

## Sympathovagal quotient and resting-state functional connectivity of control networks are related to gut *Ruminococcaceae* abundance in healthy men

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### ABSTRACT

**Introduction:** Heart rate variability (HRV), brain resting-state functional connectivity (rsFC), and gut microbiota (GM) are three recognized indicators of health status, whose relationship has not been characterized. We aimed to identify the GM genera and families related to HRV and rsFC, the interaction effect of HRV and rsFC on GM taxa abundance, and the mediation effect of diet on these relationships.

**Methods:** Eighty-eight healthy, young Colombian men were included in this cross-sectional study. HRV metrics were extracted from 24-hour Holter monitoring data and the resting functional connectivity strength (FCS) of 15 networks were derived from functional magnetic resonance imaging. Gut microbiota composition was assessed using the sequences of the V3-V4 regions of the 16 S rRNA gene, and diet was evaluated using a food frequency questionnaire. Multivariate linear regression analyses were performed to evaluate the correlations between the independent variables (HRV metrics and FCS) and the dependent variables (GM taxa abundance or alpha diversity indexes). Mediation analyses were used to test the role of diet in the relationship between HRV and GM.

**Results:** The sympathovagal quotient (SQ) and the FCS of control networks were positively correlated with the abundance of the gut *Ruminococcaceae* family and an unclassified *Ruminococcaceae* genus (*Ruminococcaceae\_unc*). Additionally, the interaction between the FCS of the control network and SQ reduced the individual main effects on the *Ruminococcaceae\_unc* abundance. Finally, reduced habitual fiber intake partially mediated the relationship between SQ and this genus.

**Conclusion:** Two indicators of self-regulation, HRV and the rsFC of control networks, are related to the abundance of gut microbiota taxa in healthy men. However, only HRV is related to habitual dietary intake; thus, HRV could serve as a marker of food choice and GM composition in the future.

### 1. Introduction

Heart rate variability (HRV) reflects the bidirectional interaction between the heart and the central nervous system (CNS) mediated by the autonomic nervous system (ANS). HRV has been proposed as an index of psychophysiological self-regulation (Smith et al., 2020). Higher HRV is related to better self-rated physical and mental health (Jarczok et al.,

2015), while low HRV is related to several physical and mental diseases and an increased all-cause mortality risk (Jarczok et al., 2022).

The sympathovagal quotient (SQ) is a frequency-domain HRV metric initially meant to reflect the ratio between sympathetic and parasympathetic (vagal) heart innervation, reflected by the low-frequency (LF) and high-frequency (HF) metrics, respectively. The SQ is used as an indicator of sympathovagal modulation of the heart. Its increase has

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been related to a variety of physical and mental health disorders, such as weight gain (Costa et al., 2019) and psychiatric stress-related disorders (Schneider and Schwerdtfeger, 2020). In contrast, a decrease in SQ has been associated with psychological well-being (Shiga et al., 2021).

Another critical aspect of human physiology is the gut microbiota (GM), mainly because its impact on metabolism, the systemic immune response, and the nervous system. Consequently, some GM taxa are protective, and others increase the risk of several chronic diseases (Chen et al., 2021). The abundance of GM taxa is variable among individuals and functionally complex, and it is influenced by many intrinsic and extrinsic factors (Falony et al., 2016). An approach to simplifying GM complexity is the categorization of GM taxa into a small number of groups based on the predominance of specific taxa known as enterotypes (Arumugam et al., 2011).

Previous studies addressing the HRV-GM relationship have consistently found that HRV is related to changes in alpha diversity and abundance of the *Lachnospiraceae* and *Ruminococcaceae* families, which belong to the *Firmicutes* phylum (Michels et al., 2019; Mörkl et al., 2022; Tsubokawa et al., 2022). However, methodological flaws limit the scope of some of these studies. For instance, HRV data came from short electrocardiographic (ECG) recordings (Tsubokawa et al., 2022), which are considered unreliable, or the authors did not adjust for medical treatment or other confounders when needed (Mörkl et al., 2022). Finally, the studies so far have been descriptive and have not explained the mechanisms behind the observed HRV-GM association.

Diet is an important factor that may be involved in the HRV-GM relationship. GM composition is significantly modulated by food, and HRV has been associated with many aspects of diet in a bidirectional way, as an indicator of self-regulation ability in food choices and an index of the physiological effects of nutrient intake in the body (Maier and Hare, 2017; Young and Benton, 2018). The neural mechanisms linking HRV with food choices involve prefrontal areas. Maier and Hare (Maier and Hare, 2017) found that subjects with higher HRV were able to resist taste temptation better than those with lower HRV, which was correlated with a reduced representation of taste value in the ventromedial prefrontal cortex (vmPFC). The vmPFC interacts with the dorsolateral prefrontal cortex (dlPFC) to represent food healthiness (Wilson et al., 2023). Additionally, the grey matter volume of the vmPFC and dlPFC predicts cognitive regulation during dietary self-control tasks (Schmidt et al., 2018). Interestingly, both the vmPFC and dlPFC are well-established regulators of HRV (Matusik et al., 2023), and anchored nodes of two correlated large-scale resting-state networks (RSNs), the default mode network (DMN), and the central executive network (CEN), respectively. The DMN is involved in self-referential cognitive processes, and the CEN is involved in executive function (EF). Their interaction is mediated by the salience network (SN) (Bressler and Menon, 2010). Due to its heterogeneity, Yeo et al. subdivided the CEN network into sub-networks (Yeo et al., 2011). One has top-down control over the dorsal attention network (Control A), whereas the other has a stronger connection to the DMN (Control B). Control A drives attention toward task-relevant perceptual information and away from task-irrelevant stimuli and thoughts. In contrast, Control B is activated when attention is driven away from perceptual information and directed toward introspective processes (Dixon et al., 2018).

Extensive cross-sectional studies have investigated the correlation between resting-state functional connectivity (rsFC) and GM in healthy adults from a Chinese cohort (Cai et al., 2021; Zhang et al., 2022; Zhu et al., 2022). The authors reported that structural and functional magnetic resonance imaging (MRI) at rest and under EF tasks correlated with alpha diversity indexes and GM enterotypes. Despite the wide characterization of this relationship, its interpretation is difficult because the enterotype approach does not allow identifying specific taxa related to brain connectivity. Interestingly, some brain areas and RSNs associated with their enterotypes, such as DMN and CEN, are involved in food choices, which raises a question about the role of diet in the correlation between GM, brain connectivity, and EF.

