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Ketogenic diet is less effective in ameliorating depression and anxiety in obesity than Mediterranean diet: A pilot study for exploring the GUT-brain axis

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ABSTRACT

Obesity is associated with depressive symptoms due to biological and psychological factors. Dietary interventions, including the Ketogenic (Keto) and Mediterranean (Med) diets, impact weight loss and mental health differently. While the Keto diet promotes rapid weight loss by increasing ketone body levels, its effects on mental health, particularly in individuals with obesity, remain unclear. This exploratory pilot study explores the impact of both diets on depression and impulsiveness, focusing on the gut-brain axis. Sixty-four participants (Body Mass Index 30–45 kg/m^2, ages 18–65) were randomly assigned to follow one of the two diets for three months. Due to attrition, 37 participants (Med n = 23; Keto n = 14) completed the study. Depression and impulsivity scores were evaluated before and after the intervention. Stool samples were collected for microbiota analysis, and faecal transplants were performed in healthy mice. Brain and serum metabolites in recipient mice were analysed using High-Resolution Magic Angle Spinning (HR-MAS) and Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. The Med diet showed greater improvement in depression scores compared to the Keto diet, while the latter was associated with reductions in impulsivity (urgency subscale). However, faecal transplants from the Keto group induced anxiety-like behaviours in recipient mice, which correlated with significant microbiota and metabolite changes. The Keto group exhibited increased levels of taurine, alanine, and betaine in the brain, and threonine levels were correlated with behavioural changes. These findings suggest that the Med diet offers more consistent short-term benefits related to depressive symptoms, while the Keto diet modulated impulsivity. The animal model findings highlighted the role of diet-induced microbiota changes and metabolite alterations in the gut-brain axis. Long-term studies in a larger population are needed to tailor dietary interventions, essential for optimizing mental and physical health in obesity.

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1. Introduction

Obesity has lately been highlighted as a worldwide health pandemic due to the massive increase in the number of cases in the last decades. According to the World Health Organisation (WHO; 2025. Obesity and overweight.), over 300 million people suffer from obesity, of which 115 million people have serious obesity-related health problems. Evidence-based obesity treatment combines behavioural interventions, nutrition, physical activity, pharmacotherapy, and metabolic/bariatric procedures, depending on the severity of the disease (Bray et al., 2016). Although the significant development of obesity pharmacotherapies, such as the Glucagon-Like Peptide 1 (GLP-1) agonists, is bringing new attention to body weight loss (Li et al., 2024), specialist still relies on hypocaloric diets to fight obesity due to the side effects and high cost of these new drugs (Melson et al., 2024). Among these hypocaloric diets, the Mediterranean (Med) diet is the best option for cardiovascular health (Martínez-González et al., 2019); however, its modest effect on body weight loss (Poulimeneas et al., 2020) demands different dietary programs. Lately, the Ketogenic (Keto) diet has been adopted by many nutritionists in their day-to-day practice due to its promising effect on body weight loss (Volek et al., 2024). One of the most challenging aspects of a dietary program is the participant's adherence to the nutritional requirements (Krishnan et al., 2020), which is directly linked to the intervention's success. Rutinary food preparation could make participants lose interest in a highly demanding diet and could increase anxiety, finally affecting the participant's well-being (Solomou et al., 2023). Many studies have pointed out that obesity is linked to depression in a bidirectional relationship.

While obesity could lead to depression due to social stigma, discrimination and low self-esteem (Emmer et al., 2020), depression may also contribute to obesity due to eating behaviour impairments and loss of interest in doing physical activities (Erden et al., 2023; Victoria-Montesinos et al., 2023). Some biological mechanisms have been associated with these diseases, such as low-grade chronic inflammation and hormonal imbalance (Capuron et al., 2016; Ge et al., 2018; Lu, 2007; Schachter et al., 2018) which finally affect brain chemistry dysregulating some neurotransmitters important for mood regulation, appetite and energy balance such as serotonin and dopamine (Belujon and Grace, 2017; Moncrieff et al., 2022). These events create a vicious cycle that is hard to break.

Emerging research highlights the importance of the gut-brain axis in obesity (Schachter et al., 2018; Mela et al., 2025). The gut microbiota, the diverse community of bacteria in the digestive tract, can influence brain function and emotional regulation via immune, endocrine, and neural pathways (Liang et al. 2022). Dietary interventions have been demonstrated to alter gut microbiota composition (Gutiérrez-Repiso et al., 2019), impacting physical and mental health. Notably, Keto diets have shown promise in reducing inflammation, promoting weight loss, and potentially improving emotional well-being by altering gut microbiota and raising ketone body levels (Ang et al., 2020; El-Zein, 2022). In fact, ketone bodies and their relationship with depression are a hot topic in the fields of neuroscience due to their multiple beneficial effects in related mechanisms (Omori et al., 2023). Among those, the anti-inflammatory effects and neuroprotective properties have been highlighted as the most influential for mood disorders (Omori et al., 2022; Puchalska and Crawford, 2017). While some studies have suggested that ketone bodies may exert mood-modulation or antidepressant effects, others have reported inconsistent or null findings, highlighting the need for further investigation into their role in depression (Decker et al., 2025; Dong et al., 2025; Ren et al., 2025). In addition, most of these studies have been done in diagnosed cases, not in people with obesity, where the long-term effects of the Keto diet on mental well-being have not been elucidated yet.

Considering all the information described above, it seems plausible that finding the appropriate diet intervention for people with obesity, which ensures adherence to the dietary program, will increase the success ratio of the intervention. Therefore, this study aims to better understand the effects of two different diet interventions (Med and Keto diets) on the participant's mental well-being, specifically by analysing their depressive and impulsive state, and the role of microbiota diet-related changes in those processes.

2. Material and methods

2.1. Human subjects

Sixty-four healthy participants with obesity from both sexes enrolled in this study. The recruitment was performed by the outpatient clinics of the Endocrinology and Nutrition Department at Virgen de la Victoria University Hospital in Malaga, where all participants provided written informed consent before enrolment by EU laws (provisions of Regulation (EU) 2016/679 General Data Protection and Organic Law 3/2018 of Personal Data protection and guarantee of digital rights and Law 14/2007 on Biomedical Research). Inclusion criteria: aged between 18 and 65 years old and body mass index (BMI) between 30 and 45 kg/m2. Exclusion criteria: pregnancy or breastfeeding period; being recently diagnosed with celiac, Crohn's or any disease which could alter the nutritional status, allergies or food intolerances; as well as being treated with antibiotics and probiotics. This is a sub-study based on a secondary outcome from the one registered at ClinicalTrials.gov (NCT04453150). The study was approved by the Ethics Research Committee of Malaga (1/2019-P14 approved on 31/01/2019) and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. In this manner, the current study represents a prospective assessment of the trial, aimed to evaluate the impact of two hypocaloric diets, a Mediterranean and a Ketogenic diet, on the depressive and impulsive state in patients with obesity.

This study used a per-protocol analysis, including only participants who completed the full 3-month dietary intervention and provided valid data for key outcomes (e.g., Beck Depression Inventory (BDI) and Urgency, Premeditation, Perseverance, Sensation Seeking (UPPS) assessments) at the two time-points. This approach was chosen to ensure the integrity of the outcome measures and accurately assess the effects of the interventions in adherent participants. Fig. 1 shows the CONSORT flow diagram including participant withdrawing. For this sub-study, only participants with quality data at baseline and post—intervention periods in at least one of the two main variables, BDI and/or UPPS questionnaires, were included. Thus, from 64 volunteers, 37 were finally considered: 23 for the Mediterranean diet (BDI: 18; UPPS: 18) and 14 for the Ketogenic diet (BDI: 12, UPPS: 9).

2.2. Human dietary interventions

The participants followed two different hypocaloric diets for 3 months, particularly Med diet and Keto diet. Participants were randomly assigned to one of the interventions, Med or Keto diet, in a 1:1 ratio through a block randomization, using a computer-generated random allocation sequence without additional stratification, to ensure balanced group sizes during the recruitment process.

