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ORIGINAL ARTICLE

Glutamate interactions with obesity, insulin resistance, cognition and gut microbiota composition

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Abstract

Aims To investigate the interactions among fecal and plasma glutamate levels, insulin resistance cognition and gut microbiota composition in obese and non-obese subjects.

Methods Gut microbiota composition (shotgun) and plasma and fecal glutamate, glutamine and acetate (NMR) were analyzed in a pilot study of obese and non-obese subjects ($n=35$). Neuropsychological tests [Trail making test A (TMT-A) and Trail making test B (TMT-B)] scores measured cognitive information about processing speed, mental flexibility and executive function.

Results Trail-making test score was significantly altered in obese compared with non-obese subjects. Fecal glutamate and glutamate/glutamine ratio tended to be lower among obese subjects while fecal glutamate/acetate ratio was negatively associated with BMI and TMT-A scores. Plasma glutamate/acetate ratio was negatively associated with TMT-B. The relative abundance (RA) of some bacterial families influenced glutamate levels, given the positive association of fecal glutamate/glutamine ratio with Corynebacteriaceae, Coriobacteriaceae and Burkholderiaceae RA. In contrast, Streptococaceae RA, that was significantly higher in obese subjects, negatively correlated with fecal glutamate/glutamine ratio. To close the circle, Coriobacteriaceae/Streptococaceae ratio and Corynebacteriaceae/Streptococaceae ratio were associated both with TMT-A scores and fecal glutamate/glutamine ratio.

Conclusions Gut microbiota composition is associated with processing speed and mental flexibility in part through changes in fecal and plasma glutamate metabolism.

Keywords Microbiota · Metabolomics · Glutamate · Trail making test · Cognition

Introduction

Gut microbiota is known to produce a wide repertoire of compounds that may potentially play an important role in brain function (the so called gut microbiota-brain axis) and behavior. This axis is a bidirectional communication system and involves neuronal, endocrine, immune and metabolic pathways, although there is still a long way to decipher its physiology [1]. There is some evidence that bacterial commensals may regulate neurotransmission, neurogenesis, brain inflammatory status and activation of the hypothalamic–pituitary–adrenal [2]. Microbiota is characterized by its important plasticity, changing dramatically and rapidly in response to diet [2]. The neurochemicals produced by both multicellular organisms and prokaryotes, such as serotonin, GABA or glutamate,

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are considered part of the language that uses the gut–microbiota–brain system [3].

Glutamate, the main neurotransmitter of the central nervous system, is a non-essential amino acid that plays a key role in the development and function of normal brain activity, regulating communication between neurons and brain plasticity. Glutamate is a key factor involved in memory and learning [3, 4]. When glutamate concentrations are low in the brain gray matter, evaluated using magnetic resonance spectroscopy, there is a poorer performance in several cognitive tests, like the trail making test-A (TMT-A) [5]. This is a neuropsychological test that provides information about visual and motor processing speed, while trail making test B (TMT-B) provides information about flexibility and executive function. In both tests, shorter times to completion indicate better performance [6, 7].

Biochemically, glutamate is close to the entry point of inorganic nitrogen, there are two pathways in enteric bacteria: glutamate by the action of glutamine synthetase and glutamate synthase from glutamine, or by the enzyme glutamate dehydrogenase (GDH) from 2-oxoglutarate. Glutamine Synthetase/Glutamate-glutamine-oxoglutarate aminotransferase [GS/GOGAT] cycle into organic nitrogen metabolism [8, 9]. Acetate and butyrate are the products that result from the fermentation of glutamate [10], being acetate the most common short chain fatty acids in the human colon, produced in the large intestine by anaerobic intestinal microbiota through fermentation of non-digestible carbohydrate [11]. Glutamine plasma level was significantly higher after weight loss compared with their baseline values in a 3-week weight loss program [12], and other studies have confirmed increased levels of plasma glutamine levels after body mass reduction [13, 14]. In fact, plasma glutamine/glutamate ratio was associated with insulin resistance (HOMA-IR) [15].

As glutamate is also central to nitrogen metabolic circuits of enteric bacteria, and some bacterial species (Bacteroides thetaiotaomicron) have been associated with increased glutamate levels [16, 17], we hypothesized that glutamate levels in plasma and feces could be influenced by the composition of the gut microbiota. As we described altered TMT-A scores in subjects with obesity in a previous study [14], we explored the potential associations of TMT-A with glutamate levels and gut microbiota composition. We explored glutamate/glutamine ratio as a proxy measure of glutamate generation and glutamate/acetate ratio evaluating glutamate degradation.

Methods

Subjects

We recruited 35 subjects (19 obese and 16 non-obese subjects that were similar in age and sex) at the Endocrinology Service of the Hospital Universitari Dr. Josep Trueta (Girona, Spain) from January to September 2012. Inclusion criteria were age from 30 to 65 years, and ability to understand study procedures. Systemic diseases, infection in the previous month, serious chronic illness, > 20 g ethanol intake/day, or use of medications that might interfere insulin action were exclusion criteria. The institutional review board approved the study protocol and all subjects gave written informed consent, after the nature and potential risks for the study were explained to them.

Analytical methods

Fasting plasma glucose, insulin, HOMA-IR, HDL-cholesterol, fasting triglycerides, plasma LBP and C-reactive protein concentration measurement were performed as previously described [18].