Taken together, these studies suggest that the relationship between HRV, rsFC, and GM significantly impacts health status. However, this interaction needs to be better characterized by addressing some methodological limitations of previous studies, evaluating the relationship between HRV, rsFC, and GM in the same subjects, and exploring the mediation effect of diet on this relationship. Therefore, in this cross-sectional study, we aimed to (1) identify the GM genera and families related to HRV and rsFC, (2) determine whether HRV and rsFC interact in their influence on GM taxa abundance, and (3) explore the mediation effect of diet in the HRV-GM and rsFC-GM relationships. We hypothesize that HRV and rsFC are related to gut microbiota composition, and diet mediates this relationship.

## 2. Materials and methods

### 2.1. Study population selection

This cross-sectional study included Colombian healthy men aged 21–40 years. Exclusion criteria were self-reported medical conditions, current or long-term use of any medication, vegan or vegetarian diet, left-handedness, presence of heart devices or any other device not compatible with MRI, and drug abuse assessed using the Alcohol, Smoking, and Substance Involvement Screening Test scale (ASSIST) version 1.1.66. We also asked for recent exposure to several microbiota modifiers such as antibiotics, probiotics, and recent infectious diseases, among others and when they were present, a suitable washout time was taken before inclusion in the study. All subjects underwent physical examination to obtain anthropometric and blood pressure measurements. Biological samples, 24-hour Holter and resting-state functional magnetic resonance imaging (rsfMRI) data were collected within four weeks.

This study was approved by the Research Ethics Committee of the Faculty of Medicine at the University of Antioquia (minute 007 of May 11, 2017), in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki in 1975 and Resolution 8430 issued by the National Ministry of Health of Colombia in 1993. All the participants provided written informed consent.

### 2.2. Health parameters

#### 2.2.1. Anthropometric and blood pressure measurements

Blood pressure was the average of three consecutive measurements taken in the left arm supported in a table at the heart level. The waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference. The body mass index (BMI) was calculated from weight and height measurements obtained using a calibrated digital scale and a stadiometer, respectively.

#### 2.2.2. Biochemical parameters

All measurements were performed in fresh serum from peripheral blood. High-sensitivity C-reactive protein, high-density lipoprotein, total cholesterol, and glycated hemoglobin levels were quantified using Dimension® Flex® reagent cartridges (DF34, DF48B, DF27, and DF105A, respectively).

### 2.3. Holter acquisition and analysis

A Custo Flash 510 V model monitor was installed in volunteers between 7 am and 10 am, and it was removed the following day around the same time to ensure a minimum ECG recording of 21 h. The monitor performed recordings in three channels every  $2.5 \text{ ms} \pm 0.1\%$  per channel, with a quantification amplitude of  $5.6 \mu\text{V}/\text{Bit} \pm 1\%$  of 10 bit, a response frequency range of 0.05–45 Hz, and a resistance  $\geq 10 \text{ M}\Omega$  filtered at 50 Hz 80 dB. Volunteers were asked to perform their usual life activities during recording but were instructed to avoid physical

exercise. They were also asked to log their daily activities. HRV time- and frequency-domain metric calculations were performed using the ANS diagnostic module of the Custo diagnostics Holter ECG (Customed Inc., Germany). The HRV metrics used for the analyses were the standard deviation of NN intervals (SDNN), the root mean square of successive differences between normal heartbeats (RMSSD), the natural logarithms of LF and HF (ln LF and ln HF), and SQ as ln (LF/HF).

#### 2.4. Resting-state functional MRI acquisition and graph connectivity analysis

Scanning was performed using an Ingenia 3 T Philips MRI scanner with a 16-channel phased-array rigid head coil. A high-resolution 3D T1-weighted sequence (175 axial slices; TR = 7807 ms; TE = 3593 ms; FOV = 256 mm × 256 mm; matrix size = 256 × 256; voxel size = 1 mm × 1 mm × 1 mm, FA = 8°) and rs-fMRI for 10 min (300 volumes; 36 slices; repetition time, TR = 2000 ms; echo time, TE = 30 ms; flip angle, FA = 70°; field of view, FOV = 112 mm × 112 mm; matrix size = 72 × 72; voxel size = 2 mm × 2 mm × 3 mm) were acquired. The volunteers were instructed to keep their eyes closed and remain still.

The Configurable Pipeline for the Analysis of Connectomes (C-PAC) version 1.8.1 was used to pre-process the MRI data. The structural images were skull-stripped, segmented into the main cerebral tissues, and constrained into the individual subject space. Functional images were realigned, motion-corrected, and skull-stripped. The global mean intensities were normalized, the nuisance signals were regressed-out (scanner drift, physiological noise, and head motion signals), the volumes with head motion higher than 0.3 mm were interpolated, and the time series were bandpass-filtered (0.01–0.1 Hz). Anatomical and functional images were normalized to the 2 mm Montreal Neurological Institute (MNI) 152 space. The pre-processed functional average time series of 385 non-limbic regions were extracted from the 400 Schaefer's cortical parcellation and assigned to one of the Yeo 17 networks (Yeo et al., 2011). The areas within the two limbic Yeo-17 networks were excluded from the analyses because of the low signal-to-noise ratio of the frontal orbital areas. The functional connectivity matrix was calculated as pairwise Pearson's correlation between the time series and z-scored. The matrix was thresholded to retain the strongest 10% weight, and the negative weights were set to 0. The resting-state functional connectivity strength (FCS) of RSNs was measured using the node strength connectivity metric (Stg) derived from the graph theory approach, which provides information about the weight of the correlation between a node and the rest of the cortex. Using the Brain Connectivity Toolbox, Stg was computed as the sum of the link weights connected to a node. Afterward, the average Stg was calculated dividing by the number of connected nodes in each network.

#### 2.5. Stool collection, bacterial DNA extraction, and sequencing

Volunteers collected stool samples in a plastic sterile container refrigerated until delivery within the next two hours. Fresh stool samples were used for DNA extraction using the Stool Nucleic Acid Isolation Kit (Norgen Biotec Corp.). DNA was quantified using a NanoDrop™ 2000-Thermo Scientific™ spectrophotometer. Before DNA sequencing, the samples were normalized to a final concentration of 10 ng/μL. DNA sequencing libraries were prepared and sequenced using a 300 bp paired-end Illumina MiSeq protocol at Macrogen Inc. (Seoul, Republic of Korea). The V3-V4 hypervariable regions of the bacterial and archaeal 16 S rRNA genes were amplified using the primers Bakt\_341F (5'-CCTACGGGNGGCWGCAG-3') and Bakt\_805R (5'-GACTACHVGGGTATCTAATCC-3'). The forward and reverse primers contained Illumina adapter, pad, and linker sequences.