All participants received dietary counselling from expert nutritionists and written support materials and menus with the specific dietary plan for adherence during the intervention (including detailed daily meals, specifying food portions) at the baseline visit. The meals were tailored to achieve an energy deficit of 600 kcal/day, based on the estimated energy requirements calculated by the Harris-Benedict formula (Harris and Benedict, 1918). The adherence to the diet was monitored at each face-to-face visit, through interim telephone calls, constant contact with the nutritionists for doubts or incidents through an instant message app during the study, and through the evaluation of capillary ketonemia levels weekly (Freestyle Optium Beta-Ketone, Abbot Laboratories SA, Chicago, IL, USA). The composition of those diets is detailed below:

a) The Med diet was performed with a 600 Kcal/day caloric deficit and the following caloric distribution: 45 % carbohydrates, 35 % fat, and 20 % protein. Mediterranean dietary pattern is based on olive oil as the primary fat source; regular vegetables (2 servings/day), fruits (3 servings/day), legumes (3 servings/week) and fish (3 servings/week) intake; red meat/sausages reduced intake (<2 times/week); and dairy product, sugary drinks and factory-made pastries limited intake (<1 time/week). This dietary program recommended at least four meals/day (breakfast, lunch, evening snack and dinner).

b) The Keto diet had a caloric deficit of 600 Kcal/day and the following caloric distribution: 5 % carbohydrates, 65 % fats and 30 % proteins of high biological value.

Moreover, a daily physical activity program was recommended for all participants, which included 250 min/week of fasting walk and strength training as a minimum goal.

2.3. Human depression and impulsivity assessments

The Beck Depression Inventory (BDI) and the UPPS Impulsive Behaviour Scale were performed by the participants in a self-report

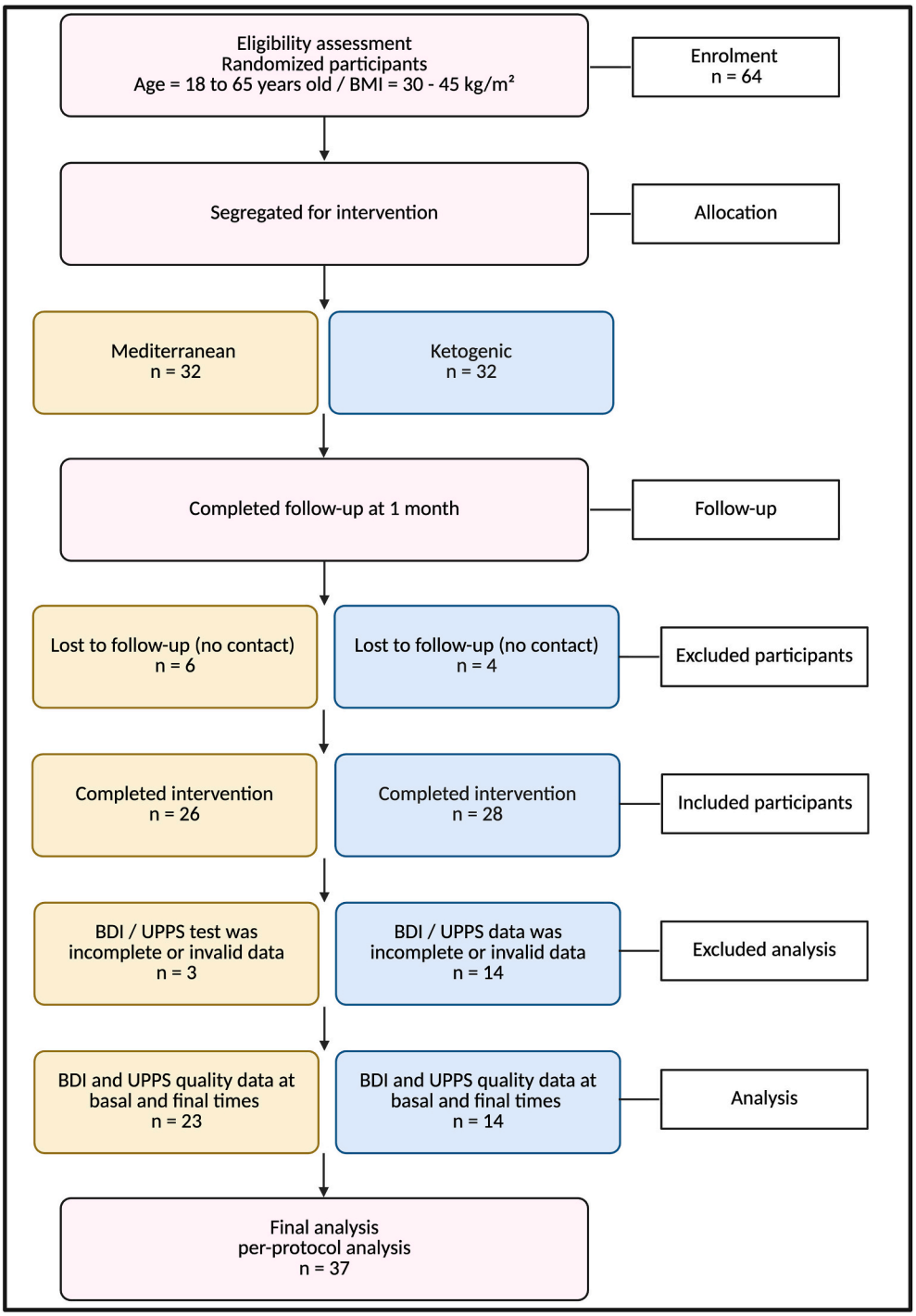

Fig. 1. Participant flow diagram. Sixty-four healthy participants with obesity (32 per group, aged 18–65, Body Mass Index (BMI) 30–45 kg/m^2) were randomized into 3-months diet intervention: Mediterranean (Med) and Ketogenic (Keto). Faecal and blood sample collection, and cognitive assessments (BDI: Beck Depression Inventory; UPPS: Impulsive Behaviour Scale) were performed.

assessment before and after dietary intervention.

BDI consists of 21 multiple-choice questions, each corresponding to a particular symptom of depression ranging from mood-related issues to physical symptoms. Each question offers four possible responses scored from 0 (absence of symptom) to 3 (severe symptoms). Only those participants with the information available and complete for all the items and for both sample times were included in the current sub-study.

UPPS analyse different facets of impulsivity, identifying impulsivity as a multi-dimensional construct: 1) Urgency: rashly acting when experiencing negative emotions; 2) Lack of premeditation: acting without considering the consequences of the actions; 3) Lack of perseverance: difficulty in remaining focused on tasks; and 4) Sensation seeking: pursue potentially dangerous experiences. The test consists of 20 items rated on a 4-point scale (1 = strongly disagree, 4 = strongly agree). The scores for each dimension provide insight into an individual's impulsive behaviour profile. High scores in specific dimensions may indicate a greater propensity for risky behaviours or difficulties with emotional regulation. Only those participants with the information available and complete for all the items were included in the current sub-study. The improvement in the individuals' cognitive performance was found by subtracting the score priot diet intervention from the score obtained at the post-intervention (Δ=final-basal condition).

2.4. Human sample collection

Blood and stool samples were collected from all the participants in overnight fasting conditions (before breakfast) during the follow-up session. Blood samples were used for biochemical analysis. Walnut-sized stool samples were collected in a sterile wide-mouth flask and promptly frozen at −80 °C until further analysis.

