstract
The APOE ε4 allele (APOE4) is a known risk factor for neurodegenerative and cardiovascular diseases, but its link to body composition and metabolism remains debated. The gut microbiota influences host metabolism and immunity, yet its relationship with APOE genotype in healthy individuals is not well understood. The objective of this work was to examine associations between APOE genotype and gut microbiota composition and function in healthy adults, focusing on microbial and metabolic differences related to the APOE4 allele. Seventy-seven healthy Spanish adults were genotyped for APOE. Fecal microbiota profiles were assessed by 16 S rRNA gene sequencing, and predicted functions were inferred using PICRUSt2. Body composition (DEXA) and physical activity (accelerometry) were also measured. APOE4 carriers exhibited subtle shifts in microbiota composition, including a five-fold reduction in *Megamonas* and lower abundance of the *Eubacterium brachy* group—both linked to energy harvest and adiposity—compared to APOE3 homozygotes. An uncharacterized *Puniceicoccaceae* genus was enriched in APOE4 carriers. Although *E. brachy* group abundance correlated with adiposity, no significant differences in body composition were observed. Functional predictions showed APOE4-associated microbiota enriched in pathways for carotenoid biosynthesis and trehalose metabolism, and depleted in tryptophan biosynthesis, propionate production, and multidrug resistance mechanisms. APOE4 carriers harbor gut microbiota with distinct taxonomic and functional features, potentially reflecting adaptations to metabolic and oxidative challenges. These findings underscore the relevance of the gut microbiome in shaping APOE4-associated phenotypes and warrant further investigation into its mechanistic contributions to health and disease.

Keywords APOEε4 allele, Gut microbiota, Metabolic function, Healthy adults, Body composition

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Introduction

Apolipoprotein E (apoE) is a key structural component of several plasma lipoproteins, including chylomicron remnants, very low-density lipoproteins (VLDL), and a subclass of large high-density lipoproteins (HDL). Its role in these lipoproteins is critical for their clearance from circulation, as apoE functions as a high-affinity ligand for LDL and VLDL receptors (Getz and Reardon 2009). apoE is primarily synthesized in the liver, but its expression is also observed in the brain, muscle, kidney, adipose tissue, and immune cells (Driscoll and Getz 1984). apoE is encoded by the APOE gene, which exists in three allelic forms: ε2, ε3, and ε4 (Huang et al. 2004). These alleles result in six genotypic combinations in humans: three homozygous (APOE2/E2, APOE3/E3, and APOE4/E4) and three heterozygous (APOE3/E2, APOE4/E3, and APOE4/E2) genotypes. While the APOE4 allele is well established as a genetic risk factor for neurodegenerative and cardiovascular diseases, its association with obesity remains controversial (Alagarsamy et al. 2022). Research investigating the relationship between metabolic syndrome, obesity, and APOE genotype has demonstrated that APOE4 carriers exhibit higher plasma cholesterol levels, regardless of their body mass index (BMI). However, elevated triglyceride levels and reduced HDL concentrations were specifically observed in APOE4 carriers with overweight or obesity (Torres-Perez et al. 2016). Additionally, APOE4 carriers tend to have a lower BMI compared to APOE3 and APOE2 carriers, with APOE2 carriers showing the highest BMI levels (Tejedor et al. 2014).

Given the established links between lipid metabolism, obesity, and gut microbiota composition, the role of APOE4 in modulating the gut microbiome has garnered increasing scientific interest. The gut microbiota of individuals with obesity differs significantly from that of lean individuals, exhibiting an enhanced ability to extract energy from the diet and thereby contributing to greater caloric availability for the host (Turnbaugh et al. 2006). A hallmark of obesity-associated gut microbiota is an increased *Firmicutes/Bacteroidetes* ratio, along with a reduced abundance of genera such as *Bacteroides*, *Prevotella*, *Roseburia*, *Faecalibacterium*, and *Ruminococcus*, while genera such as *Streptococcus* and *Clostridium* are more prevalent (Haro et al. 2017). Obesity-related gut dysbiosis is also characterized by a strong correlation between mucin-degrading bacteria, host adiposity, and obesity-related metabolic disturbances (Nehra et al. 2016). There is robust evidence linking alterations in gut microbiota composition and function with metabolic disorders commonly associated with obesity, including insulin resistance, atherosclerosis, and low-grade chronic inflammation (Kobyliak et al. 2015). One of the key factors influencing gut microbiota composition is an individual's genetic background (Goodrich et al. 2014). A genome-wide association study (GWAS) investigating the relationship between common single nucleotide polymorphisms (SNPs) and gut microbiota composition identified associations between microbial taxa and the APOE gene (Bonder et al. 2016). Furthermore, studies conducted in both animal models and patients with Alzheimer's disease (AD) have reported a correlation between the presence of the E4 allele and specific microbial taxa (Cammann et al. 2023; Hou et al. 2021; Tran et al. 2019; Zajac et al. 2022). However, these studies have primarily focused on elderly AD patients, whose gut microbiota may be altered due to age and disease-related factors such as medication use and other comorbidities. Additionally, these investigations have not accounted for obesity as a potential modulator of gut microbiota composition.

Given that previous research has linked APOE4 to specific microbial taxa in AD patients and animal models, these findings may be influenced by disease-related confounding factors. To address this limitation, this study aims to determine whether similar associations exist in individuals without Alzheimer's disease. Specifically, we investigated the relationship between the APOE4 allele and gut microbiota composition in a healthy population, free from neurodegenerative disease-associated influences. This approach provides a more precise understanding of the baseline impact of APOE4 on the gut microbiome and enables an assessment of whether body composition is associated with gut microbiota profiles in APOE4 carriers.

Materials and methods

Study population

With the objective of investigate the association between the APOE4 allele and gut microbiota composition in healthy adults, independent of neurodegenerative disease, and exploring whether body composition is linked to microbiota profiles in APOE4 carriers, participants were recruited among employees of the Universidad Europea de Madrid (Madrid, Spain), including teaching, research, administrative and maintenance staff, through internal announcements according to the following inclusion criteria: Caucasian men or premenopausal women aged 18 to 45 years, with a body mass index (BMI) between 25 and 30 kg/m^2. Exclusion criteria included the presence of any pathology (current or within the six months prior to the study), a history of gastrointestinal surgery, antibiotic use within three months prior to enrolment, smoking, consumption of prebiotics, probiotics, or dietary supplements, a vegetarian or vegan diet, and pregnancy or lactation. To ensure participants were metabolically healthy, they were asked about the results of their most recent annual blood test, routinely performed as part of occupational health assessments

at the Universidad Europea de Madrid. Only individuals who reported having normal results, including lipid profile, were considered eligible for inclusion.