Metabolomic analysis

For metabolomic analyses, plasma and feces were collected after an overnight fast and stored at − 80 °C until NMR measurement. For analysis, plasma samples were thawed on ice. 300 µl of 10% D_2O buffer (0.04% NaN_3, 140 mM Na_2HPO_4, 5 mM TSP, pH 7.4) were pour into 300 µl of plasma sample. Then, 550 µl of the sample was transferred to a 5-mm NMR tube for analysis. Feces, were treated after thawing samples on ice. 100 mg of sample were mix with 1 ml 0.1 M PBS (pH 7.4) and submitted to 3 freeze/thaw cycles with liquid nitrogen. Then, sample was centrifuge at 12,000*g* for 20 min. Supernatant was filtered through a 0.22 µm filter, collected and stored at − 80 °C until NMR measurement. For feces extracts, 1H NMR spectra were acquired at 27 °C on a Bruker AVI-600 using a 5 mm TCI cryoprobe and processed using Topspin3.2 software (Bruker Biospin). ^{1}H 1D NMR spectra with water presaturation (25 Hz) and a noesy mixing time of 10 ms were acquired with 256 free induction decays (FIDs). 64 k data points were digitalized over a spectral width of 30 ppm for an optimal baseline correction. A 4 s relaxation delay was incorporated between FIDs. The FID was multiplied by an exponential function with a 0.5 Hz line broadening factor. For plasma, a Carr–Purcell–Meiboom–Gill (CPMG) spin-echo pulse sequence, which generates spectra edited by T2 relaxation times with reduced signals from high molecular weight species and improved resolution of low molecular weight metabolite resonances, was acquired with a total of 16 accumulations and 72 K data points over a spectral width of 16 ppm. A 4-s relaxation delay was included between FIDs and a water presaturation pulse of 25 Hz was applied.

The parameters for 2D experiments were 512 increments in t1 and 32 FIDS for total correlation spectroscopy (TOCSY) experiments with MLEV pulse sequence, and 256

t1 increments and 96 FIDS for HSQC (Heteronuclear Single Quantum Correlation) experiments. Both experiments had a relaxation delay of 1.5 s and were acquired in the phase-sensitive mode. The mixing time for TOCSY spectra was set to 65 ms.

Glutamate and glutamine metabolites were assigned to the signals in the ^{1}H-NMR using 2D experiments, human metabolome database [19] and the biological magnetic resonance bank database [20]. Spectra were normalized to total intensity to minimize the differences in concentration and experimental error during the extraction process. Optimal integration regions were defined for each metabolite, an attempt being made to select the signals without overlapping. Integration was performed with global spectra deconvolution in MestreNova 8.1.

Gut microbiota composition

Stool specimens were obtained from patients using sterile containers and were immediately frozen in liquid nitrogen and stored at − 80 °C until analysis. Samples were processed individually using the Fast DNA Spin Kit for faeces (MP Biomedicals, Solon, OH). Briefly, a frozen aliquot (400 mg) of each sample was added to a 2 mL tube containing 825 µL sodium phosphate buffer, 275 µL of pre-lysis solution and Lysing matrix E, a mixture of ceramic and silica particles designed to efficiently lyse all stool microorganisms. Each extraction tube was agitated twice for 40 s using a Fast Prep FP120 instrument at a speed setting of 6 to ensure proper extraction of fungal DNA, a crucial point in the methodology. Tubes were cooled on ice between the different agitation procedures. DNA extraction was then carried out following the manufacturer's instructions. The quantity and quality of isolated DNA was determined with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

Applying the following parameters: min_length: 50, trim_qual_right: 20, trim_qual_type: mean, and trim_qual_window: 20. R1 and R2 from Illumina sequencing where joined using *fastq-join* from each-tools suite. Three files per sample were obtained, one of joined pairs of reads and two of not joined reads. The fastq files were converted into fasta files using the 'fastq_to_fasta' tool from the FastX-Toolkit program. Those files were filtered from human contamination using bowtie2program [21].

The unaligned files, i.e., those that did not map against the human genome, were the input files of a BLASTn search against a customized bacterial database (Bacteria_2015_06_09) consisting of the human microbiome and the bacterial genomes downloaded from the NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/genomes/human microbiome/Bacteria/ and updated to July 2014 and June 2015, respectively). The best hits of the BLASTn output files were extracted, converted into contingency tables and transformed into BIOM format to be used as input files of the Quantitative Insights Into Microbial Ecology (QIIME) open-source software pipeline version 1.9.0, that implemented the RA calculations of the bacterial hits at different taxonomical levels. This pipeline was also used to estimate the alpha diversity or diversity within samples, through the calculation of the Shannon diversity index of the samples, using the following parameters for rarefactions: 20 steps of 40 iterations each, comprised between 100 and 213,096 reads (the number of reads of the smallest sample) with increments of 10,654 reads per step. Diversity was calculated for the first and second visit of the subjects. Beta diversity or diversity among samples was also estimated to generate Principal Coordinates Analysis (PCoA) of the samples, from Bray- Curtis dissimilarity and Canberra distance matrices.