#### 2.6. Gut microbiota taxonomy bioinformatic analysis

The sequences were analyzed using Mothur's version 1.44 pipeline

according to the MiSeq standard operating procedure (SOP). Briefly, paired-end reads were assembled using Mothur's tool "make.contigs" and then aligned to the SILVA 16 S rRNA reference database version v138\_1. VSEARCH algorithm was used to remove chimeric sequences. The non-bacterial lineages were removed. Mothur's subroutine "dist.seqs" was used to cluster reads into operational taxonomic units (OTUs) at a distance limit of 0.03. Data were normalized with the "total group" method, and rare OTUs with less than three sequences were removed. Phylogenetic classification was performed using the Ribosomal Database Project (RDP) classifier tool (80 bootstrap threshold). A Biological Observation Matrix (BIOM) file was generated and imported into the R environment to perform statistical analyses using the PHYLOSEQ and MICROBIOME packages. The normalized counts of each bacterial taxon were calculated using the median value of the read counts in R software. The frequencies of the most common phyla and families were determined from these counts. Alpha diversity indices were estimated using the R package PHYLOSEQ.

#### 2.7. Habitual diet estimation

Habitual food intake over a year was assessed using an online version of a self-reported semi-quantitative food intake frequency questionnaire (CFIA) developed locally (Monsalve Álvarez and González Zapata, 2011). We calculated dietary energy and macronutrient values from this questionnaire using an in-house developed R script.

#### 2.8. Assessment of potential confounders

Depression and anxiety were self-assessed using an online validated Spanish version of the Zung scale (Jaramillo-Toro et al., 2018) and the validated Spanish version of the state and trait anxiety inventory questionnaires (STAI-S and STAI-T) (Guillén-Riquelmeé and Buela-Casal, 2011). Zung raw scores were multiplied by 1.25 to obtain index scores used to determine the cut-off scores. The cut-off scores for detecting depression or anxiety were >50, >44, and > 41 for the Zung, STAI-R, and STAI-S scales, respectively (Dunstan and Scott, 2019). Physical activity was evaluated using the short Spanish version of the International Physical Activity Questionnaire (IPAQ).

The presence of intestinal parasites was examined in fresh stool samples following WHO recommendations (World Health Organization (WHO, 1994). Sample concentration was performed using 15 ml fecal parasite concentrators (Mini Parasep®, Apacor, Inc. United Kingdom). Previous SARS-Co-V2 was verified by detecting IgG and IgM using immunochromatography or chemiluminescent immunoassays.

#### 2.9. Statistical analyses

##### 2.9.1. Sample size and power analysis

Since no studies had assessed the relationship between HRV and rsFC with GM composition or diet, we assumed that we could find a correlation of at least 0.3, resulting in a sample size of 85 individuals. This calculation was performed using STATA software version 13, assuming an alpha of 0.05 and a power of 0.80. The lowest correlation found in our analyses was -0.2 between SQ and habitual monounsaturated fat (MUF) and fiber intake. The highest correlation was 0.6 in the MLR analyses between the interaction SQ-Stg\_Control B and *Ruminococcaceae\_unc*. Consequently, the power for this range of correlations, given a sample size of 88, was 0.46 and 1.0, respectively.

##### 2.9.2. Description of participant characteristics and common GM taxa

The normal distribution of quantitative variables was checked using the Shapiro-Wilk test, histograms, and Q-Q graphs. Normally distributed variables were described by mean and standard deviations, whereas non-normally distributed variables were described by median and interquartile ranges. Qualitative variables were described by frequency. These analyses were performed using IBM SPSS Statistics software

version 24. The abundance of the most common GM taxa was calculated by dividing the absolute abundance of each phylum or family by the total absolute GM abundance across the sample at the corresponding taxonomic levels.

### 2.9.3. Multivariable linear regression analyses

The relationship between GM, HRV, and rsFC was characterized in two steps. First, the Microbiome Multivariable Associations with Linear Models software version 2 (MasAlin2) was used to assess the correlation between the absolute abundance of GM families and genera (dependent variables), and the non-normalized HRV time and frequency domain metrics, and the FCS of 15 RSNs (independent variables). We set the minimum taxa abundance at five and ten for the genus and family taxonomic levels, respectively, and the minimum prevalence at 50% for both taxonomic levels. The total sum scaling normalization and log transformation methods were applied to the GM taxa counts. After the Benjamini–Hochberg procedure (BH), the other parameters were maintained as recommended by the developers. The maximum significance allowed was 0.2 after the Benjamini–Hochberg procedure (BH). MasAlin2 analyses were performed using R studio 2022.07.2 +576 version (Mallik et al., 2021).

Second, the relative abundance of each taxon identified in the previous step was calculated and used as a dependent variable to confirm their correlation with HRV metrics and the FCS of RSNs after adjusting for confounders using multiple linear regression analyses (MLR). Additional MLR analyses were performed to evaluate the correlation of alpha diversity indices with the HRV metrics and FCS of the RSNs identified in the previous step.

To evaluate the interaction effect of HRV and FCS on the relative abundance of taxa confirmed in the second step, we used MLR analyses. To avoid inflation of the coefficient and variance (VIF) in the interaction models, the interacting variables were centered by subtracting the mean from each variable. Interactions were created by multiplying the centered variables.

All MLR analyses were adjusted for age, BMI, habitual fiber intake, and Zung scale scores. The normality of dependent variables was checked before all MLR analyses using the Shapiro-Wilk test and their histogram. The Rachas test was used to verify the assumption of randomness. Data normalization of the dependent variables was performed using IBM SPSS Statistics software version 24. The covariates were chosen based on their biological plausibility. The absence of multicollinearity was verified by calculating the variance inflation factor (VIF < 5.0). Normality (Shapiro-Wilk test  $p > 0.05$ ), no autocorrelation (Durbin-Watson test  $d = 1.5–2.5$ ), and homoscedasticity (Breusch-Pagan test;  $p > 0.05$ ) of the residuals were checked for each analysis. The quality of the models was verified using the Akaike information criterion (AIC) and the adjusted R2. These MLR analyses were performed using STATA software version 13.