Sample collection

Participants were provided with a Fe-Col® Fecal Sample Collection Kit (Alpha Laboratories, Hampshire, United Kingdom) and were instructed to defecate directly onto the collection paper. Fecal samples were then transferred into a sterile tube and transported to the laboratory in an insulated ice box containing a cooling element. Upon arrival, samples were immediately stored at – 80 °C until further analysis.

DNA extraction

Human and bacterial DNA were extracted from 100 mg of fecal sample using the E.Z.N.A.® Stool DNA Kit (Omega Biotek, Norcross, GA, USA), following the manufacturer's protocol for bacterial DNA extraction, with the aid of a bead-beating homogenizer (Bullet Blender Storm, Next Advance, NY). DNA concentration and purity were assessed using the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA) and measured with an FP-8300 spectrofluorometer (Jasco, Tokyo, Japan). Bacterial DNA was used for gut microbiota analysis, while human DNA was utilized for APOE genotyping.

APOE genotyping

Allelic discrimination of APOE gene variants was performed using TaqMan PCR technology (7300 Instrument; Applied Biosystems) with Assay-On-Demand single nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems). APOE haplotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) were determined based on genotyping of rs7412 and rs429358 (E2: rs7412-T, rs429358-T; E3: rs7412-C, rs429358-T; E4: rs7412-C, rs429358-C). PCR amplification was conducted on a StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA) with the following thermal cycling conditions: an initial denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 92 °C for 15 s, annealing/extension at 60 °C for 1 min. A final step at 60 °C for 30 s was included to stabilize fluorescence signals before analysis.

Body composition measurement

Body composition was assessed using dual-energy X-ray absorptiometry (DEXA) (GE Healthcare, Madison, WI). The body composition parameters measured in this study included total body fat mass, estimated visceral adipose tissue (VAT), total muscle mass, as well as fat and muscle mass distribution in the trunk and extremities. The adiposity index (AI) was calculated as the total fat mass / height^2.

Physical activity

The physical activity levels were assessed as previously described in (Bressa et al. 2017) using an ActiSleep V.3.4.2 accelerometer (Actigraph, Manufacturing Technology Inc., Shalimar, FL). Physical activity intensity was recorded for seven consecutive days, including five weekdays and two weekend days. The collected data were processed using ActiLife software (ActiLife6, Actigraph).

Diet analysis

Dietary pattern characterization of the participants was assessed using a validated food frequency questionnaire (FFQ) consisting of 93 food items (Vioque and Gonzalez 1991), designed to estimate habitual dietary intake over the previous year.Participants reported the frequency of consumption and portion size for each item, allowing estimation of daily energy and nutrient intake, including macronutrients (carbohydrates, proteins, fats) and fiber. In addition, a 3-day dietary record was completed, covering two weekdays and one weekend day, in which participants recorded all foods and beverages consumed, including details on preparation methods, brand names, and portion sizes. This dual approach allowed us to capture both long-term dietary habits and short-term variability. Dietary data were analyzed using the Dietsource® software (version 3.0, Novartis, Barcelona, Spain), enabling detailed nutrient profiling to assess potential dietary confounders and ensure comparability between genotype groups. To further characterize the lifestyle of the participants and ensure cohort homogeneity, a structured screening questionnaire was administered to collect information on general health status, smoking habits, alcohol consumption, potential dietary restrictions and supplement use,

Short-chain fatty acids (SCFAs)

SCFAs were quantified following the method described by García-Villalba et al. (2012) (García-Villalba et al. 2012), with modifications. Fecal samples were suspended in 1% phosphoric acid and extracted using ethyl acetate. The extracted samples were analyzed using a gas chromatograph (Agilent GC system 7820 A) coupled to a mass detector (Agilent MSD 5975), equipped with a DB-Wax 121–7037LT column (Agilent Technologies). Data acquisition was performed using selective ion monitoring (SIM). SCFAs were quantified by comparing the peak area of their target ions against an 8-point external calibration curve (0.02–5.00 ppm) using reference standards (Sigma-Aldrich). 4-methylvaleric acid and 2-ethylbutyric acid were used as the internal and external standards, respectively.

Sequencing and bioinformatics

Bioinformatics analysis of 16S amplicon sequencing data was performed using the Quantitative Insights

into Microbial Ecology (QIIME2) v.2024.5 (Bolyen et al. 2019). The hypervariable regions V3 and V4 were amplified using the primer pair 341F / 806R 5′-CCTACGGGNGGCWGCAG-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′. The amplicon of 459 bp was visualized in a 0.8% agarose gel stained with ethidium bromide, bands were cut and cleaned using the MinElute Gel extraction kit (Qiagen, Hilden, Germany). DNA amplicons were sequenced on a MiSeq Illumina platform (Illumina, San Diego, CA). The 16s paired reads were imported in QIIME2 and processed with DADA2 plugin (Callahan et al. 2016) adjusting the maximum expected error threshold to 2.0 as default (Edgar and Flyvbjerg 2015) and filtering chimera with consensus method. Taxonomy classification was assigned with q2-feature-classifier plugin (Bokulich et al. 2018) using a pre-fitted sklearn-based taxonomy classifier method (Pedregosa, Fabian et al., n.d.). To construct the classifier, we extracted the sequences according to 341 F/806R primers from the SILVA 132.2 database clustered at 99% identity. Before training our classifier, we use the q2-clawback plugin (Kaehler et al. 2019) to include the species probability (weights) likely to be observed for human stool fetched with redbiom (McDonald et al. 2019) against qiita.ucsd.edu. Diversity analyses were obtained through QIIME 2's q2-diversity plugin. Beta-diversity was evaluated by calculating Bray-Curtis, Jaccard, unweighted and weighted Unifrac distance metrics. To study alpha-diversity, Observed_features, Pielou evenness, Shannon-Weave and Faith's Phylogenetic Diversity indices were calculated.