Neuropsychological assessment TMT-A and TMT-B

The TMT-A (TMT-A, greater focus on attention) consisted of a standardized page in which numbers 1 to 25 are scattered within the circles, and participants were asked to connect the numbers in order as quickly as possible. Before starting the test, a 6-item practice test was administered to the participants to make sure they understood both tasks. A maximum time of 300 s was allowed before suspending the test. The direct scores of TMT-A were the time in seconds taken to complete each task. In the same way, TMT-B (Trail B, greater focus on executive function) consisted of an alternating sequence of numbered circles and letters [6, 22]. In both tests, shorter times to completion indicate better performance.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 statistical package for Windows (IBM Corp., 188 Armonk, NY, USA) and R Commander (the R Foundation). Parameters that did not fulfill normal distribution were logarithmically transformed to improve symmetry for subsequent analyses. Descriptive results of continuous variables are expressed as mean and standard deviation (SD) or median and their interquartile range as appropriate for the distribution of variables. Analysis student unpaired *t* tests were used to evaluate the effects of obesity. Bivariate correlations (Spearman' tests) and multiple linear regression analysis were performed to test the independent associations between bacterial family relative abundances and metabolic parameters and cognitive tests. Levels of statistical significance were set at $p < 0.05$.

Results

Characteristics of the subjects in this cohort according to obesity status are shown in Table 1. HOMA-IR was significantly increased among obese participants while age and years of education did not show significant differences between groups (Table 1).

We detected two signals indicative of fecal glutamate. One of them had the cleanest signal (purity greater than 95%) while the other had a glutamine-overlapped signal (purity greater than 80%, here named glutamate*). To calculate the fecal glutamate/glutamine and glutamate/acetate ratio we used that signal with the purity greater than 95%.

Plasma glutamate/glutamine ratio, indicative of glutamate generation from glutamine metabolism, significantly and positively correlated with BMI ($r=0.418$, $p=0.017$), fasting triglycerides ($r=0.62$, $p=0.0001$) and ultrasensitive-CRP ($r=0.55$, $p=0.002$). Fecal glutamate/glutamine ratio was significantly and positively correlated with fasting glucose ($r=0.346$, $p=0.042$). Fecal glutamate/acetate ratio was negatively correlated with fasting triglycerides ($r=-0.436$, $p=0.012$) and ultrasensitive-CRP ($r=-0.414$, $p=0.025$) while fecal glutamate/acetate ratio in feces was negatively correlated with BMI ($r=-0.406$, $p=0.015$) (Table S1).

Trail making test scores are associated with fecal glutamate

TMT-A score was significantly increased among obese patients, indicative of a poorer performance (more time to complete the test) (Table 1 and Figure S1). Fecal glutamate was negatively associated with TMT-A score ($r=-0.377$, $p=0.025$) (Fig. 1a). Fecal glutamate* also significantly correlated with TMT-A ($r=-0.493$, $p=0.0026$) and TMT-B ($r=-0.38$, $p=0.026$) (Fig. 1b), while fecal acetate levels were positively associated with TMT-B ($r=0.512$, $p=0.0002$) (Figure S2A) (Fig. 2).

Fecal glutamate/glutamine ratio showed significant and negative associations with TMT-A ($r=-0.610$, $p=0.0001$), suggesting better performance with increased glutamate/glutamine ratio (Fig. 1c). Glutamate/acetate ratio in feces negatively correlated with TMT-A ($r=-0.455$, $p=0.0061$) and TMT-B ($r=-0.471$, $p=0.0050$) while glutamate/acetate ratio in plasma only positively associated with TMT-B ($r=-0.357$, $p=0.0487$) (Figure S2C).

Fecal glutamate is linked to gut microbiota composition

At family level, we analyzed the possible relationships between the relative abundance (RA) of bacterial families with both fecal and plasma glutamate and/or TMT-A/TMT-B scores. Those highlighted families are shown in figure S3A.

Of the bacterial families showing relatively high RA (over 3%), only Streptococcaceae and Coriobacteriaceae

Table 1 Clinical parameters, fecal and plasma glutamate and glutamine in association with obesity

		BMI < 30	BMI > 30	*p*
N	35	16	19	
Body Mass Index (kg/m^2)	32.657 ± 9.802	23.588 ± 3.216	40.295 ± 6.177	**< 0.0001**
Patient age (years)	51.971 ± 8.322	50.063 ± 10.415	53.579 ± 5.872	0.2427
Education (years)	15.364 ± 2.956	16 ± 3.12	14.765 ± 2.751	0.2361
HOMA-IR	2.404 ± 3.168	0.922 ± 0.418	3.712 ± 3.927	**0.0100**
Trail making test A	38.657 ± 17.555	29.625 ± 9.415	46.263 ± 19.356	**0.0027**
Trail making test B	102.588 ± 52.111	92.933 ± 50.787	110.211 ± 53.229	0.3449
Fecal glutamate (glutamine-overlapped signal) (a.u)	13.494 ± 4.796	14.901 ± 4.086	12.309 ± 5.128	0.1125
Fecal glutamate (a.u)	11.559 ± 6.193	13.734 ± 7.604	9.726 ± 4.064	0.0716
Fecal glutamine (a.u)	12.380 ± 4.687	13.101 ± 5.902	11.773 ± 3.856	0.4474
Fecal acetate (a.u)	67.827 ± 55.575	51.815 ± 48.625	81.31 ± 58.685	0.1190
Glutamate/glutamine ratio in feces	0.958 ± 0.359	1.076 ± 0.359	0.859 ± 0.337	0.0742
Glutamate/acetate ratio in feces	0.477 ± 0.444	0.622 ± 0.465	0.354 ± 0.396	0.0747
Plasma glutamate (a.u)	0.723 ± 0.153	0.705 ± 0.144	0.739 ± 0.164	0.5413
Plasma glutamine (a.u)	1.074 ± 0.173	1.109 ± 0.212	1.042 ± 0.129	0.2786
Plasma acetate (a.u)	0.209 ± 0.032	0.209 ± 0.034	0.209 ± 0.031	0.9630
Glutamate/glutamine ratio in plasma	0.683 ± 0.152	0.644 ± 0.129	0.717 ± 0.167	0.1784
Glutamate/acetate ratio in plasma	3.471 ± 0.516	3.39 ± 0.498	3.542 ± 0.536	0.4171