2.5. Animal studies

Eighteen mice (8-week male C57BL/6) were depleted of their gut microbiota using an antibiotic suspension (Ampicillin; 1 g/L) in the water bottle for 2 weeks before the faecal transplant procedure. Faecal transplants (FMT) from human samples (Med and Keto group, n = 18) were administrated to the recipient animal by oral gavage over three consecutive days after 3 days of washout from the antibiotic treatment. To maintain the microbiota transplant, a faecal boost was administered twice per week. An extra group with the faecal transplant from human samples at basal condition (before dietary intervention; n = 8) was used as reference group for behavioural data. Only male mice were used in this study to reduce variability in behavioural and microbiota responses due to sex-related hormonal fluctuations, which can confound interpretation in small experimental groups. Considering that the focus of the FMT experiments was to investigate the impact of donor-derived microbiota on host behaviour, we aimed to minimize recipient-related confounders. Animals were housed (five per cage) under controlled conditions (20 °C–22 °C, food and water ad libitum) and maintained under veterinary supervision. The local Ethics Committee reviewed and approved all experiments and animal protocols. They complied with Royal Decree, 1201/2005 (BOE n° 252) regarding the protection of experimental animals and with the Directive of the Council of the European Communities (86/609/EEC).

2.6. Open field test

To assess the response to a novel stressful environment and locomotive activity, mice were placed in the centre of the open field arena (the walls of the maze were made of white opaque Plexiglas; 40 cm long × 40 cm wide × 30 cm high) at ground level and allowed to explore for 5 min. A video recording was carried out during the experiment. Animals were food-deprived during behavioural sessions to enhance task engagement but were immediately returned to their home cages after completing the behavioural task. Various parameters of mice behaviour were analysed regarding the open field area, open zone and centre zone. The videos were analysed using computer software (Viewer, Biobserve, Germany).

2.7. Mice sample collection

At the end of the experiment period, mice were anaesthetised with sodium pentobarbital (Euthanimal) and transcardially perfused with saline. Blood samples were collected by cardiac punction before the perfusion. The brain was dissected free and used for metabolites analysis (see below).

2.8. Microbiota analysis

DNA extraction from stools was done using the QIAamp DNA stool Mini kit (Qiagen). Gut microbiota was assessed through the 16S rRNA sequencing with the 16S Metagenomics Kit (Thermo Fisher Scientific). Libraries were created using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific). Barcodes were added to each sample using the Ion Xpress Barcode Adapters kit (Thermo Fisher Scientific). Emulsion PCR and sequencing of the amplicon libraries were performed on an Ion 530 chip (Ion 530TM Chip Kit) using the Ion Torrent S5TM system and the Ion 510/520/530TM Kit-Chef (Thermo Fisher Scientific) according to the manufacturer´s instructions. The process included reference Escherichia coli samples for quality assessment. After sequencing, the individual sequence reads were filtered using Ion Reporter Software V4.0 to remove low-quality and polyclonal sequences and further translated into amplicon sequence variants (ASVs) using DADA2 with adapted parameters for Ion Torrent data within the microbiome analysis package QIIME2 2024.2 version (https://www.qiime2.org) (Bolyen et al., 2019), which will also be used for diversity analysis and subsequent taxonomic analysis through ANCOM-bc.

2.9. Metabolite quantification by 1H NMR spectroscopy

All spectra were acquired on a Bruker AVANCETM 600 MHz Spectrometer (Bruker BioSpin, Ettlingen, Germany) equipped with an *Advance III* console and either a 4 mm TXI HR-MAS probe for intact tissue analysis or a nitrogen-cooled TCI Prodigy cryoprobe for plasma samples analysis.

Water-suppressed 1H high-resolution magic angle spinning (HR-MAS) NMR spectra of intact tissue samples were acquired using a Carr-Purcell-Meiboom-Gill (*CPMG*) sequence with the following parameters: 12 kHz spectral width, 64 k data points, 64 scans, 1 ms echo time (2τ) with a total echo time of 130, and 5 s relaxation delay. Water presaturation was applied during the relaxation delay. Metabolite quantification was performed using creatine as the internal reference with the software LCModel (Provencher, 1993), as described elsewhere (Righi et al., 2009).

For plasma samples, the following acquisition sequences were used: 1D Nuclear Overhauser Effect Spectroscopy (NOESY), 1D Carr-Purcell-Meiboom-Gill (CPMG) for T2 editing of macromolecule signals, and 2D J-resolved (JRES) to aid in the identification of metabolites. Small metabolites were quantified on the CPMG spectra using the software Chenomx (v10.0, Chenomx Inc., Edmonton, Ca) and as the concentration reference, the Electronic Reference To access in-vivo Concentrations (ERETIC), as implemented in TopSpin 3.5.pl7 (ERETIC 2).

2.10. Statistical analysis

All statistical analyses were performed with SPSS 28.0.1.1, GraphPad Prism 9 and Quiime2. Kolmogorov-Smirnov and Levene tests were used to check normality and homoscedastic assumption, respectively. Data are reported as the mean ± Standard Error of the Mean (SEM), and the number of experimental individuals or repetitions is indicated in each

figure. For human studies, only datasets with basal and final parameters were included. A post-hoc power analysis was conducted based on the observed effect size between groups, indicating that the statistical power for detecting differences in BDI and UPPS outcomes was approximately 70 % at a significance level of 0.05. Statistical analysis was carried out using Student's *t*-test for two independent means or dependent means when corresponding. Repeated measures ANOVA was used when needed. Spearman correlation test was performed when required. To control for multiple comparisons, p-values were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR) method with a threshold of Q = 0.05. Only correlations that remained significant after correction were interpreted. The significance level was set at $p < 0.05$.

3. Results

3.1. The Mediterranean diet showed greater improvement in depression compared with the Ketogenic diet regardless of body weight loss outcomes

No differences were observed between volunteers of the different diets at baseline, indicating that the groups were well-balanced at the start of the intervention. Blood ketonemia, assessed to measure dietary adherence indirectly, indicates differences in ketone bodies concentration between groups at 1 and 2 months, losing the differences at 3 months, although with higher values again for the Keto diet participants (Fig. 2A). The anthropometric parameters measured in the following-up session showed a significant reduction between the basal and final state of the participant (before and after 3-month diet intervention, respectively). Notably, BMI, Waist and Hip circumferences, as well as Fat, and Muscle mass values were reduced in both dietary treatments, as well as the theoretical basal metabolic rate, although no significant differences were attributed to the type of diet (Table 1).

Although both diets succeed in the body weight loss outcomes, the Keto diet showed more significant body weight loss than the Med diet (Fig. 2B, $p < 0.05$). Regarding BDI score, both Med and Keto groups shown mild depressive symptoms at baseline (Med: 14.55 ± 6.32; Keto: 13.83 ± 10.50) which was improved after the 3-month dietary program. However, analysing the depression state between groups, we found less improvement in BDI score with the Keto diet compared to the Med diet (Fig. 2C, $p < 0.05$), suggesting that body weight loss is not directly associated with their depressive state. To explore this relationship, a Spearman correlation analysis was performed between changes in body weight and BDI scores, which revealed no significant association ($p = 0.511$), supporting the observation that weight loss may not be directly related to changes in depressive symptoms.