PICRUST2

KEGG orthologs abundances predictions have been obtained by PICRUSt2 (Langille et al. 2013) software (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) using default "max parsimony" method for hidden-state prediction and a NSTI (Nearest Sequenced Taxon Index) value of 2. PICRUSt2 wraps several tools to generate functional predictions from amplicon sequences: HMMER (Eddy 1998), EPA-NG (Barbera et al. 2019), GAPPA (Czech et al. 2020), CASTOR (Louca and Doebeli 2018) and MINPATH (Ye and Doak 2009).

Table 1 Distribution of APOE genotypes in the study population

Genotype	Frequency	%	Valid %	Cumulative %
E2/E3	0	0.0	0.0	0.0
E3/E3	60	77.9	77.9	77.9
E3/E4	17	22.1	22.1	22.1
E2/E2	0	0.0	0.0	0.0
Total	77	100	100	

The table presents the frequency and percentage distribution of different APOE genotypes among participants. Valid % represents the proportion of each genotype within the total sample, while Cumulative % indicates the cumulative frequency distribution.

Statistical analysis

Statistical analysis was carried out using QIIME2 v. 2024.5, SPSS software 21.0 (SPSS, Chicago, IL) and R statistical package 4.4.0. The statistical analysis followed an Intention-to-Treat (ITT) approach, missing values were assumed to be missing at random and were imputed using the regression method. The imputation model all available outcome variables to improve precision and reduce bias. A total of 5 imputations were generated to create multiple datasets, which were subsequently pooled following Rubin's rules. Variable normal distribution was assessed using the Shapiro-Wilk test, when normal distribution was not assumed, non-parametric tests were performed. Intergroup comparisons of variables were performed with t-test or Mann-Whitney. Microbiota abundance was estimated using and the Analysis of Compositions of Microbiomes with Bias Correction 2 ANCOM-BC2 (Lin and Peddada 2020) and Microbiome Multivariable Association with Linear Models (MaAsLin 2), (Mallick et al. 2021) was used to study the association of the microbiota with body composition variables, SCFAs, and diet, controlling for age and sex as covariates, and considering data with a q-value < 0.1 as significant.

Results

Cohort description

Seventy-seven healthy Caucasian participants (37 women and 40 man), aged between 18 and 48 years (33.6 ± 7.6 years) and body mass index (BMI) 24 ± 6 kg/m2, were recruited. The most prevalent genotype was ApoE3/3, found in 60 participants (77.9%), the ApoE3/4 genotype was present in 17 individuals (22.1%), whereas the ApoE2/3 and ApoE2/2 genotypes were absent (0%) in this sample. This distribution suggests that ApoE3 is the predominant allele, while ApoE4 is present in a smaller proportion of the population, consistent with expected frequencies in a general population of Spain (Corbo and Scacchi 1999) (Table 1).

In our healthy population, neither E2/E2 nor E2/E3 genotypes were detected, in contrast to findings of González et al. (2020), who reported a 10.23% frequency of E2/E3 in a Spanish-Iberian non-Alzheimer population, although E2/E2 was also absent (González et al. 2020).

In the ApoE3/3 genotype group, there were 31 men and 28 women, while in the ApoE3/4 genotype group, there were 9 men and 9 women. The mean age for the ApoE3/3 group was 33.57 ± 7.73 years, and for the ApoE3/4 group, it was 33.56 ± 7.23 years. No significant differences in age were observed between the two groups ($p = 0.497$).

Participants diet

Diet plays a crucial role in shaping the gut microbiota and thus is an important factor to consider in microbiota studies. To control for this variable in subsequent microbiome analyses, dietary information was collected from participants through a 3-day dietary record and FFQ. The results regarding macronutrient content show no significant differences between the two genotypic groups in terms of carbohydrate, protein, and fat intake, as well as in total energy and fiber consumption (Table 2).

Table 2 Dietary intake in ApoE3/3 and ApoE3/4 carriers

Diet	ApoE3/3	ApoE3/4	*p*
Energy intake (kcal)	2186.02 ± 663.27	1953.65 ± 705.69	0.207
Carbohydrates, (% E intake)	45.80 ± 6.65	46.11 ± 6.05	0.862
Fats (% E intake)	36.98 ± 6.31	36.00 ± 7.195	0.580
Protein (% E intake)	17.20 ± 2.89	18.00 ± 3.46	0.332
Dietary fiber (g/d)	23.99 ± 10.40	21.59 ± 10.24	0.397

Data are presented as mean ± standard deviation (SD). Energy intake is expressed in kilocalories (kcal), while macronutrient composition (carbohydrates, fats, and protein) is expressed as a percentage of total energy intake (% E intake). Dietary fiber intake is reported in grams per day (g/d).

Body composition

The comparison of body composition parameters between ApoE3/3 and ApoE3/4 carriers revealed no statistically significant differences in any of the measured variables (all p-values > 0.05) (Table 3). None of the studied parameters showed notable differences in body fat composition or lean mass between genotypes. Overall, the findings suggest that the ApoE4 genotype does not appear to be significantly associated with variations in body composition parameters in this cohort.

When the data were stratified by sex, no significant differences were observed in body composition parameters among women (Table 4). However, in men, appendicular lean mass was significantly higher in the ApoE3/3 group compared to the ApoE3/4 group.

Table 3 Body composition parameters in ApoE3/3 and ApoE3/4 carriers

Variable	ApoE3/3	ApoE3/4	*p*
BMI	23.43 ± 5.73	23.28 ± 2.54	0.910
Body fat %	23.66 ± 10.68	26.81 ± 10.94	0.736
Adiposity index	5.57 ± 2.78	6.11 ± 2.76	0.964
Android/Gynoid fat ratio	0.94 ± 0.28	0.87 ± 0.29	0.322
Estimated visceral fat (g)	341 ± 188	310 ± 205	0.548
Total fat mass (g)	17,744 ± 6510	18,937 ± 6332	0.510
Android fat mass (g)	1405 ± 751	1367 ± 679	0.854
Gynoid fat mass (g)	3244 ± 1123	3189 ± 1386	0.871
Total lean mass (kg)	47.04 ± 12.75	42.66 ± 13.47	0.217
Appendicular lean mass (kg)	20.99 ± 6.47	19.17 ± 6.54	0.305

Data are presented as mean ± standard deviation (SD). BMI: body mass index; VAT: visceral adipose tissue. Adiposity index was calculated as total fat mass/height^2.