### 2.9.4. Diet mediation analyses

To identify the macronutrients that could mediate the relationship between GM taxa with HRV and the FCS of RSNs, a 2-tail Spearman's rank correlation coefficient calculation was performed using IBM SPSS Statistics software version 24. The correlation matrix was plotted using the website <https://www.bioinformatics.com.cn>, an online data analysis and visualization platform. In this platform, a  $p$ -value equal to or below 0.05 was set as significant. Mediation analyses were performed using PROCESS macro for SPSS version 4.2 model 4. The mediator and dependent variables were normalized. We selected 5,000 bootstrap samples with a 95% confidence interval (CI 95%). The percentage contribution of the mediator was calculated as  $[a*b/(c+a*b)*100]$  (Baron and Kenny, 1986).

### 2.9.5. Sensitivity analyses

All MLR analyses were repeated by adding the following additional confounders individually in different models: physical activity,

parasites, previous infection with SARS-Cov2, level of education level, socioeconomic status, and STAI-T scale scores. Mediation analyses were repeated by increasing the bootstraps to 10,000.

## 3. Results

### 3.1. Descriptive data

In the data analyses we included 88 of 402 volunteers who completed the pre-selection questionnaire (Supplementary Figure 1). The sociodemographic, anthropometric, biochemical, physiological, and lifestyle habits and other general health characteristics of the included volunteers are described in Table 1. Unexpectedly, we found that some volunteers scored above the cutoff point on the Zung self-rating depression scale ( $n=15$ , 17.04%), State-Trait Anxiety Inventory

**Table 1**  
Demographic and clinical characteristics of participants.

Variables	n=88
<b>Demographics</b>	
Age (years), median (IQR)	30 (25.3–34.0)
<b>Level of education</b>	
Bachelor	2 (2.3)
Undergraduate	68 (77.3)
Postgraduate in progress	18 (20.5)
<b>Anthropometrics</b>	
BMI (kg/m <sup>2</sup> ), mean (SD)	24.2 (±2.6)
WHR(cm), median (IQR)	0.9 (0.8–0.9)
<b>Biochemical</b>	
HbA1c (%), median (IQR)	5.3 (5.1–5.6)
HDL (mg/dL), median (IQR)	46.0 (40.1–52.1)
Total cholesterol (mg/dL), mean (SD)	180.5 (±34)
Total cholesterol: HDL (mg/dL), median (IQR)	3.8 (3.1–4.6)
hs-CRP (mg/L), median (IQR)	1.0 (0.1–1.4)
<b>Cardiovascular</b>	
Heart rate (beats/min), mean (SD)	70.1 (±7.8)
SBP (mmHg), mean (SD)	114.3 (±9.4)
DBP (mmHg), mean (SD)	70.3 (±7.0)
SDNN (ms), median (IQR)	84.3 (71.6–98.6)
RMSSD (ms), median (IQR)	55 (45.4–73.7)
Ln LF (ln ms <sup>2</sup> ), mean (SD)	7.5 (±0.5)
Ln HF (ln ms <sup>2</sup> ), mean (SD)	6.6 (±0.8)
SQ (ln ms <sup>2</sup> ), mean (SD)	0.9 (±0.4)
<b>Lifestyle habits</b>	
<b>Habitual diet</b>	
Caloric intake (Kcal), median (IQR)	2350.2 (1775.9–2888.3)
Protein (gr), median (IQR)	83.6 (64.8–102.2)
Total fat (gr), median (IQR)	79.8 (58.9–103.6)
Carbohydrates (gr), median (IQR)	304.3 (224.8–396.3)
Fiber intake (gr), median (IQR)	17.9 (12.6–25.1)
<b>Other health parameters</b>	
<b>Zung scale score</b>	
>50, n (%)	15 (17.0)
<b>STAI-T score</b>	
>44, n (%)	14 (15.9)
<b>STAI-S score</b>	
>41, n (%)	3 (3.4)
<b>Parasites</b>	
Presence n (%)	42 (47.7)
<b>Previous COVID-19<sup>a</sup></b>	
Yes n (%)	11 (12.5)

Values are expressed in percentages (%), mean ± standard deviation (SD), or median (IQR, interquartile range). BMI; body mass index, WHR, waist-to-hip ratio; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; SBP, systolic blood pressure, DBP, diastolic blood pressure; SDNN, the standard deviation of the NN intervals; RMSSD, root mean square of successive differences between normal heartbeats; LnLF, the natural logarithm of low-frequency power; LnHF, natural logarithm high-frequency power; SQ sympathovagal quotient, the natural logarithm of the LF /HF ratio. STAI-T and STAI-S, State-Trait Anxiety Inventory (STAI) form Y; trait and state subscales, respectively.

<sup>a</sup> Previous infection with SARS-Cov-2 verified with anti-SARS-Cov2 antibodies quantification.

trait subscale (STAI-T) (n=14, 15.9%), State-Trait Anxiety Inventory state subscale (STAI-S) (n=3, 3.4%), and both Zung and STAI scales (n=13, 14.8%), despite the reported absence of psychiatric diagnoses and treatments during the pre-selection process. The most common phyla identified were *Bacteroidetes* (56%) and *Firmicutes* (39%). The most abundant families were *Prevotellaceae* (33%), *Bacteroidaceae* (15%), *Ruminococcaceae* (14%) and *Lachnospiraceae* (11%).

3.2. Higher SQ and functional connectivity strength of control networks were related to higher *Ruminococcaceae* abundance at genus and family taxonomic levels

We found a positive and statistically significant correlation between the SQ and FCS of control networks A and B (Stg\_Control A and Stg\_Control B) and the relative abundance of the *Ruminococcaceae* family and the *Ruminococcaceae\_unc* genus (Fig. 1). After adjustment for confounders, these correlations remained, and Stg\_Control A had a positive and statistically significant correlation with the Shannon index (Table 2). The average whole-brain FCS across participants and the localization of the control A and B networks are shown in Supplementary Figure 2. Brain regions included in the control A and B networks are listed in Supplementary Table 1.