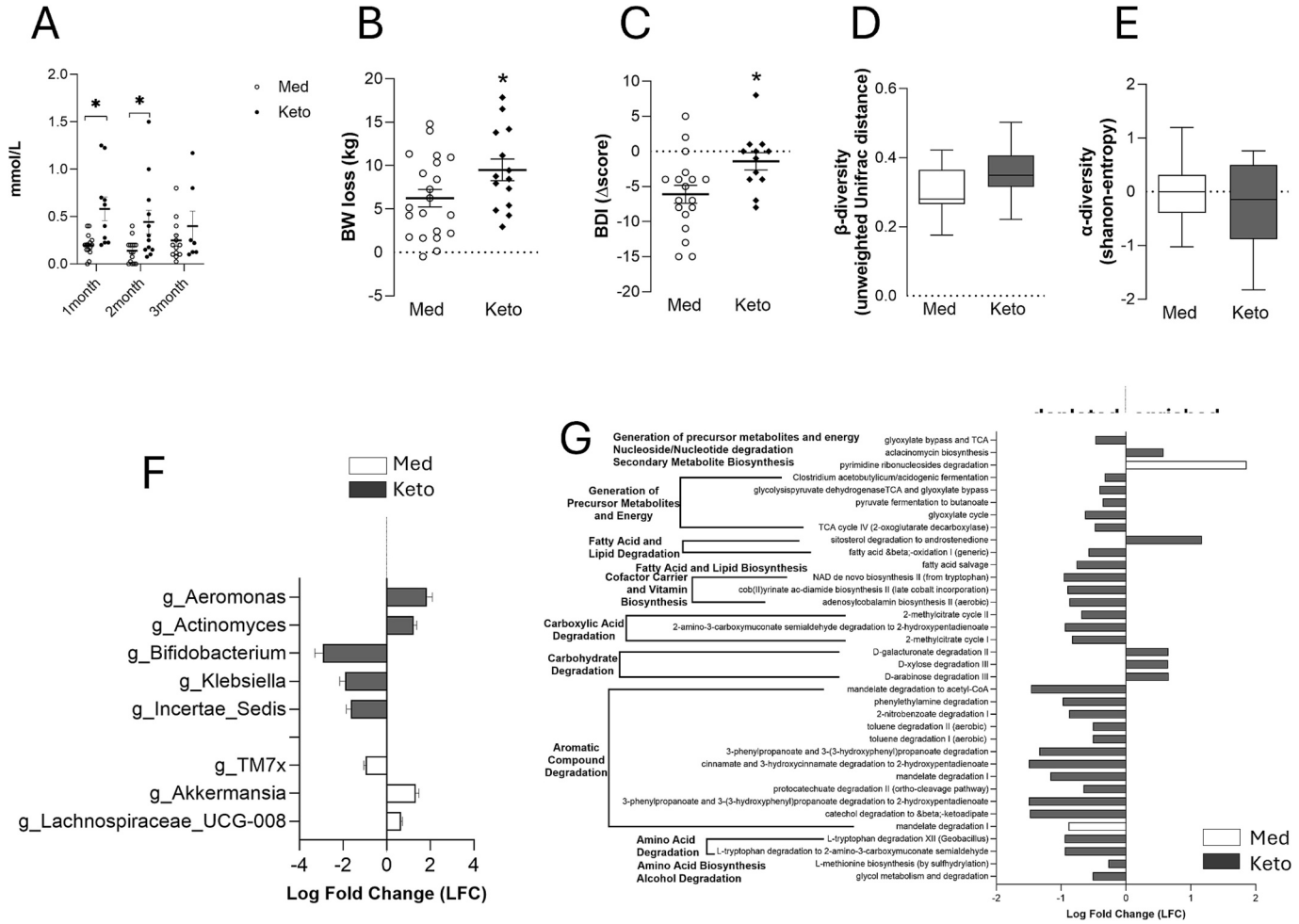

Fig. 2. 3-months diet intervention reduced body weight in people with obesity and improved their depression index. A. Ketone bodies concentrations. B. Body weight (BW) loss in kilograms (kg) was higher in the Ketogenic (Keto) group compared to the Mediterranean (Med). C. Beck's Depression Inventory (BDI) score difference (Δ=final-basal condition) was higher in Keto group compared to Med. D. β-diversity (unweighted unifrac distance) did not reach significant value between groups. E. Microbiota α-diversity (Shannon_PD index) did not reach significant value between groups. F. Significant changes in enriched and depleted bacteria genera due to diet intervention. G. Significant microbiota-related Metacyc inferred pathways by PiCRUSt. Data are expressed as the mean (± SEM). Student's t-test for independent samples was used for statistical analysis (BW: n = 14–21; BDI: n = 12–18). *$p < 0.05$. Microbiota data are expressed as log fold change ± SE, and ANCOM_bc was used for microbiota enrichment and PiCRUSt for funtional analysis (n = 11–16).

Table 1
Clinical and metabolic parameters for patients with obesity who completed the study and with impulsivity and depression data assessment before and after 3 months with Mediterranean or Ketogenic diets.

	BASAL		FINAL	
	Med	**Keto**	**Med**	**Keto**
Age (years)	47.30 ± 2.25	44.71 ± 3.50		
Gender (W/M)	18/5	14/0		
BMI (Kg/m^2)	36.44 ± 0.91	36.41 ± 1.07	**34.02 ± 0.94*****	**32.86 ± 1.02*****
Waist (cm)	111.14 ± 2.89	112.86 ± 3.12	**100.52 ± 2.48*****	**100.71 ± 4.17*****
Hip (cm)	124.62 ± 1.87	127.86 ± 2.50	**119.29 ± 2.10*****	**120.43 ± 2.80*****
Fat mass (%)	40.75 ± 0.91	42.65 ± 1.15	**38.63 ± 0.92*****	**40.33 ± 0.93*****
Muscle (Kg)	55.97 ± 2.52	53.73 ± 1.04	**54.35 ± 2.56*****	**50.37 ± 1.09*****
TBMR (Kcal/d)	1806.43 ± 80.58	1719.92 ± 44.47	**1738.85 ± 79.88*****	**1604.23 ± 41.58*****
CF (ppm)	73.80 ± 2.22	79.58 ± 2.70	73.81 ± 2.49	**76.83 ± 2.79*****
Chol (mg/dl)	188.33 ± 5.46	200.62 ± 9.08	190.95 ± 6.78	194.08 ± 7.34
HDL (mg/dl)	50.86 ± 2.33	52.15 ± 3.44	49.52 ± 2.00	48.92 ± 2.93
LDL (mg/dl)	115.05 ± 5.72	124.08 ± 8.45	117.9 ± 5.85	123.85 ± 6.95
Trig (mg/dl)	112.19 ± 7.77	122.08 ± 16.00	117.71 ± 8.74	106.77 ± 11.53
Apoa1 (mg/dl)	139.5 ± 20.01	166.00 ± 6.48	168.07 ± 8.71	154.64 ± 8.28
Apob (mg/dl)	98.33 ± 4.54	104.18 ± 7.73	94.2 ± 6.48	100.91 ± 5.54
Glucose (mg/dl)	91.95 ± 2.09	92.85 ± 2.80	95.62 ± 2.82	96.46 ± 1.99
Urea (mg/dl)	35.38 ± 1.85	36.58 ± 3.26	37.48 ± 2.30	36.42 ± 3.14

Data are means ± SEM. BASAL: Before diet; FINAL: after diets; W/M: Women/Men; BMI: body mass index; TBMR: theoretical basal metabolic rate; CF: cardiac frequency; ppm: pulse per minute; Trig: triglycerides; Chol: cholesterol; HDL: high density cholesterol; LDL: low density cholesterol; Apoa1: apolipoprotein a1; Apob: apolipoprotein b; Med: Mediterranean diet; Keto: Ketogenic diet. Dependent sample *t*-test comparison was done for analysis between the same group and independent sample Student's *t*-test comparisons were done for different groups. Moreover, Repeated measures ANOVA were done for multiple comparison test between the groups. ***p < 0.005 vs basal.

Regarding gut microbiota, microbial populations showed a trend to change with both diets (unweighted Unifrac distance; Fig. 2D, p = 0.07), with significant distance changes in the Keto diet but no other major changes in their diversities (Fig. 2E). Med diet increased the abundance of the genera *Akkermansia and Lachnospiraceae_UCG-008* and decreased *TM7x abundance*, while the Keto diet increased the genera *Aeromonas,* and A*ctinomyces* and depleted the genera *Incertae_Sedis*, *Klebsiella,* and *Bifidobacterium* (Fig. 2F, p < 0.05). To understand the inferred metabolic pathways, we performed the PICRUst analysis. This analysis revealed that the Med diet only affected two pathways (PWY-1501, PWY-7209). In comparison, the Keto diet affected 34 pathways (29 depleted and 5 enriched routes), most belonging to the Aromatic Compound Degradation function (Fig. 2G, p < 0.05).