Physical activity

The analysis of physical activity levels stratified by sex and ApoE genotype (ApoE3/3 vs. ApoE3/4) revealed no statistically significant differences in either light physical activity (LPA) or moderate-to-vigorous physical activity (MVPA) in both women and men. In women, the average time spent in light physical activity was 839 ± 315 min/day for the ApoE3/3 group and 800 ± 340 min/day for the ApoE3/4 group ($p = 0.450$), indicating no significant difference whereas the mean of MVPA was 364 ± 130 min/day for the ApoE3/3 group and 357 ± 122 min/day for the ApoE3/4 group ($p = 0.741$). Regarding men the ApoE3/3 group had LPA (909 ± 286 min/day) compared to the ApoE3/4 group (828 ± 372 min/day), this difference was not statistically significant ($p = 0.146$). For MPA In men, the values were 339 ± 117 min/day for the ApoE3/3 group and 325 ± 139 min/day for the ApoE3/4 group, again with no significant difference ($p = 0.460$).

Microbiota analysis

The presence of the E4 allele in the studied population is associated with significant alterations in three gut microbial taxa, as identified by ANCOM-BC (Analysis of Compositions of Microbiomes with Bias Correction). Notably, the abundance of *Megamonas* exhibits a nearly fivefold reduction, while the *Eubacterium brachy* group decreases by approximately twofold (Fig. 1). In contrast, a significant increase is observed in the genus *Incertae sedis* 18,

Table 4 Body composition parameters in ApoE3/3 and ApoE3/4 carriers stratified by sex

	ApoE3/3 W	ApoE3/4 W	*p*	ApoE3/3 M	ApoE3/4 M	*p*
BMI	23.11 ± 2.52	22.44 ± 6.28	0.760	23.44 ± 2.70	25.05 ± 2.74	0.127
Body fat %	35.20 ± 3.70	30.21 ± 9.00	0.121	18.42 ± 9.06	22.62 ± 5.82	0.103
Adiposity index	7.94 ± 1.39	6.72 ± 2.61	0.196	4.28 ± 2.58	5.61 ± 1.93	0.101
Android/Gynoid fat ratio	0.82 ± 0.14	0.74 ± 0.23	0.305	0.92 ± 0.39	1.10 ± 0.20	0.059
Estimated visceral fat (g)	319 ± 150	268 ± 170	0.436	301 ± 257	397 ± 184	0.218
Total fat mass (g)	22,621 ± 3963	18,088 ± 6921	0.954	14,792 ± 6050	17,499 ± 6307	0.283
Android fat mass (g)	1552 ± 535	1230 ± 630	0.190	1160 ± 798	1529 ± 813	0.257
Gynoid fat mass (g)	3875 ± 1450	3717 ± 1071	0.739	2419 ± 839	2908 ± 1050	0.231
Total lean mass (kg)	38.62 ± 4.34	35.40 ± 9.32	0.331	46.69 ± 18.18	55.68 ± 6.51	0.181
Appendicular lean mass (kg)	16.71 ± 2.55	14.92 ± 4.11	0.235	21.63 ± 8.41	25.50 ± 3.51	0.046

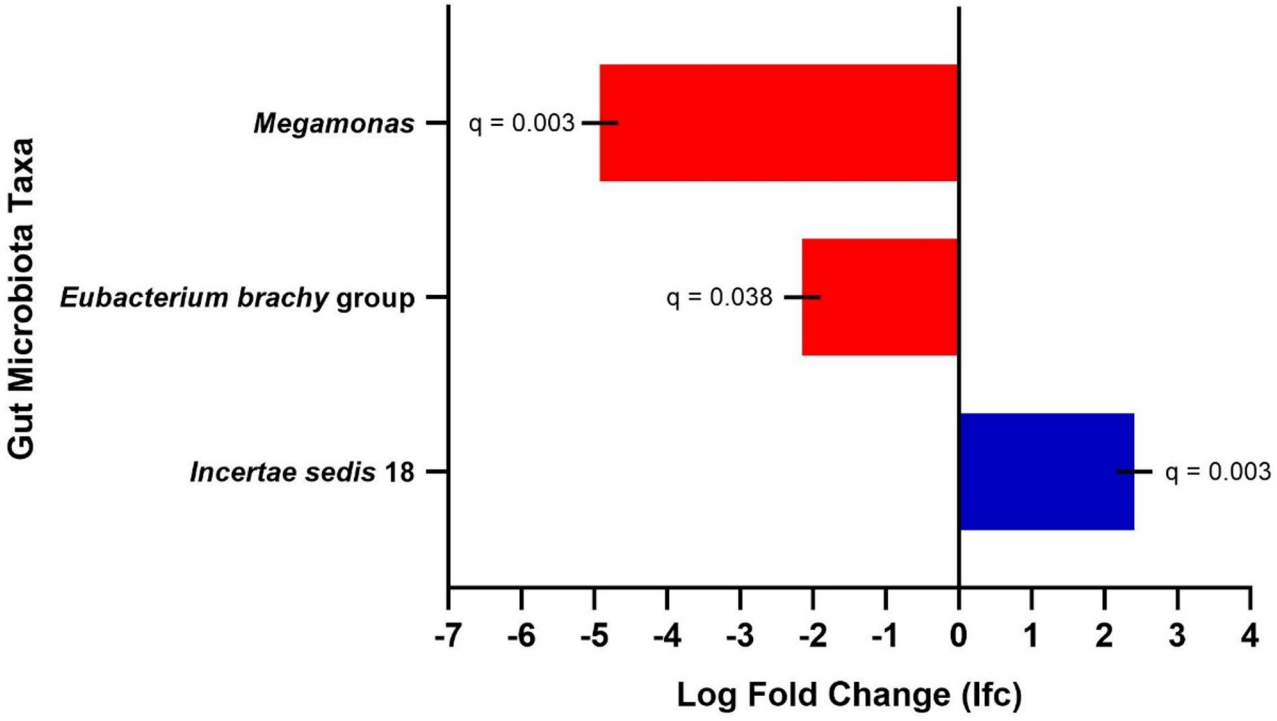

Fig. 1 Changes in gut microbiota composition associated with the presence of the E4 allele. The log2 fold change (lfc) in the relative abundance of three microbial taxa identified by ANCOM-BC is shown. q = adjusted p-value