3.3. The interaction between SQ and FCS of control B network reduced their main effect on *Ruminococcaceae\_unc* genus abundance

The interaction SQ-Stg\_Control B was correlated with the *Ruminococcaceae\_unc* genus abundance but showed a negative coefficient, indicating a reduction in the main effect of each independent variable. In contrast, the SQ-Stg\_Control A interaction was not related to the *Ruminococcaceae* family or genus relative abundance (Table 3).

3.4. Habitual fiber intake mediates the relationship between SQ and *Ruminococcaceae\_unc* genus abundance

SQ showed a weak significant negative correlation with habitual fiber intake ( $\rho=-0.21, p=0.05$ ) and MUF ( $\rho=-0.22, p=0.04$ ). The decreased habitual intake of these macronutrients was also correlated with a decrease in the relative abundance of the *Ruminococcaceae* family and *Ruminococcaceae\_unc* genus (Fig. 2a). Furthermore, the mediation analysis showed that fiber intake mediated the relationship between SQ and *Ruminococcaceae\_unc* genus with a 12.2% indirect effect [indirect effect c:  $\beta=0.03, 95\% \text{ IC } (0.002-0.12)$ ] (Fig. 2b).

3.5. Sensitivity analyses

All significant MLR analyses shown in Tables 2 and 3 survived the addition of confounders except for the correlation between control A and Shannon index, which lost significance after adding all confounders except socioeconomic status (data not shown). Mediation analyses remained significant after 10,000 bootstrapping [indirect effect c:  $\beta=0.05, 95\% \text{ IC } (0.002-0.12)$ ].

4. Discussion

In this study, we found that (1) SQ and the FCS of Control A and B networks are positively related to the abundance of gut *Ruminococcaceae* taxa, (2) SQ and Stg\_Control B antagonize each other in their influence on *Ruminococcaceae\_unc* genus abundance, and (3) the effect of SQ on *Ruminococcaceae\_unc* genus abundance is partially mediated by reduced fiber intake.

4.1. Positive correlation between SQ and *Ruminococcaceae* taxa abundance

Only one study has explored the correlation between SQ and GM in adult men (Tsubokawa et al., 2022). This study reported that SQ

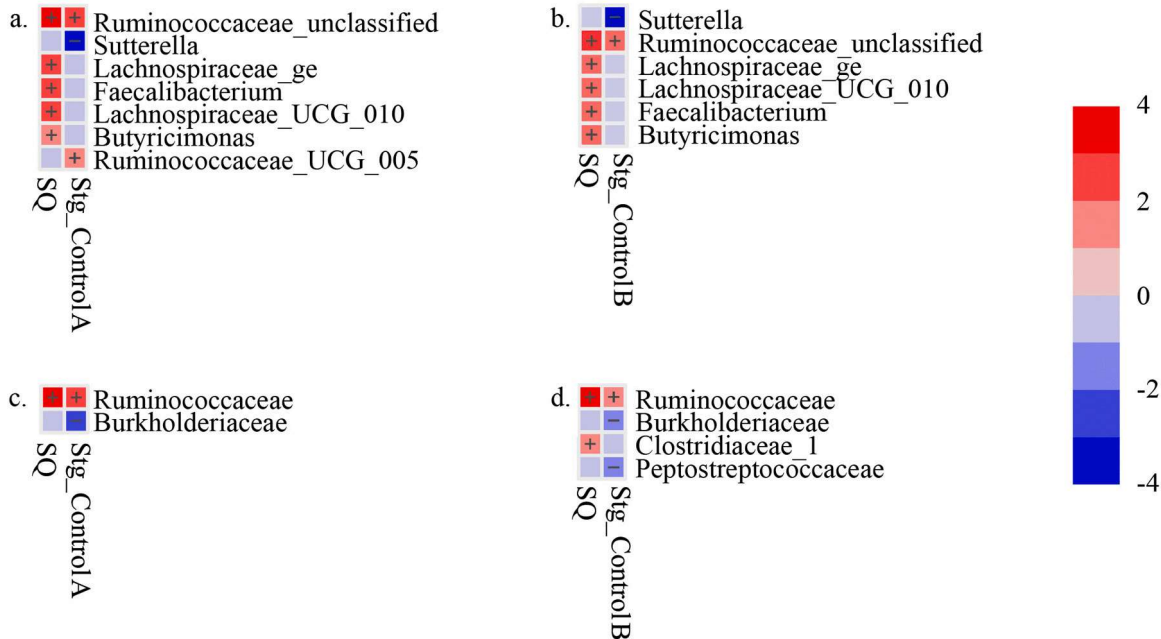


Fig. 1. Heatmaps of significant correlations between the sympathovagal quotient (SQ) and the functional connectivity strength (FCS) of control networks and gut microbiota taxa abundance. a-b. At the genus taxonomic level, SQ and FCS of control A and B networks were correlated to an increased abundance of an unclassified genus belonging to the *Ruminococcaceae* family (*Ruminococcaceae\_unc*). c-d. At the family taxonomic level, SQ and FCS of control A and B networks were correlated to an increased abundance of the *Ruminococcaceae* family. Stg\_Control A and Stg\_Control B, FCS of control A and B. Colors indicate the size effect calculated by the following formula:  $[-\log(qval)] * \text{sign}(\text{coefficient})$ . The plus and minus signs indicate the direction of the correlations. FDR, false discovery rate calculated with BH procedure. Significant q-values after BH procedure ( $q < 0.2$ ).

**Table 2**

Multivariate linear regression analyses between HRV, the FCS of control networks, and GM composition.