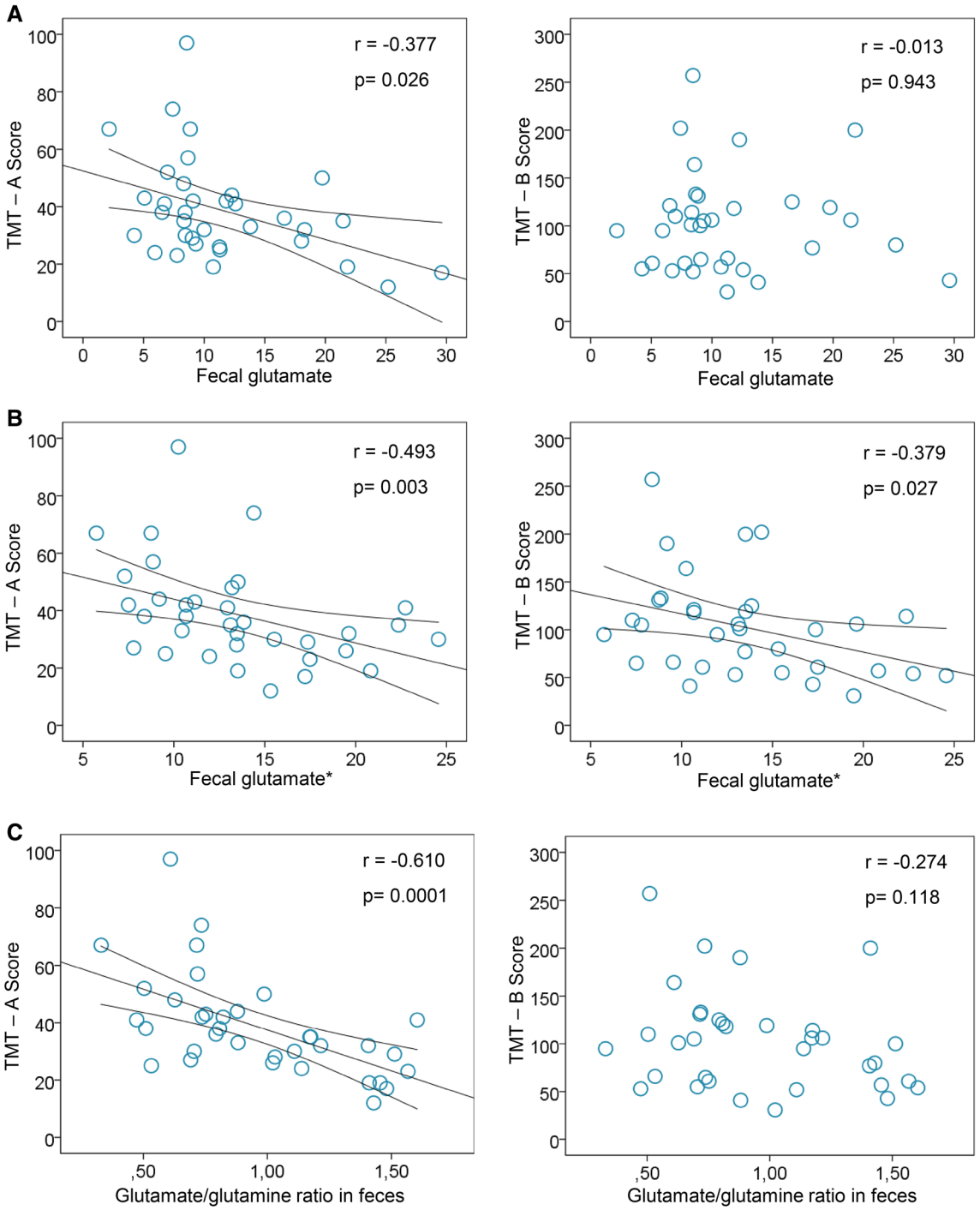

Fig. 1 Bivariate correlations between TMT and fecal glutamate. Fecal glutamate vs. TMT-A and TMT-B (**a**). Fecal glutamate* (Fecal glutamate (glutamine-overlapped signal)) vs. TMT-A and TMT-B (**b**). Glutamate/glutamine ratio in feces vs. TMT-A and TMT-B (**c**)

were significantly associated with TMT, fecal glutamate or plasma glutamate levels. Interestingly, only Streptococaceae RA was significantly increased among obese participants (Table S2). Streptococaceae RA was negatively correlated with fecal glutamate* ($r = -0.458$, $p = 0.016$) and positively associated with TMT-A score ($r = 0.48$, $p = 0.009$) (Fig. 3a) (Fig. 4a). Coriobacteriaceae RA showed significant correlation with plasma glutamate ($r = 0.615$, $p = 0.0011$) and TMT-B ($r = -0.445$, $p = 0.0226$) (Fig. 4b). In addition, Coriobacteriaceae RA was significantly associated with fecal glutamate* ($r = 0.446$, $p = 0.0198$) and showed a trend with TMT-A ($r = -0.325$, $p = 0.09$). Coriobacteriaceae RA