Table 5 Short-chain fatty acid (SCFA) concentrations in ApoE3/3 and ApoE3/4 carriers

Genotype	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Isobutyric acid	Isovaleric acid	Total SCFA
E3/E3	164.86 ± 79.08	85.67 ± 44.06	103.58 ± 70.68	22.87 ± 11.88	15.49 ± 10.69	17.93 ± 2.41	420.85 ± 193.67
E3/E4	152.95 ± 63.36	74.54 ± 25.09	96.36 ± 45.34	24.25 ± 12.73	13.93 ± 7.88	20.03 ± 4.72	390.07 ± 126.87
p	0.564	0.313	0.686	0.676	0.572	0.947	0.531

Data are presented as mean ± standard deviation (SD) and expressed in µmol/g of feces.

Table 6 Correlation between *E. brachy* group abundance and body composition parameters

		Correlation coefficient	q
*E. brachy*_group	Total fat mass (g)	0.404355	0.045306
*E. brachy*_group	Gynoid fat mass (g)	0.438997	0.045306
*E. brachy*_group	Adiposity index	0.397278	0.048242

a member of the *Puniceicoccaceae* family (Fig. 1). These findings highlight a substantial impact of the ApoE4 genotype on gut microbiota composition.

Short chain fatty acids

The comparison of fecal SCFA concentrations between ApoE3/3 and ApoE3/4 carriers revealed no statistically significant differences in any of the measured SCFAs (all p-values > 0.05) (Table 5).

Correlation and multivariate associations

Spearman correlation analysis revealed that, among the three genera associated with the presence of the E4 allele, the *Eubacterium brachy* group was positively correlated with several body composition parameters, particularly those related to fat mass (Table 6). To further explore these associations, MaAsLin 2 analysis was used to identify significant relationships between the abundance of microbial taxa that differed between the two genotype groups and body composition parameters. The results from MaAsLin 2 confirmed the findings from the correlation analysis, revealing a positive association between the *Eubacterium brachy* group and gynoid fat mass ($\beta = 1.06$, $q = 0.027$). This suggests that this bacterial taxon may play a role in body fat distribution.

PICRUST

The PICRUSt2 pipeline was used to predict the functional gene content of the gut microbiota in individuals with ApoE3/E3 and ApoE3/E4 genotypes. We identified significant differences in the predicted functional profile between ApoE3/E3 and ApoE3/E4 groups. Four activities associated with the carotenoid biosynthesis pathway exhibited significantly higher abundance in ApoE4

carriers compared to non-carriers: carotene desaturase (EC:1.3.99.26), phytoene desaturase (neurosporene-forming, zeta-carotene-forming, and lycopene-forming; EC:1.3.99.28, EC:1.3.99.29, EC:1.3.99.31) (Fig. 1A). These carotenoid desaturases are crucial critical for the conversion of phytoene into more oxidized carotenoids, leading to the synthesis of lycopene, β-carotene, and other carotenoids. Moreover, we also observed a major abundance of the EC:2.4.1.216 (trehalose 6-phosphate phosphorylase) and EC:3.5.1.90 (adenosylcobinamide hydrolase) whereas the enzymes EC:2.1.3.1 (methylmalonyl-CoA carboxytransferase) and EC:3.1.1.17 (gluconolactonase) had less representation in the microbiota of ApoE4 carriers (Fig. 2A). Regarding the KEGG Orthology (KO) we found more abundance of the K08260 (adenosylcobinamide hydrolase), K11004 (ATP-binding cassette, subfamily B, bacterial HlyB/CyaB), K06955 (uncharacterized protein), K06876 (phrB; (6–4)DNA photolyase), K07046 (L-fucono-1,5-lactonase), K12516 (bigA; putative surface-exposed virulence protein) and less presence of the K13501 (indole-3-glycerol phosphate synthase), K18925 (paired small multidrug resistance pump), K18924 (paired small multidrug resistance pump), K11623 (two-component system, NarL family, sensor histidine kinase), K17489 (methylmalonyl-CoA carboxyltransferase 12 S subunit), K01053 (gluconolactonase) in the ApoE4 carriers in comparison to non-carriers (Fig. 2B).

Discussion

Genetic factors play a crucial role in shaping gut microbiota composition and function. Previous studies conducted by our research team have demonstrated that the VDR gene TaqI (rs731236) polymorphism influences microbial diversity and composition in Caucasian populations. González-Soltero et al. (2024) reported that individuals carrying different variants of this polymorphism exhibit distinct microbial profiles, with significant differences in bacterial diversity and functional pathways. Given the well-established role of the vitamin D receptor (VDR) in immune modulation and gut homeostasis, these findings suggest that genetic variation in VDR could contribute to host-microbiome interactions, potentially impacting metabolic and inflammatory responses. Likewise, our group has previously explored the influence of PPARD and PARGC1A polymorphisms on gut microbiota composition, highlighting their role in metabolic regulation and energy homeostasis (Bailén et al. 2022). These findings emphasize the complexity of host-genome interactions with gut microbiota and their potential relevance in metabolic health.