In addition, we analysed the impulsivity of these participants through the UPPS scale, which at baseline levels suggest a moderate level of impulsivity in both groups (Med: 38.78 ± 5.79; Keto: 41.54 ± 7.76). We found a decrease in the final score after 3 months of the Keto diet compared to the Med diet (Fig. 3.A, p < 0.05), suggesting less impulsivity than the one under the Med diet. In order to elucidate if any of the dimensions of this test were affected more by the diet, we split the score according to those dimensions. Only the urgency dimension significantly differed between the Med and Keto groups, showing lower scores in the Keto group (Fig. 3C, p < 0.05).

3.2. *Ketogenic diet-related changes increased anxiety behaviour in healthy mice through the GUT-Brain axis*

In order to understand if those microbiota diet-related changes were responsible for those mood changes observed in the individuals under the different diets, an open field test was performed in recipient mice of the faecal transplant from both experimental groups. This test evaluated anxiety-like behaviour and locomotive activity in a novel stressful environment.

Animals under the Keto diet transplant (Keto) demonstrated significant impairment in locomotion (Fig. 4A-C): outer track length ($p < 0.05$), inner track length ($p < 0.05$), and centre track length ($p < 0.05$) compared to Med diet transplant. Animals that show more stereotypic

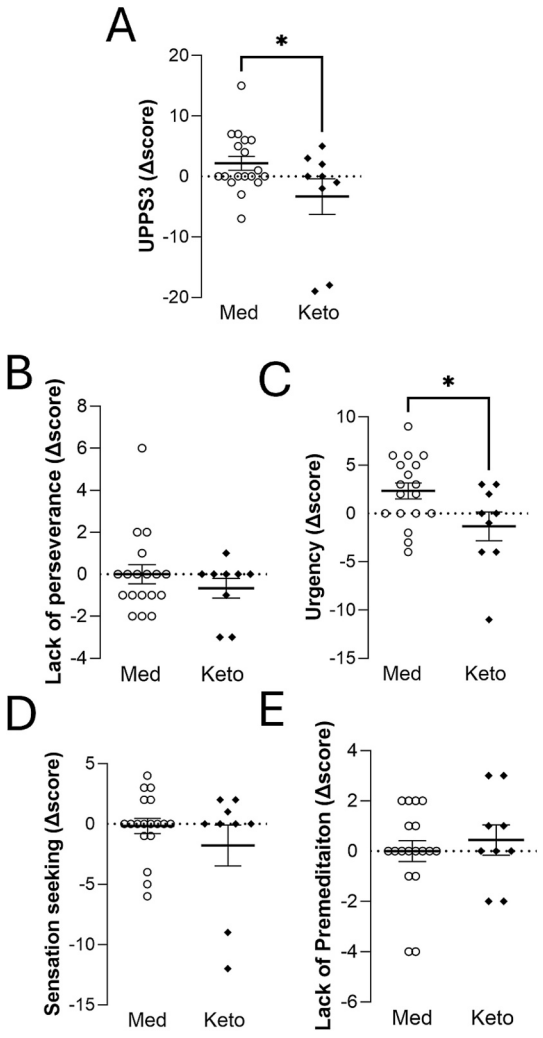

Fig. 3. Ketogenic diet intervention improved impulsive behaviour in people with obesity. UPPS impulsive behaviour test was performed in participants with obesity before and after the 3-month diet intervention (Med: mediterranean diet; Keto: Ketogenic diet) and the improvement (Δ=final-basal condition) of the different dimension of the test was analysed (B-E). A. Higher decreased on UPPS score was found in the Keto group compared to Med. B. No significant differences were found between groups in Lack of perseverance. C. Higher decreased on Urgency dimension score was found in the Keto group compared to Med. D. No significant differences were found between groups in sensation seeking. E. No significant differences were found between groups in Lack of premeditation. Data are expressed as the mean (± SEM). Student's t-test was used for statistical analysis (n = 9–18). *p < 0.05.

characteristics are considered to be anxious or stressed (Fig. 4D-F): head-stretches ($p < 0.05$), tail motion ($p < 0.05$), and head-bob movement ($p < 0.01$) depicted significantly by Keto mice compared to Med mice. Additionally, anxious mice avoid open spaces (Fig. 4H, I). Consequently, Keto mice significantly spent less time in the inner part, also increasing their latency to enter the inner part, compared to Med mice ($p < 0.05$). At the same time, no such differences were observed in the outer part (Fig. 4G). Furthermore, Keto mice significantly made less entrance to the inner central part (Fig. 4J; $p < 0.05$) while also exhibiting lower rearing frequency (Fig. 4K; $p < 0.05$) when compared to Med mice.

Microbiota analyses were performed in mice cecum samples to understand the microbiota established after the faecal transplant and how the diversity of that microbiota could be related to the result in the mice's metabolism and behaviour performance (Fig. 5). Qualitative differences between groups were found according to the unweighted Unifrac distances analysed (Fig. 5A, $p < 0.05$), while no changes were found according to its quantitative version. Besides, we found a higher richness and a tendency to increase the microbiota biodiversity in Med-recipient mice (Fig. 5B; $p = 0.06$). Analysing the microbiota composition genera abundance, we found 2 enriched (*Flavonitractor and Erysipelatoclostridium*) and 20 depleted (*DTU014, Anaerostipes, Eubacterium_siraeum, Victivallaceae, Rikenellaceae_RC9, Lachnospiraceae_UCG-006, Blautia, Butyricicoccus, Harryflintia, Victivallis, NK4A214, Bilophila, Anaeroplasma, Turicibacter, Clostridia_vadinBB60, Bifidobacterium,* and 4 uncultured: from the families *Lachnospiraceae*, *Oscillospiraceae*, *Eggerthellaceae*, and *Prevotellaceae*) genera in Keto-recipient mice compared to Med-recipient mice (Fig. 5C; $p < 0.05$). PiCRUSt analysed revealed multiple changes in the inferred metabolic pathways of bacteria. Indeed, 48 pathways were enriched and 13 depleted in the Keto recipient mice microbiota. These differences were mainly focused on degradation routes, with aromatic amino acids and carbohydrates as primary compounds affected. Moreover, biosynthesis pathways, such as tetrapyrrole and other cell structures and cofactor biosynthesis compounds, were also affected (Fig. 5D; $p < 0.05$).

A Spearman correlation test was performed to understand the relationship between microbiota composition and animal behaviour (Fig. 5 E). Some of the genera correlated with some behaviour parameters analysed, including *Alistipes* and *Muribaculaceae* as the most associated with the behavioural outcomes. *Alistipes* and *Muribaculaceae* abundances were positively correlated with the inner part ($p < 0.05$), acceleration ($p < 0.01$), spending time in the explored areas ($p < 0.01$), and number of explored areas ($p < 0.05$ and $p < 0.01$; respectively), while showing negative correlation with inner/outer ratio ($p < 0.05$, in the case of *Alistipes*), and the mobility rate ($p < 0.01$). *Oscillibacter* abundance was negatively correlated with acceleration ($p < 0.05$), spending time in the explored areas ($p < 0.05$), and the number of areas explored ($p < 0.05$). *Butyricimonas* abundance was positively correlated with inner/outer ratio ($p < 0.05$) and the mobility rate ($p < 0.05$), while negatively correlated with the spending time in the inner zone ($p < 0.05$), acceleration ($p < 0.05$) and spending time in the explored areas ($p < 0.05$).

3.3. Microbiota diet-related changes modify brain and serum metabolites associated with depression and anxiety symptoms

Searching for the link between microbiota diet-related changes and brain function in our animal model, brain and serum metabolism was analysed using HRMAS and ^{1}H NMR, respectively.