Dependent variables	Independent variables	Model results					
		$\beta$	<i>p</i> -value	95% CI	Prob F	R <sup>2</sup>	Adjusted R <sup>2</sup>
<i>Ruminococcaceae_unc</i> genus <sup>a</sup>	SQ	<b>0.41</b>	<b>&lt;0.01</b>	<b>0.19–0.64</b>	<b>&lt;0.01</b>	<b>0.19</b>	<b>0.15</b>
	Stg_Control A	<b>0.08</b>	<b>&lt;0.01</b>	<b>0.03–0.13</b>	<b>&lt;0.01</b>	<b>0.17</b>	<b>0.12</b>
	Stg_Control B	<b>0.08</b>	<b>&lt;0.01</b>	<b>0.03–0.13</b>	<b>0.01</b>	<b>0.18</b>	<b>0.13</b>
<i>Ruminococcaceae</i> family <sup>b</sup>	SQ	<b>2.97</b>	<b>&lt;0.01</b>	<b>1.23–4.70</b>	<b>&lt;0.01</b>	<b>0.22</b>	<b>0.18</b>
	Stg_Control A	<b>0.64</b>	<b>&lt;0.01</b>	<b>0.27–1.01</b>	<b>&lt;0.01</b>	<b>0.23</b>	<b>0.19</b>
	Stg_Control B	<b>0.50</b>	<b>0.01</b>	<b>0.13–0.88</b>	<b>&lt;0.01</b>	<b>0.19</b>	<b>0.14</b>
Shannon index	SQ	0.12	0.04	0.01–0.24	0.06	0.12	0.07
	Stg_Control A	<b>0.03</b>	<b>0.02</b>	<b>0.01–0.06</b>	<b>0.03</b>	<b>0.13</b>	<b>0.08</b>
	Stg_Control B	0.02	0.15	-0.01–0.04	0.13	0.10	0.04
Chao1 index <sup>c</sup>	SQ	-97.54	0.20	-248.64–53.56	0.11	0.10	0.05
	Stg_Control A	15.02	0.37	-18.11–48.14	0.15	0.09	0.04
	Stg_Control B	14.42	0.38	-17.99–46.83	0.15	0.09	0.04
Inverse Simpson index <sup>a</sup>	SQ	0.15	0.02	0.02–0.29	0.05	0.13	0.07
	Stg_Control A	0.03	0.07	-2.44e-03–0.05	0.10	0.10	0.05
	Stg_Control B	0.01	0.43	-0.02–0.04	0.26	0.07	0.02

SQ, sympathovagal quotient LF/HF; Stg\_Control A, FCS of Control A network; Stg\_Control B, FCS of Control B network. Models were adjusted by age, BMI, habitual fiber intake, and Zung depression scale scores. CI, confidence interval, Prob. F: *p*-value for the F statistic showing that at least one  $\beta$  coefficient differed from 0. Bold indicates significant correlations that met all the MLR analysis assumptions (*p*<0.05).

<sup>a</sup> Natural logarithmic normalized abundance.

<sup>b</sup> Squared root normalized abundance.

<sup>c</sup> Fractional ranking normalized values.

**Table 3**

Multivariate linear regression analyses of the interaction between HRV and FCS of control networks and its relationship with GM taxa abundance.

Dependent variables	Interaction Model	Independent variables	Model					
			$\beta$	<i>p</i> -value	95% CI	Prob. F	R <sup>2</sup>	Adjusted R <sup>2</sup>
<i>Ruminococcaceae_unc</i> genus <sup>a</sup>	SQ-Stg_Control A	SQ	0.43	<0.01	0.18–0.67	<0.01	0.29	0.22
		Stg_Control A	0.06	0.01	0.01–0.11			
		SQ-Stg_Control A	-0.03	0.26	-0.08–0.02			
	SQ-Stg_Control B	SQ	<b>0.48</b>	<b>&lt;0.01</b>	<b>0.24–0.72</b>	<b>&lt;0.01</b>	<b>0.31</b>	<b>0.25</b>
		Stg_Control B	<b>0.07</b>	<b>&lt;0.01</b>	<b>0.02–0.11</b>			
<i>Ruminococcaceae</i> family <sup>b</sup>	SQ-Stg_Control B	SQ-Stg_Control B	<b>-0.05</b>	<b>0.03</b>	<b>-0.87 to -4.50e-03</b>			
		SQ	0.47	<0.01	0.19–0.76	<0.01	0.27	0.20
		Stg_Control B	0.07	0.02	0.01–0.12			
	SQ-Stg_Control A	SQ-Stg_Control B	-0.04	0.08	-0.09–5.48e-03			
		SQ	0.39	0.01	0.10–0.67	<0.01	0.26	0.20
		Stg_Control A	0.07	0.01	0.02–0.13			
		SQ-Stg_Control A	-0.02	0.62	-0.08–0.05			

SQ, sympathovagal quotient LF/HF. Stg\_Control A, FCS of Control A network; Stg\_Control B, FCS of Control B network. SQ-Stg\_Control A and SQ-Stg\_Control B indicate interaction between those variables obtained by multiplying the centered variables. Models were adjusted by age, BMI, habitual fiber intake, and Zung depression scale scores, Prob. F: *p*-value for the F statistic showing that at least one  $\beta$  coefficient was different from 0. Bold indicates significant correlations (*p*<0.05).

<sup>a</sup> Natural logarithmic normalized abundance.

<sup>b</sup> Squared root normalized.

correlated positively with the abundance of *Lachnospiraceae inserta sedis* genus. In line with this result, we also found a positive correlation between SQ and the abundance of an undetermined genus of *Lachnospiraceae* (*Lachnospiraceae\_ge*). However, *Ruminococcaceae\_unc* was the only genus correlated with the SQ and FCS of RSNs.

Interestingly, the *Ruminococcaceae* and *Lachnospiraceae* families are the most abundant families in the gut environment (80%) and are phylogenetically and functionally closely related (Biddle et al., 2013). Two contrasting health effects have been assigned to these families. On the one hand, they are important fiber degraders that produce around 85% of total butyrate, which has positive effects on health, mainly through its anti-inflammatory effect (Siddiqui and Cresci, 2021). On the other hand, the increased abundance of some genera and species of the same families is related to increased production of trimethylamine (TMA), a precursor of liver-derived trimethylamine-N-oxide (TMAO) (Fu et al., 2020). This metabolite contributes to the formation of atherosclerotic plaques and increases the risk of cardiovascular and cerebrovascular adverse events (Wang et al., 2021).