In the context of APOE4 and gut microbiota, understanding genetic contributions to microbial composition is essential. This study examines for the first time the influence of APOE genotype on gut microbiota in cognitively healthy adults. Our findings demonstrate that the APOE4 allele is associated with distinct gut microbiome compositions and functions, even in a young, healthy population. The most pronounced taxonomic difference was a markedly lower abundance of the genus *Megamonas* in APOE4 carriers approximately five-fold less than in non-E4 individuals. This result is noteworthy because *Megamonas* has been linked to metabolic outcomes: higher *Megamonas* levels are associated with overweight and obesity (Palmas et al. 2021), and greater adiposity in adults correlating positively with body mass index (BMI) and fat mass (Lauw et al. 2023). Elevated *Megamonas* has also been reported in children with obesity (Maya-Lucas et al. 2019) and in adults with non-alcoholic fatty liver disease (Zhou et al. 2022), and its levels decrease following a mediterranean hypocaloric weight-loss intervention (Pisanu et al. 2020). The reduced *Megamonas* in APOE4 carriers observed here aligns with clinical studies reporting that APOE4 carriers tend to have lower body weight and fat mass than non-carriers in certain populations. For example, women with mild cognitive impairment who carry APOE4 exhibit lower adiposity than those without APOE4 (Ando et al. 2022), and APOE4 has been associated with a more favorable lean mass in individuals with normal BMI and a lower android-to-gynoid fat ratio among overweight and obese individuals (Ozen et al. 2022). Our findings align with previous observations in AD patients where *Megamonas* abundance was significantly lower in individuals with progressive cognitive impairment compared to those without progression. Notably, the progressive group had twice the percentage of APOE4 carriers (Yang et al. 2023). Another study reported that APOE4 was associated with reduced *Megamonas* abundance in AD patients compared to cognitively healthy individuals (Hou et al. 2021). However, we did not find a significant association between *Megamonas* abundance and body composition parameters. This discrepancy could be due to several factors of this study, the small sample size, particularly the low number of APOE4 carriers, which reflects the relatively low prevalence of the APOE*ε4 allele in the general healthy population. Besides, the younger age of the studied population may influence their nutritional and behavioral habits and impact in the final body composition. Furthermore, there are very few studies investigating the relationship between APOE genotype and body composition parameters in healthy individuals. It is also possible that the impact of *Megamonas* on body composition becomes more pronounced over time, particularly in aging individuals or those with metabolic alterations.

Beyond *Megamonas*, we identified other taxa changes that support this link. APOE4 carriers showed a depletion of the *Eubacterium brachy* group, a strictly anaerobic genus in the *Firmicutes* phylum known for

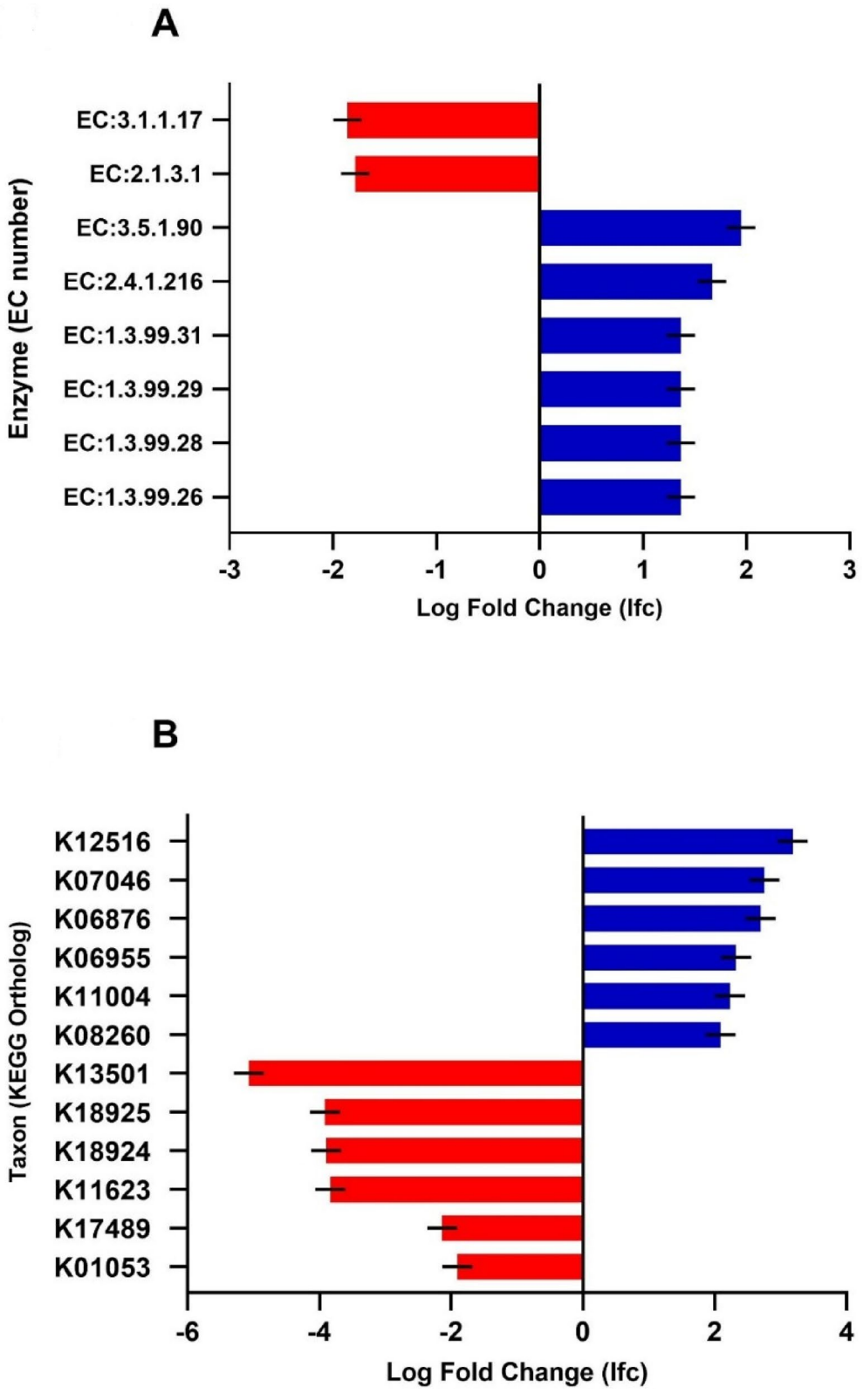

Fig. 2 Differential abundance of inferred microbial functions (EC numbers and KEGG Orthologs). Bar chart showing differences in enzyme abundance (Enzyme Commission, EC numbers) **A** and KEGG Ortholog (KO) functions **B** between ApoE4 and ApoE3 carriers. The log-fold change (lfc) represents the relative increase (blue bars) or decrease (red bars) of each function in ApoE4 compared to ApoE3. Error bars indicate the standard error of the log-fold change

fermenting complex polysaccharides and contributing to caloric extraction (Mukherjee et al. 2020). Information on *E. brachy* is limited, but bacteria from this genus have been associated with obesity and higher fat deposition in humans. In our cohort, *E. brachy* abundance correlated positively with several measures of body fat, mirroring prior reports that higher *Eubacterium* levels accompany greater adiposity (Pinart et al. 2021). Thus, the lower *E. brachy* in APOE4 carriers further indicates a gut microbiome skewed toward a lower capacity for energy harvest, consistent with the relatively lower fat mass observed in APOE4 individuals in previous studies (Ando et al. 2022; Ozen et al. 2022). On the other hand, an uncharacterized genus in the family *Puniceicoccaceae* was enriched in APOE4 carriers. Little is known about this taxon's function and its health implications; to date it has only been noted in a study of Parkinson's disease progression (Aho et al. 2019).