Regarding brain metabolism, a significant increase was found in Alanine, Taurine and Betaine concentration (Fig. 6A-C; $p < 0.05$), with a trend to decrease in Glycine in the Keto group (Fig. 6D; $p = 0.05$). However, in serum analysis, only Taurine concentration showed a similar increase in the Keto group (Fig. 6E; $p < 0.05$) with no changes in the rest of the analytes mentioned above (data non shown). In addition, the Keto group also showed a significant increase in Histidine and Methionine serum concentration (Fig. 6F&G; $p < 0.05$) with a decrease in succinate concentration (Fig. 6H; $p < 0.05$).

A Spearman correlation test was performed to elucidate the impact of those metabolite changes on animal behaviour and microbiota changes (Fig. S1). Only brain Threonine was correlated with two different behavioural parameters (Fig. S1A); being positively correlated with spending time in the explored areas ($p < 0.05$) and negatively correlated with the frequency of frozen events ($p < 0.05$). In contrast, significant correlations were found between specific bacteria genus involved in those metabolite changes (Fig. S1B). Although some bacteria genera were positively correlated with serum metabolites, such as *Prevotellaceae NK3B31 group* with 1,3-Dihydroxiacetone ($p < 0.05$), 3-Methyl-2-oxovalerate ($p < 0.05$), Acetone ($p < 0.05$), Fumarate ($p < 0.05$), Phenylalanine ($p < 0.05$) and Tyrosine ($p < 0.05$); the greater impact of microbiota changes was in brain metabolism. The most striking changes were observed in *Bifidobacterium* which correlate negatively with Lactate ($p < 0.05$), N-AcetylAspartate (NAA; $p < 0.05$) and N-AcetylAspartate + N-Acetylaspartylglutamate (NAA + NAAG; $p < 0.05$).

4. Discussion

Lifestyle interventions have been highlighted as the most efficient strategy for losing weight and improving the quality of life for people living with obesity. However, getting any conclusion about the best approach to counteract the deleterious effect of the disease and reducing the risk of developing any of its comorbidities is even more difficult due to its complexity. Although the Keto diet has been postulated as a promising strategy for reducing body weight and improving the inflammatory state of the individual, the long-term effects still need to be clarified. This pilot study, part of a clinical trial, showed for the first time how a 3-month dietary intervention affects the emotional well-being of participants with obesity differently depending on the type of diet administered more than the body weight loss related to the specific diet. In fact, the Keto diet, the most effective diet for losing weight, had the slightest improvement in BDI score, considering the basal condition before the diet intervention. According to the literature, the Med diet provides more benefits in terms of depressive symptoms due to better quality nutrients included in the regimen than a regular hypocaloric diet (Martínez-González and Sánchez-Villegas, 2016; Opie et al., 2018). Although some studies point out that the ketosis induced by the Ketogenic diet could improve depression symptoms due to the upregulated GABAergic signalling produced by ketone bodies increase (Calderón et al., 2017; Qiao et al., 2024), this issue has been unexplored yet. It is worth mentioning that our results, although came from a discrete small sample size, showed short-term changes with both dietary interventions, where the Med diet induced the greatest improvement. Another unexplored effect of dietary interventions in Obesity is the impulsivity that generates in the individuals undergoing those regimens, which could predict the success of a dietary intervention. Although, the Keto diet showed a worse depressive symptoms scenario than the Med diet, it was successful in impulsivity, showing greater improvements in the urgency subscale score, which could explain the greater body weight loss compared to the Med diet. This fact supports previous studies showing reduced impulsivity in individuals with high blood levels of β-hydroxybutyrate, one of the most abundant ketone bodies after Keto diet intervention(Campbell et al., 2023).

Many studies have corroborated that microbiota-related changes affect depression and anxiety disorders in human and animal studies (Hou et al., 2022). In fact, we found in this pilot study differences in microbiota abundance depending on the 3-month diet intervention although functional analysis revealed that most of these short-term changes were related to metabolism and energy efficiency. To understand this evidence alone, we performed faecal transplantation in healthy mice using samples from Med and Keto groups at the end of the dietary intervention. Interestingly, we found less locomotion and exploration, more stereotypic movements, and inner space avoidance in the Keto recipient mice, which is linked to anxiety/depression symptoms

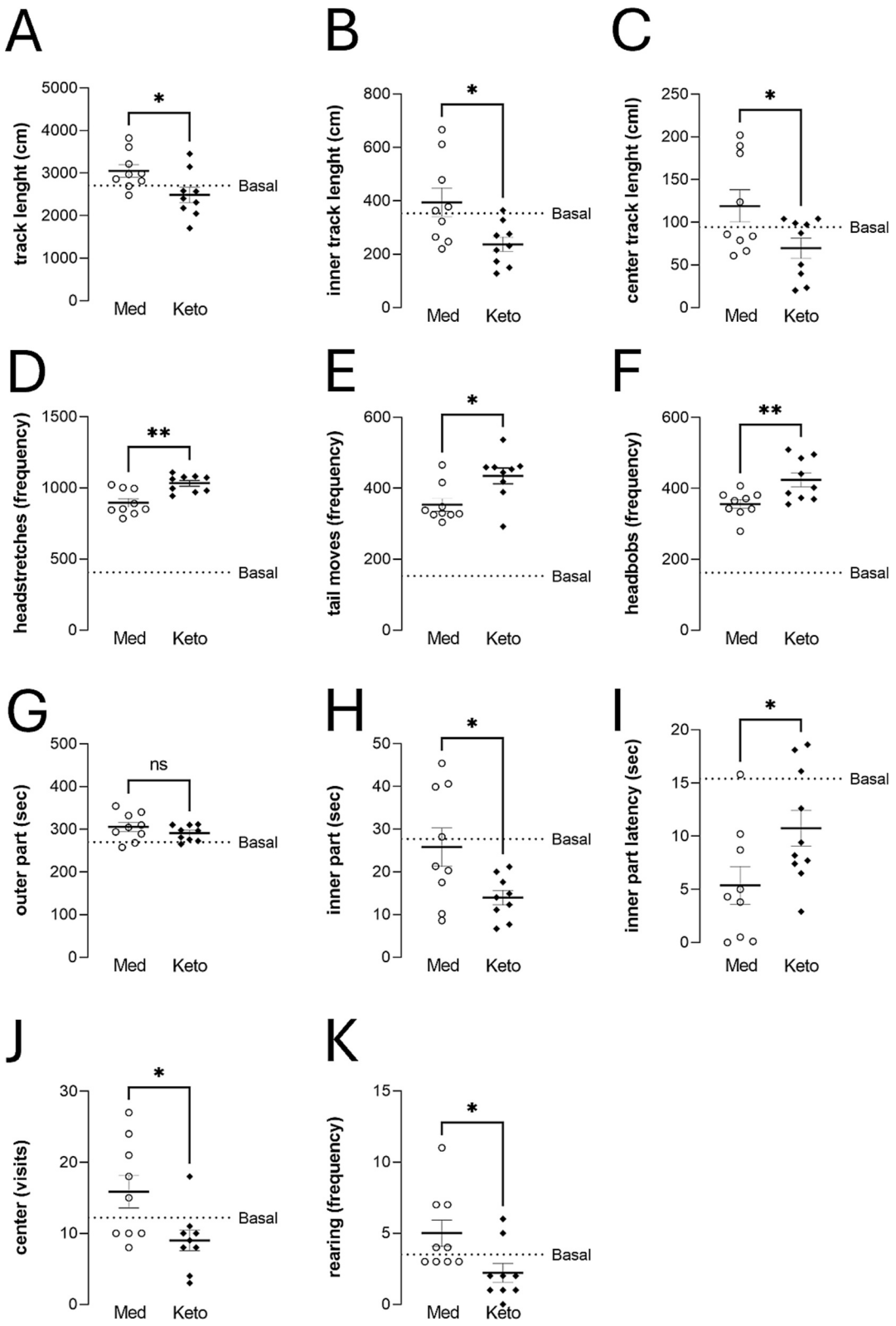

Fig. 4. Anxiety/Depression features were higher in the Keto-recipient mice compared to Med group. A faecal transplant from the human participant before (Basal status: dot line) and after diet intervention (Med: Mediterranean recipient mice; Keto: Ketogenic recipient mice) was performed in healthy recipient mice (C57BL/6J) to better understand the effect of microbiota changes in anxiety and depression parameter. Open Field paradigm was used for recording animal behaviour. Keto recipient mice travelled less distance in centimetres (cm) than the Med group in the total arena surface (A), inner part of the arena (B) and centre part of the arena (C). Regarding stereotypic movements, Keto group showed more head-stretches (D), tail moves (E) and head-bobs (F) than Med group. No differences were found in spending time in the outer part of the arena (G). Keto recipient mice showed avoidance for the inner part spending less time in seconds (sec) in the inner part of the arena (H) and showing longer latency to enter the centre (I). Keto recipient mice showed less exploration by less visit to the centre (J) and lower rearing frequency (K). Data are expressed as the mean (± SEM). Student's t-test was used for statistical analysis (n = 9). *$p < 0.05$, **$p < 0.001$.