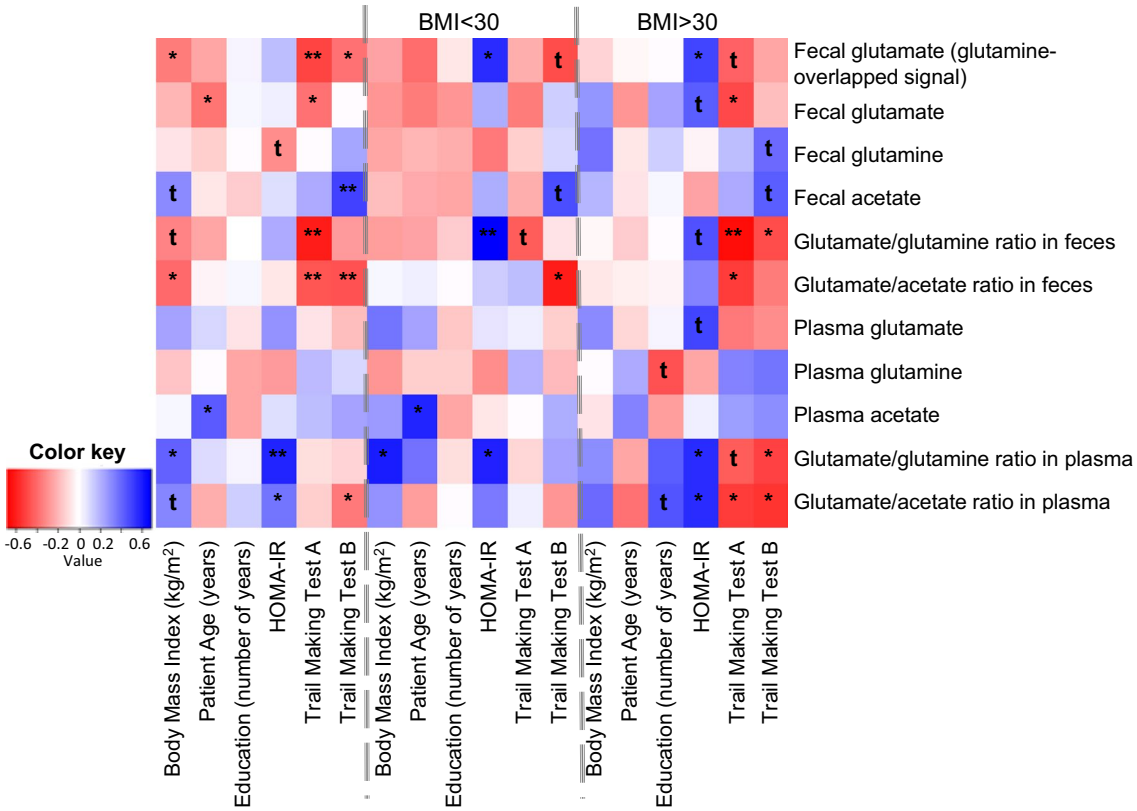

Fig. 2 Bivariate correlations between plasma and fecal glutamate and glutamine and clinical parameters. (*t* for trend $0.05 < p < 0.1$, $*p < 0.05$ and $**p < 0.01$)

was positively associated with fecal glutamate/glutamine ratio ($r = 0.30$, $p = 0.012$), Coriobacteriaceae RA was also significantly and positively correlated with glutamate/acetate ratio in plasma ($r = 0.517$, $p = 0.0081$) and showed a trend with fecal glutamate/acetate ratio ($r = 0.379$, $p = 0.0516$) (Fig. 3a) (Figure S2C).

Of the other families with relatively high RA (over 0.06%), only Corynebacteriaceae and Burkholderiaceae RA had a significant association with both fecal glutamate and TMT-A (Figure S3B). Corynebacteriaceae RA was positively correlated with fecal glutamate ($r = 0.389$, $p = 0.0450$), fecal glutamate/glutamine ratio ($r = 0.47$, $p = 0.013$) and negatively correlated with TMT-A ($r = -0.399$, $p = 0.039$) (Fig. 3a) (Fig. 4d). Burkholderiaceae RA was positively correlated with fecal glutamate ($r = 0.40$, $p = 0.035$) fecal glutamate/glutamine ratio ($r = 0.451$, $p = 0.018$) and negatively correlated with TMT-A ($r = -0.42$, $p = 0.026$) (Fig. 3a) (Fig. 4e).

Of note, fecal glutamate/glutamine ratio was positively correlated with Coriobacteriaceae ($r = 0.300$, $p = 0.0129$), Corynebacteriaceae ($r = 0.471$, $p = 0.0131$), Burkholderiaceae ($r = 0.451$, $p = 0.0182$). On the other hand, glutamate/glutamine ratio in feces was negatively correlated with Streptococcaceae ($r = -0.568$, $p = 0.0020$) (Fig. 3a).