In addition to taxonomic differences, our study provides insight into functional alterations in the gut microbiome associated with APOE4. Using PICRUSt2 to infer metagenomic functional potential, we found that the microbiota of APOE4 carriers could be metabolically different. One finding was the enrichment of carotenoid biosynthesis pathways in APOE4-associated microbiota. Multiple carotenoid-producing enzyme genes (e.g., EC 1.3.99.26/28/29/31, involved in β-carotene and lycopene biosynthesis) were predicted to have a higher abundance in APOE4 carriers. This suggests an elevated microbial capacity to synthesize carotenoids in the gut. We interpret this as a potential compensatory mechanism as APOE4 carriers are known to have lower circulating carotenoid and fat-soluble vitamins levels than APOE3 carriers likely due to differences in lipid transport and higher carotenoid clearance rates (Huebbe et al. 2016; Ma et al. 2021; Sanchez-Muniz et al. 2009). Our findings suggest that the gut microbiome of APOE4 individuals may adapt to compensate for lower systemic carotenoid levels by enhancing microbial carotenoid production locally. The microbiota plays a crucial role in vitamin bioavailability, not only by metabolizing and modifying vitamins but also by influencing their absorption in the gut. Additionally, the composition and function of the gut microbiome are, in turn, shaped by vitamin availability and dietary supplementation (Barone et al. 2022). Further supporting this idea, there is a study in mice with a genetic deficiency in retinoid metabolism has shown that, despite having similar fecal bacterial taxa, their microbial functional composition differs significantly depending on whether they were on a vitamin A-sufficient or vitamin A-deficient diet. This suggests that vitamin availability can drive microbiome functional shifts, even in the absence of major taxonomic changes (Honarbakhsh et al. 2021). Carotenoids have antioxidant properties, and enhanced microbial production might help counteract oxidative stress in the gut environment. This is particularly relevant given that APOE4 status and AD are associated with elevated oxidative stress and inflammation (Mirzaei et al. 2024). It remains to be determined whether these predicted functions lead to measurably higher luminal or circulating carotenoid levels, or even changes in oxidative stress levels in specific regions of APOE4 carriers.

Another notable functional difference was observed in carbohydrate metabolism, specifically trehalose utilization. APOE4 carriers showed a potential increased abundance of the enzyme trehalose-6-phosphate phosphorylase (EC:2.4.1.216) in their gut microbiota. Trehalose is a dietary disaccharide that has garnered attention for its neuroprotective effects—it can induce autophagy, reduce amyloid-β accumulation, and mitigate neurodegenerative processes in experimental models of AD (Liu et al. 2020). There is also evidence that trehalose's benefits might be partly mediated by the gut microbiome or gut-brain signaling (Khalifeh et al. 2020). Our finding of enhanced trehalose metabolism by APOE4-associated microbes suggests that more trehalose may be broken down by the microbiota in APOE4 carriers, potentially reducing its bioavailability to the host raising the possibility that alterations in trehalose metabolism within the gut microbiota could impact brain health, particularly in ApoE4 carriers.

We also found differences in vitamin B12 (cobalamin) related pathways that may have functional implications. An enzyme involved in the bacterial cobalamin salvage pathway, adenosylcobinamide hydrolase (EC:3.5.1.90; K08260), was more abundant in the predicted metagenome of APOE4 carriers. In gut bacteria, this enzyme helps specifically in the cobalamin salvage pathway. The upregulation of this pathway could indicate that the microbiota of APOE4 carriers experiences relative vitamin B12 scarcity and is adjusting by increasing B12 recycling. This idea is supported by a study showing that after B12 supplementation bacteria downregulate their own B12 biosynthesis (Kelly et al. 2019). Vitamin B12 plays a crucial role in various physiological processes relating to brain health, including homocysteine/methionine metabolism, nerve function, energy production, and synaptogenesis (Lauer et al. 2022). AD has been linked to higher homocysteine levels only in non APOE4 carriers, suggesting possible differences in B-vitamin handling (Lin et al. 2025). Additionally, B12 is necessary in propionate synthesis (Kelly et al. 2019) serving as cofactor for the methylmalonyl-CoA carboxytransferase (EC:2.1.3.1). Interestingly, we noted a lower abundance of a propionate-producing enzyme (methylmalonyl-CoA carboxytransferase) in APOE4 carriers' microbiota. We measured fecal SCFA and found no significant difference

in propionate levels between APOE4 and APOE3 groups, suggesting that any microbial B12 functional differences were not large enough to alter propionate fecal levels.

Another inferred enzyme with reduced representation in the microbiota of APOE4 carriers was gluconolactonase (EC:3.1.1.17, K01053), which plays a role in carbohydrate metabolism and NADPH production. A decrease in this enzyme could impair the antioxidant capacity of both the microbiome and the host. Notably, higher levels of NADPH oxidase 2 and increased oxidative stress have been observed in patients with neurodegenerative diseases (Loffredo et al. 2020), suggesting a potential link between APOE4-associated microbiome alterations and oxidative stress-related neurodegeneration.