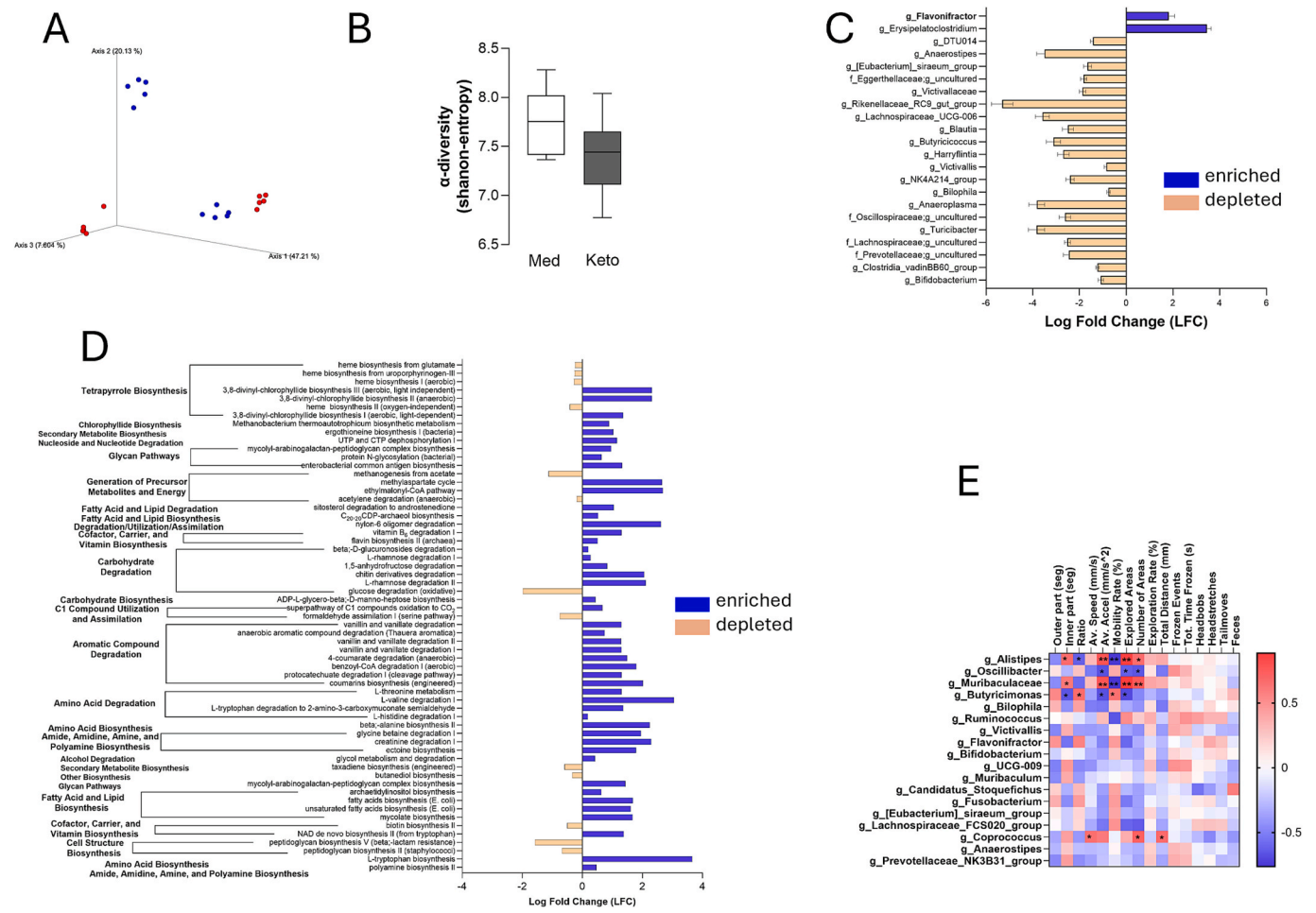

Fig. 5. Microbiota diet-related changes were established in the faecal transplant mice model. A. Microbiota β-diversity (PCoA with unweighted Unifrac distances) established in the recipient mice depending on the different dietary programs (Med: mediterranean recipient mice; Keto: Ketogenic recipient mice). Individuals are represented by different colours (blue: Med; red: Keto). B. Microbiota α-diversity (Shannon_PD index) established in the recipient mice depending on the different dietary programs. C. Microbiota abundance differential analysis at genus level between Keto and Med recipient mice. D. Microbiota-related Metacyc differential analysis inferred by PiCRUSt. E. Relative abundance microbiota correlations with recipient mice behaviour. Heatmap represents positive (red) or negative (blue) correlation between microbiota abundance with animal behaviour parameters. Data are expressed as log fold change ± SE, ANCOM_bc was used for differential abundance of microbiota analysis (n = 9). Spearman test was run for correlation analysis. *p < 0.05, **p < 0.01.

(Bouguiyoud et al., 2022; von Mücke-Heim et al., 2022). Specific beneficial bacteria, such as *Faecalibacterium*, *Eubacterium, Lactobacillus*, and *Bifidobacterium*, are often reduced in individuals with depression (Liu et al., 2023; Yang et al., 2020). Indeed, *germ-free* animal studies revealed depression-like behaviour in mice due to the absence of gut microbiota, but also faecal microbiota transplantation from individual with major depression disorder in healthy mice induce similar depressive phenotype (Zheng et al., 2016). These bacteria produce short-chain fatty acids (SCFAs), which protect the brain and regulate inflammation inducing Treg development by histone deacetylases inhibition (Rasouli-Saravani et al., 2023). Our results about microbiota changes in recipient mice corroborated this fact since among all the different genres depleted by the Keto transplant; we found *Eubacterium and Bifidobacterium*. In addition, the Keto recipient group showed *Flavonifractor* enriched. This genus has been associated with depressive symptoms in different studies (Coello et al., 2021; Gao et al., 2023), highlighting its potential influence on gut-brain axis dynamics. One of the main metabolites produced by this genus is flavonoids (Rodriguez-Castaño et al., 2020) which play an important role in tryptophan metabolism, the essential neurotransmitter in depression (Correia and Vale, 2022; Kurniati, Hirai, and Egashira, 2022; Rolinski, Martin, and Birch, 2008). In fact, analysing the functional implication of those microbiota changes, we found that depleted genera in Keto-recipient mice were associated with glucose metabolism pathway which it is the primary energy source under typical dietary conditions to maintain brain homeostasis and therefore brain functions (Zhang et al., 2021). To further analyse these events, metabolites were measured in serum and brain samples of the animal model. Although, some changes were found in different metabolite concentrations due to the faecal transplant, Taurine concentration was the most consistent, showing an increase in serum and brain in the Keto-recipient mice. Taurine has been reported as neuroprotective (Oh et al., 2020), which, together with Betaine, have been shown beneficial effects in depression (Jangra et al., 2023; Ko et al., 2024; Liu et al., 2024; Pierro et al., 2015). In fact, different studies have been shown the reduction in taurine concentration in mice with a depression-like behaviour (Zhu et al., 2023) and how taurine and betaine supplementation can protect the brain from the deleterious effect of an obesity-induced diet (Ko et al., 2024). These results could indicate a compensatory mechanism to counteract the deleterious effects of depressive symptoms in brain metabolism. In addition, correlations between serum and brain metabolites with mice behavioural parameters brought attention to Threonine brain concentration since it is highly correlated with some of the parameters studied. Threonine is essential for neurotransmitter synthesis as a precursor for metabolites like glycine and serine, with glycine also shown in our study supporting mood regulation via N-methyl-D-aspartate (NMDA) receptors (Peineau et al., 2009). Altered threonine levels