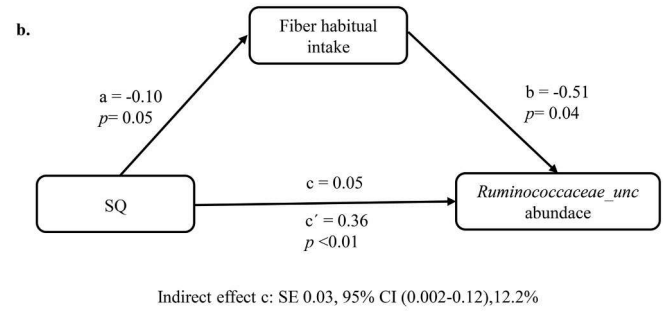
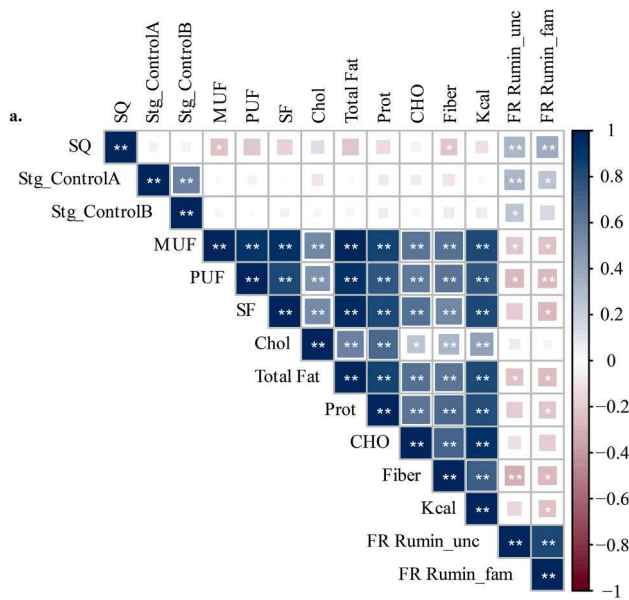
Overall, SQ is positively correlated with the abundance of genera belonging to the *Ruminococcaceae* and *Lachnospiraceae* families,

suggesting a detrimental effect on health based on the negative implications of high SQ and the potential profile of harmful metabolites originating from the increased abundance of some members of these taxa. However, characterization of these unclassified genera and their metabolites is necessary to confirm this assumption.

#### 4.2. Positive correlation between the FCS of control networks and *Ruminococcaceae* taxa abundance

We found that an increased abundance of *Ruminococcaceae* taxa was related to an increased FCS of control A and B networks. The increased FCS indicates higher rsFC of nodes within these networks with the rest of the brain cortex.

Three publications derived from the analyses of the relationship between brain connectivity and GM in a Chinese cohort of healthy individuals found a relationship between alpha diversity and the *Ruminococcaceae* enterotype with brain structural and functional connectivity as well as with EF tasks (Cai et al., 2021; Zhang et al., 2022; Zhu et al., 2022). They found that higher FCS of CEN nodes correlated with worse performance on behavioral inhibition (go/no go) and attention tasks



**Fig. 2.** Diet correlation and mediation analyses. a. Spearman correlation heatmap to assess the relationship between habitual diet, the sympathovagal quotient (SQ), the functional connectivity strength (FCS) of control A and B networks and gut microbiota taxa abundance. SQ showed negative and significant correlations with habitual monounsaturated fat intake (MUF) ( $\rho = -0.22, p = 0.04$ ) and habitual fiber intake ( $\rho = -0.21, p = 0.05$ ). The relative abundance of *Ruminococcaceae* unclassified genus (FR Rumin\_unc) and family (FR Rumin\_fam) was correlated to reduced intake of all macronutrients except carbohydrates (CHO) and Cholesterol (Chol). PUF, polyunsaturated fat; SF, saturated fat; Prot, Proteins; Kcal, kilocalories. Stg\_Control A and Stg\_Control B, FCS of control A and B networks. Colors indicate correlation strength and direction according to the bar on the right side. Asterisks indicate significance: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ . b. Mediation effect of habitual fiber intake in the relationship between SQ (X) and the relative abundance of *Ruminococcaceae\_unc* genus (Y). Indirect effect significance after 5000 bootstraps. The percentage of indirect effect was calculated as  $[a*b/(c'+a*b)*100]$ . Values of habitual fiber intake and *Ruminococcaceae\_unc* genus abundance were natural logarithmic normalized.

(digit span) (Cai et al., 2021; Zhang et al., 2022). These findings suggest that worse performance in EF is related to higher FCS in brain areas related to inhibitory control. Interestingly, they also found that FCS of CEN nodes mediated the relationship between GM and these inhibitory control tasks. Furthermore, they found connectivity differences between the *Prevotella* enterotype and *Ruminococcaceae* enterotype in men (Zhu et al., 2022). In consonance with these findings, we found a correlation between the abundance of *Ruminococcaceae* and the FCS of control A and B networks in men. Nonetheless, results from these studies are difficult to compare with ours owing to the extensive methodological differences in brain connectivity analyses.

In summary, the positive correlation between the FCS of control networks and the abundance of *Ruminococcaceae* may be related to reduced inhibitory control. Nevertheless, this needs direct confirmation by exploring the relationship between the FCS of control networks, performance in inhibitory control tasks, and abundance of *Ruminococcaceae*.

#### 4.3. Interaction between the SQ and the FCS of control B network in their relationship with *Ruminococcaceae\_unc* genus abundance

Our results showed that when SQ was high, the relationship between Stg\_Control B and *Ruminococcaceae\_unc* abundance was reduced, and when Stg\_Control B was high, the relationship between SQ and *Ruminococcaceae\_unc* genus abundance was reduced. This might indicate negative feedback between SQ and the FCS of the control B network, with consequent changes in their relationship with *Ruminococcaceae\_unc* genus abundance.

The control B network contains the dlPFC, a brain region consistently involved in HRV regulation (Matusik et al., 2023). This brain region has lateralized influence over HRV, an activation of the right dlPFC has been associated with increased sympathetic tone. In contrast, the activation of the left dlPFC increases parasympathetic tone. A clinical trial showed that anodal high-definition tDS (HD-tDCS) on the left dlPFC increased LF

and SQ in healthy young adults (Gu et al., 2022).

Placing our interaction findings in the context of the reported lateralized influence of the dlPFC on HRV, it is tempting to speculate that activation of the control B network, which contains the right dlPFC, will contribute to increasing SQ by increasing the sympathetic tone. However, when the SQ reaches a threshold, the sympathetic tone decreases through a reduction in the right dlPFC activity or increased activation of the left dlPFC, leading to a reduction of SQ. Consequently, the reduction of SQ and the right dlPFC activity would be related to a lower abundance of *Ruminococcaceae\_unc* genus.

Since SQ and Stg\_Control B did not show a significant correlation in our analysis, the bottom-up branch of this hypothetical feedback loop is likely indirect and could involve the vmPFC. The indirect modulation of the dlPFC through the vmPFC driven by heart signaling has been suggested by the following two observations: First, activation of the vmPFC is induced by heartbeat-evoked responses (HERs) (Azzalini et al., 2021). Second, increased connectivity between the vmPFC and several other areas, including the dlPFC, can be induced by HRV biofeedback, which involves the modification of HRV by pace breathing (Schumann et al., 2021).