The APOE4-associated microbiota had a lower predicted capacity for synthesizing tryptophan and its downstream metabolites. The enzyme indole-3-glycerol phosphate synthase (essential for tryptophan biosynthesis in bacteria) was among the most depleted functions in APOE4 carriers. Gut bacteria can convert tryptophan into serotonin and a variety of indole derivatives that influence intestinal barrier integrity, immune modulation, and even metabolic signaling. Notably, certain microbial tryptophan metabolites such as indolepropionic acid and indolelactic acid have antioxidant and anti-inflammatory effects (Roager and Licht 2018), and anti-obesogenic properties as observed with tryptamine (Lee et al. 2022). Again, these results support the idea that APOE4 carriers are less protected against oxidative stress and the associated tissular damages. Beyond metabolism of nutrients and neuroactive compounds, several microbial pathways related to environmental stress resistance and potential virulence were altered in APOE4 carriers, suggesting that APOE4 may modulate the composition and functional capacity of the gut microbiota, potentially altering host-microbe interactions in a manner that affects intestinal homeostasis, microbial colonization dynamics, and susceptibility to pathogenic challenge. We found a significant reduction in bacterial multidrug resistance (MDR) efflux pumps functions in APOE4-associated microbiota. These small MDR pumps (SMR family K18924 and K18925) help bacteria expel toxic substances, including antibiotics and oxidative byproducts (De Gaetano et al. 2023). Similarly, we observed a decrease in a bacterial nitrate-responsive sensor kinase (NarL family K11623) in APOE4 microbiota. In enteric bacteria, nitrate sensors are key for switching to nitrate respiration under low-oxygen conditions and can regulate virulence factors in pathogens like *E. coli* and *Salmonella* (Gushchin et al. 2021).

Conversely, a few predicted functions suggested enhanced colonization factors or catabolic capabilities in APOE4 microbiota. We detected higher levels of a gene (K11004) coding for an ATP-binding cassette (ABC) transporter subunit, analogous to the *Escherichia coli* HlyB protein, which is involved in secreting hemolysin and related toxins (Moussatova et al. 2008), also other bacteria such as *Vibrio parahaemolyticus*, *Enterobacter cloacae*, and *Morganella morganii* possess homologous type I secretion systems to export their toxins, contributing to intestinal disorders (Anlauf et al. 2024). Additionally, the APOE4 group showed increased abundance of the BigA protein (K12516), a surface-associated adhesin implicated in bacterial biofilm formation (identified in uropathogenic *E. coli* strains) and possibly gut colonization (Allsopp et al. 2010). We also noted the enrichment of a fucose metabolism enzyme the L-fucono-1,5-lactonase (K07046) in APOE4 carrier's microbiota. Fucose is a sugar abundantly present in the intestinal mucus (as part of fucosylated glycans). Microbes that can liberate and utilize fucose can gain a competitive advantage and often contribute to gut health by producing SCFAs and by outcompeting pathogens for nutrients. However, L-fucose can also serve as an energy source for enteropathogens such as *Campylobacter jejuni* (Pickard and Chervonsky 2015).

Conclusion

In summary, our study suggests that the presence of an APOE4 allele influences gut microbiota composition and metabolic potential in healthy adults. The association of APOE4 with a leaner-associated microbiota might partly explain why APOE4 carriers often exhibit lower BMI or distinct fat distribution. APOE4 carriers are at a higher risk for Alzheimer's disease (AD), and our findings suggest that the gut microbiota of APOE4 individuals may exhibit distinct metabolic characteristics that could influence AD risk.

Limitations and future directions

One limitation of this study is the small sample size, particularly the low number of APOE4 carriers, which reflects the relatively low prevalence of the APOE*ε4 allele in the general healthy population. This limits statistical power and may contribute to variability in the results. On the other hand, there are very few studies investigating the relationship between APOE genotype and body composition parameters in healthy individuals, and therefore this study contributes to increasing the knowledge in this field. Most existing research has focused on older individuals with Alzheimer's disease, where factors such as neuroinflammation, metabolic changes, and gut dysbiosis may confound results. All these circumstances make direct comparisons with our findings challenging.

Future studies should aim to increase cohort sizes, explore a broader range of age groups, and conduct longitudinal analyses to assess how APOE genotype interacts

with gut microbiota over time. It will also be critical to perform metabolomic profiling to directly measure microbial-derived metabolites, such as carotenoids, SCFAs, and tryptophan derivatives, in APOE4 carriers.

Abbreviations

ABC	ATP-binding cassette
AD	Alzheimer's disease
AI	Adiposity index
ANCOM-BC	Analysis of compositions of microbiomes with bias correction
APOE	Apolipoprotein E
BMI	Body mass index
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
EC	Enzyme Commission
FFQ	Food Frequency Questionnaire
GC	Gas chromatography
GWAS	Genome-wide association study
HDL	High-density lipoprotein
ITT	Intention-to-treat
KO	KEGG ortholog
LPA	Light physical activity
MaAsLin	Microbiome Multivariable Association with Linear Models
MDR	Multidrug resistance
MVPA	Moderate-to-vigorous physical activity
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NSTI	Nearest Sequenced Taxon Index
PCR	Polymerase chain reaction
PICRUSt2	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
QIIME2	Quantitative insights into microbial ecology 2
RNA	Ribonucleic acid
SCFA	Short-chain fatty acid
SD	Standard deviation
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
VAT	Visceral adipose tissue
VDR	Vitamin D receptor
VLDL	Very low-density lipoprotein

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Author contributions
CB contributed to formal analysis, methodology, software, and writing—review & editing. RGS was responsible for formal analysis, funding acquisition, investigation, methodology, validation, writing—original draft, and writing—review & editing. MT contributed to investigation, methodology, and writing—review & editing. SC participated in formal analysis, funding acquisition, investigation, methodology, validation, writing—original draft, and writing—review & editing. BG was involved in validation, writing—original draft, and writing—review & editing. ML contributed to investigation, funding acquisition, validation, writing—original draft, and writing—review & editing.

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Data availability
The samples included in this study are part of the NCBI BioProject (Accession No. PRJNA647292) and are associated with the following SRA accession numbers: SRS5367680, SRS5367682–SRS5367684, SRS5367687, SRS5367689, SRS5367691, SRS5367694–SRS5367698, SRS5367700–SRS5367703, SRS5367706–SRS5367707, SRS5367709–SRS5367710, SRS5367712–SRS5367718, SRS5367720–SRS5367722, SRS5367725–SRS5367728, SRS5367730–SRS5367732, SRS5367735, SRS5367737–SRS5367741, SRS5367743–SRS5367746, SRS5367748–SRS5367757, SRS5367759–SRS5367768, SRS5367771, SRS5367774–SRS5367775, SRS5367778–SRS5367781, SRS5367784–SRS5367785, SRS5367787.The data obtained and / or analyzed in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Research Ethics Committee of the Community of Madrid (CEIm-R) (Ref: 47/560280.9/18) and registered on ClinicalTrials.gov (Accession No. NCT02901912). Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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