We then explored the possible associations with family RA ratios. Fecal glutamate/glutamine ratio correlated with Coriobacteriaceae/Streptococcaceae ratio (C/S ratio) ($r = 0.60$, $p = 0.0008$), Corynebacteriaceae/Streptococcaceae ratio (CY/S ratio) ($r = 0.64$, $p = 0.0003$), Burkholderiaceae/Streptococcaceae ratio (B/S ratio) ($r = 0.61$, $p = 0.0007$). TMT-A showed association with CY/S ratio ($r = -0.519$, $p = 0.0055$), C/S ratio ($r = -0.551$, $p = 0.0029$) and B/S ratio ($r = -0.494$, $p = 0.0088$). C/S ratio was associated with fecal glutamate/acetate ratio ($r = 0.455$, $p = 0.0171$) and showed a trend with TMT-B ($r = -0.348$, $p = 0.0810$) (Fig. 3b).

Burkholderiaceae RA/Coriobacteriaceae RA ratio (B/C ratio) was associated with plasma glutamate ($r = -0.59$, $p = 0.0016$), plasma glutamate/acetate ratio ($r = -0.543$, $p = 0.005$) and showed a trend with TMT-B ($r = 0.34$, $p = 0.08$) (Fig. 3b).

Burkholderiaceae + Coriobacteriaceae/Streptococcaceae ratio ((B + C)/S ratio) was significantly correlated with fecal glutamate/glutamine ratio ($r = 0.614$, $p = 0.0007$), with fecal glutamate/acetate ($r = 0.454$, $p = 0.0175$) and with TMT-A score ($r = -0.5640$, $p = 0.0022$) (Fig. 3b).

Finally, we performed multivariate linear regression analysis. The variance of TMT-A score was independently

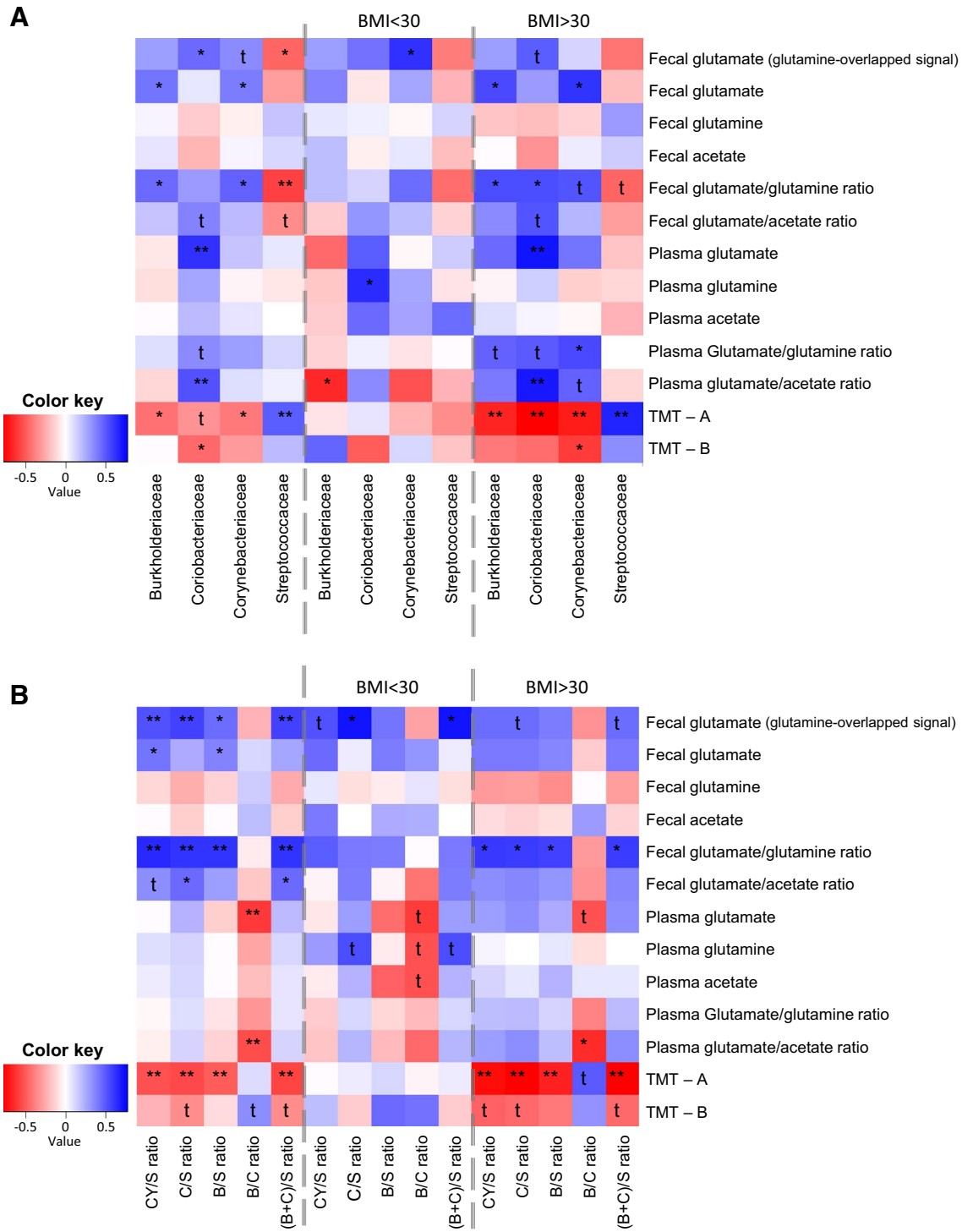

Fig. 3 Bivariate correlations between plasma and fecal glutamate and glutamine and relative abundance of bacterial families (**a**) and bacterial family ratios (**b**). (*t* for trend $0.05 < p < 0.1$, $*p < 0.05$ and $**p < 0.01$)

explained by Streptococcaceae, Coriobacteriaceae, Corynebacteriaceae, and Burkholderiaceae RA even after controlling for age and BMI (Table S3A). Furthermore, the variance of TMT-A score and TMT-B score were independently explained by B/C ratio even after controlling for age and BMI (Table 2b and Table S3B).