Finally, in the top-down branch of this hypothetical feedback system, the control B network regulation of HRV would also be indirect. It has been shown that control networks interact with the DMN anchored in the vmPFC, which directly influences the SN. This network, together with the cingulate cortex, appears to have the direct and strongest modulation of HRV (Duggento et al., 2017).

Behaviorally, one point of convergence of HRV and control networks is their relationship with self-control and EF. Increased HRV has been consistently correlated with better EF, especially cognitive inhibition and flexibility (Magnon et al., 2022). Interestingly, the reduction of self-control and its connection with HRV and control networks has been shown in eating behavior disorders. For example, changes in HRV or rsFC in control networks have been linked to bulimia nervosa (Peschel et al., 2016; Wang et al., 2020). Moreover, clinical interventions that

increase HRV or modify right dlPFC function, such as HRV biofeedback and tDCs, have been shown to contribute to the treatment of some of these disorders (Ester and Kullmann, 2022; Scolnick et al., 2014). Finally, significant changes in GM composition have been described in eating disorders (Carbone et al., 2021).

Taken together, SQ and Stg\_Control B appear to antagonize each other in their relationship with *Ruminococcaceae\_unc* genus abundance, possibly through a negative feedback loop initiated by HERs. In addition, reduced self-control in food choices could explain the relationship between SQ-Stg\_Control B and *Ruminococcaceae\_unc* genus abundance.

#### 4.4. Mediation effect of fiber intake in the relationship between SQ and *Ruminococcaceae\_unc* genus abundance

Our previous discussion and mediation analysis results suggest that SQ influences the abundance of the *Ruminococcaceae\_unc* genus through food decision-making. Considering that HRV is generated by the neural integration of multiple top-down signals coming from emotional, cognitive, and behavioral CNS networks and bottom-up signals coming from the immune system and chemoreceptors, mechanoreceptors, and metaboreceptors of peripheral internal viscera, HRV reflects a broad psychophysiological regulation (Smith et al., 2020). In addition, HRV also reflects the signaling from the heart to the brain, where HERs influence emotions, behavior, and cognition after being integrated with other visceral information (Azzalini et al., 2021). An example of the influence of visceral signaling on brain function is the feeding behavior in which the internal state of hunger influences visual, planning, motor behavior, and memory to achieve food intake.

Evidence suggests that low HRV, measured by the SDNN, could influence food decisions by modifying the activation of the vmPFC and its interaction with the dlPFC toward the selection of unhealthy food (Maier and Hare, 2017; Wilson et al., 2023). In addition, a recent study showed that men who had high saturated fat (SF) intake also had low fiber intake, and this was related to an increased abundance of the TMA producer genus *Anaerotruncus*, which belongs to the *Ruminococcaceae* family (Bailén et al., 2020). This genus has been related to increased TMAO levels in human studies (Franck et al., 2022). In contrast, men with low SF intake also had high fiber intake, which was related to the increased abundance of *Ruminococcaceae UCG-014* genus (Bailén et al., 2020). This suggests that while some *Ruminococcaceae* genera increase in the presence of low fiber and high SF, others increase in the opposite situation. Since *Ruminococcaceae\_unc* is an unclassified genus, we do not know its metabolic profile. However, its correlation with low fiber intake suggests that it might be involved in the reduced production of beneficial metabolites and an increased generation of detrimental metabolites such as TMA. Finally, if the *Ruminococcaceae\_unc* genus is involved in TMAO production, its relationship with an increased SQ might be explained by the reported rise in sympathetic innervation to the heart mediated by this metabolite (Meng et al., 2019).

In summary, SQ seems to reflect the broad influence of the psychophysiological status on food choice more than the FCS of the Control B network. The mediation of habitual fiber intake in the relationship between SQ and *Ruminococcaceae\_unc* suggests that this genus is not fibrinolytic but instead participates in the production of other metabolites such as TMAO, which might perpetuate the increased SQ by strengthening the sympathetic input to the heart.

## 5. Strengths and limitations

To our knowledge, this is the first study to evaluate the relationship between three well-established health status indicators, HRV, rsFC, and GM. Our sample was homogenous and was controlled for confounders that might influence these health indicators. Therefore, our sample of healthy men allowed us to perform a reliable description of the influence of HRV and rsFC on GM. On the other hand, this study has several limitations: (1) due to its cross-sectional design, we cannot draw any

causal relationships; (2) our selection of young, healthy men from a region of Colombia does not allow us to expand the conclusions to other populations with different sex, age, and region of origin; (3) the assumption of a negative relationship between the FCS of control networks and inhibitory control was not directly tested in our sample; (3) we did not measure beneficial or detrimental metabolites in our participants and its relationship with HRV and rsFC; (4) the mediation effect of diet was exploratory, and it should be measured in future interventional studies.

## 6. Conclusion

Two indicators of self-regulation, HRV and the rsFC of control networks, are related to the abundance of gut microbiota taxa in healthy men. However, only HRV is related to habitual diet intake; thus, HRV should be analyzed in future studies as a marker of food choice and GM composition. The wide use of wearable devices that measure HRV daily and the availability of therapeutic interventions that directly improve HRV, such as biofeedback, supports the need to continue investigating the HRV's relationship with food choices and GM profiles. Advances in this field will contribute to decreasing the prevalence of disorders characterized by reduced self-control in dietary decisions, a shared characteristic of several chronic disorders.

### CRedit authorship contribution statement

**Juan C. Calderón:** Resources, Supervision, Writing – review & editing. **Jaime Gallo-Villegas:** Formal analysis, Writing – review & editing. **Juan F. Alzate-Restrepo:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Jazmin X. Suarez-Revelo:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Gabriel Castrillón:** Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Daniel A. Vargas-Tejada:** Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Valentina Hawkins-Caicedo:** Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Ana Lucía Miranda-Angulo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Juan D. Sánchez-López:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The authors have deposited gut microbiota raw sequences into the Sequence Read Archive (SRA) with the BioProject accession number PRJNA1000574. Neuroimages were deposited into the Openneuro repository <https://openneuro.org/git/3/ds004648>.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2024.107003](https://doi.org/10.1016/j.psyneuen.2024.107003).

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