The variance of fecal glutamate/glutamine ratio was independently predicted by Corynebacteriaceae RA and CY/S

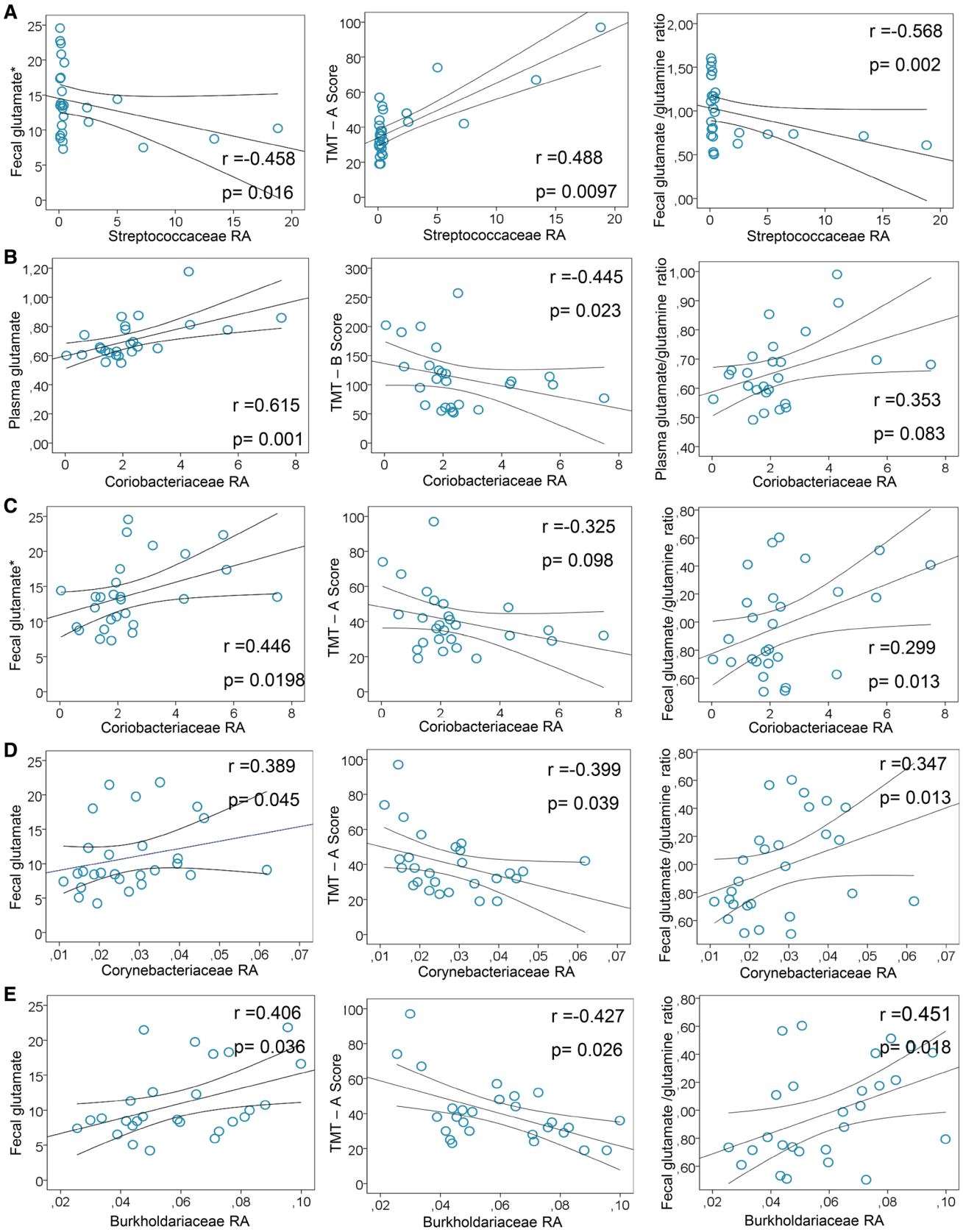

Fig. 4 Bivariate correlations graphs between fecal glutamate. TMT and the relative abundance of several bacterial families

Table 2 Multiple linear regression analysis to predict the contribution of bacterial families to TMT-A

TMT-A	β	p	TMT-A	β	p	TMT-A	β	p	TMT-A	β	p
(A)											
Patient age	0.143	0.27	Patient age	0.202	0.213	Patient age	0.13	0.414	Patient age	0.241	0.112
BMI (kg/m^2)	0.113	0.425	BMI (kg/m^2)	0.539	0.003	BMI (kg/m^2)	0.554	0.002	BMI (kg/m^2)	0.388	0.014
Streptococcaceae RA	0.705	<0.0001	Coriobacteriaceae RA	− 0.432	0.014	Corynebacteriaceae RA	− 0.486	0.006	Burkholderiaceae RA	− 0.501	0.002
Adjusted R^2	0.603		Adjusted R^2	0.367		Adjusted R^2	0.409		Adjusted R^2	0.454	
P value	<0.0001		P value	0.004		P value	0.002		P value	0.001	
(B)											
Patient age	0.206	0.233	Patient age	0.184	0.288	Patient age	0.218	0.208	Patient age	0.206	0.233
BMI (kg/m^2)	0.423	0.019	BMI (kg/m^2)	0.336	0.064	BMI (kg/m^2)	0.312	0.093	BMI (kg/m^2)	0.421	0.019
C/S ratio	− 0.318	0.069	CY/S ratio	− 0.345	0.061	B/S ratio	− 0.336	0.072	(B + C)/S ratio	− 0.320	0.068
Adjusted R^2	0.284		Adjusted R^2	0.291		Adjusted R^2	0.283		Adjusted R^2	0.285	
P value	0.013		P value	0.012		P value	0.014		P value	0.013	

ratio. Other models indicated that fecal glutamate/glutamine ratio variance was explained by (B + C)/S and C/S ratios, Coriobacteriaceae RA, and BMI (Table S4).

Discussion

The plasma profile of metabolites in obese subjects has recently disclosed differences in glutamine and glutamate levels in association with insulin resistance [23]. In fact, plasma glutamate was strongly associated with BMI [24]. Previous studies showed an increased level of glutamine plasma after the body mass reduction [13, 14]. We here confirm positive associations of glutamate/glutamine ratio in plasma with BMI and glutamate/acetate ratio in plasma showed association tendency with BMI. Furthermore, plasma glutamate/glutamine ratio and glutamate/acetate ratio in plasma were associated with HOMA-IR.

Decreased cognitive flexibility in obese individuals is well known [25]. In the present study, we found that fecal glutamate was associated with a decrease in the time required to complete TMT-A, which implies better cognitive function, better performance of visual and motor processing speed, while plasma glutamate/glutamine ratio was associated with improvements in TMT-B scores in the obese group. Glutamate in plasma has been suggested to participate in the glutamate homeostasis of the brain, since its accumulation produces neurotoxicity [26]. In agreement with current results, negative associations between brain glutamate concentration at gray matter (using MR) and TMT-A (5) have been described. In this sense, polymorphisms of the CADM2 gene have been described in association with decreased glutamate, generating a loss of cognitive flexibility [27, 28]. Fecal glutamate/acetate ratio was also associated with TMT-A and TMT-B, while that ratio in plasma was associated with TMT-B score. Therefore, greater proportion of glutamate relative to acetate could be due to decreased catabolism of glutamate, both in plasma and in feces and could indicate better performance in the cognitive tests.

Interestingly, the relative abundance of several bacterial families was associated with glutamate levels. In fact, several bacterial strains such as Corynebacterium glutamicum, Brevibacterium lactofermentum, and Brevibacterium flavum have been extensively used for the industrial fermentative production of glutamate [29]. Corynebacterium glutamicum was isolated in the search to identify a natural glutamate producer in 1956 [30] and widely used as an industrial workhorse for the production of amino acids and various bio-based chemicals [31]. We found that the RA of Corynebacteriaceae, Coriobacteriaceae (phylum Actinobacteria), and Burkholderiaceae (phylum Proteobacteria) were associated with higher fecal glutamate/glutamine ratio. We have also found that Coriobacteriaceae RA was

positively associated with plasma glutamate and negatively with TMT-B. In the literature, on the other hand, the relative abundance of Bacteroides thetaiotaomicron has been described to be linked to lower plasma glutamate levels, maintaining glutamate in a relatively narrow range [24]. We have not explored gut microbiota composition at the species level.

While Burkholderiaceae, Coriobacteriaceae, and Corynebacteriaceae were associated with better performance in the TMT-A, Streptococaceae RA was associated with impairment in the performance of TMT-A, with relative increased RA in the obese group.

Among families of bacteria associated with TMT and fecal and plasma glutamate C/S ratio and CY/S ratio were most consistently associated with TMT-A and its associated glutamate/glutamine ratio in feces. Interestingly, B/C ratio did not remain significantly associated with impairment of TMT-B after controlling for patient age and BMI.

Our study has evaluated multiple associations among gut microbiota composition, cognitive tests and fecal and plasma metabolites. It is important to state that the cross-sectional design is a limitation of our study, and also precludes to extrapolate these findings to the general population.

In summary, current findings suggest that the microbiota could affect the levels of fecal glutamate and acetate, resulting in a better performance in cognitive tests. Whether the modulation of fecal metabolites through changes in diet composition may impact cognitive function should be explored in future studies.

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Author contributions MEPB, MSM and MAR researched the data, performed the statistical analysis and wrote and edited the manuscript. CC and LR researched the data and performed neuropsychological assessment TMT-A and TMT-B MP-S, PG-C and JRH researched the data, performed the 1H-NMR for plasma and feces metabolomic analysis and contributed to the writing and editing of the manuscript. VP-B, AM performed the gut microbiota composition analysis and contributed to the writing of the manuscript. JMM-N, EC-I, RS, JRH, WR contributed to the discussion and reviewed the manuscript. JMF-R Carried out the conception and coordination of the study, contributed to statistical analysis and writing the manuscript and directly participated in the execution of the study. JMF-R is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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