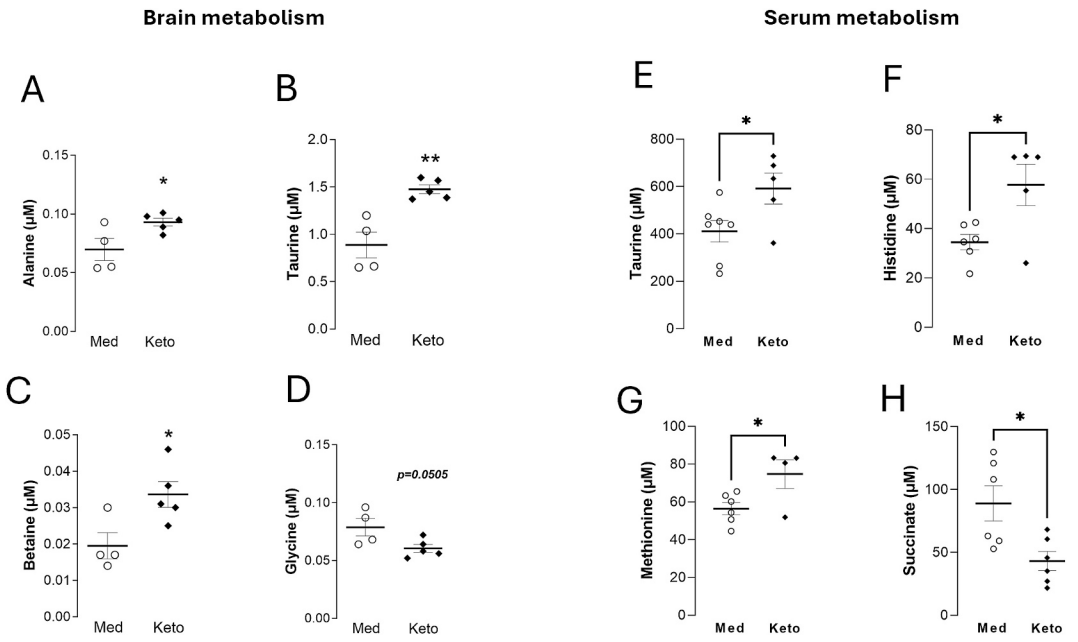

Fig. 6. Brain and serum metabolism modification by the faecal transplant in healthy mice. A faecal transplant from the human participant after diet intervention (Med: Mediterranean diet; Keto: Ketogenic diet) was performed in healthy recipient mice (C57BL/6J) to better understand the effect of microbiota changes in Anxiety and depression metabolites. Keto recipient mice showed an increase in Alanine (A), Taurine (B) and Betaine (C) concentration while tending to reduce Glycine concentrations (D) in the brain. Regarding serum metabolites, Keto recipient mice showed an increase in Taurine (E), Histidine (F) and Methionine (G) while reducing Succinate (H) concentrations. Data are expressed as the mean (± SEM). Student's t-test was used for statistical analysis (n = 5). *p < 0.05, **p < 0.001.

can increase pro-inflammatory cytokines, such as interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) (Dong et al., 2025; Yu et al., 2024), commonly elevated in depression (Elgellaie et al., 2023). It also plays a critical role in maintaining gut barrier integrity, and low levels may lead to gut dysbiosis (Mao et al., 2011). This gut imbalance can trigger inflammation and disrupt the gut-brain axis, which corroborates our results above-mentioned. Although the correlation between threonine and microbiota changes did not reach significant values, it is worth noting that the concentration of this metabolite in the brain was negatively correlated with the abundance of the bacterial genres present in Keto-recipient mice.

In conclusion, our findings highlight the multifaceted effects of dietary interventions on depression and impulsive symptoms in a pilot study involving individuals with obesity. While the Ketogenic diet demonstrated greater weight loss, it showed limited improvement in depressive symptoms compared to the Mediterranean diet. The pilot study outcomes suggest that microbiota changes induced by these diets significantly influence the gut-brain axis, impacting both mood and impulsivity. Due to the gender imbalance and restriction to a cohort of Spaniards living with obesity, the findings may primarily reflect diet effects in women with obesity and should be interpreted with caution when generalising across sexes and different populations (e.g., normoweight, ethnicity …). Because we focused on participants who adhered to the dietary protocols and completed the key outcome assessments, we could not generalise our findings since this may result in overestimating the intervention effects, particularly if participants who experienced fewer benefits were more likely to withdraw. Future studies with larger, more balanced cohorts and intention-to-treat analyses are warranted to validate these exploratory results. The observed alterations in metabolite levels further underline the critical role of these biochemical pathways in depression and anxiety. While the FMT outcomes suggest a potential association between microbiota diet-related changes and behavioural performance, it is important to note that these findings do not establish a causal relationship. Additional mechanistic studies are needed to directly confirm the causal role of specific microbial taxa or metabolites. These preliminary and exploratory short-term results emphasise the need for long-term studies to explore tailored dietary strategies that also consider mental health outcomes in obesity.

5. Limitations of the study

The dietary interventions were conducted over a 3-month period, which may not capture the long-term effects of the Ketogenic and Mediterranean diets on depressive and impulsive symptoms and gut microbiota composition. Given that the Ketogenic group included only female participants, sex imbalance is a potential confounder that may influence group differences, particularly in psychological and behavioural outcomes. In addition, to avoid bias by the day of the week or the time for the test assessment, a specific schedule was fixed to collect all the data, making the task unsuitable for everyone, which finally reduced the experimental sample size. There is a risk of expectancy or placebo effects influencing participants' responses based on the lack of a blinded design and the reliance on self-reported outcomes (BDI and UPPS), which may limit the objectivity of the observed psychological changes. We acknowledge that the dropout rate, particularly in the Ketogenic group, could introduce bias, as well as the lack of objective adherence biomarker for the Mediterranean diet. Although a particular emphasis on the direct contact with the patients throughout the intervention was done, the absence of a specific tool to assess the dietary adherence as well as the physical activity could limit the interpretation of the results. Regarding animal studies, we encountered two limitations. Firstly, although antibiotic-treated animals are a validated model for faecal microbiota transplants, they were not a fully germ-free animal model, and some results could be hampered. However, to validate the effectiveness of our approach, we analysed microbial depletion in our antibiotic-treated animals and confirmed that over 80 % of bacterial genera were successfully depleted. While not entirely germ-free, this level of depletion provides a robust and practical model for evaluating microbiota-driven effects. Secondly, we only could test some parameters of the animal behaviour due to the complexity of the study. While the open field test provides valuable insights into anxiety-like behaviour, impulsivity, and locomotion in mice, it cannot fully replicate the

complexity of human psychological processes, particularly in relation to gut-brain axis interactions. Further behavioural tests might be performed for detailed phenotyping of these animals.

CRediT authorship contribution statement

Virginia Mela: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Nadia Suyin Ortiz Samur:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Akshay Kumar Vijaya:** Investigation, Formal analysis. **Vanesa Jiménez Gálvez:** Methodology, Investigation. **María Luisa García-Martín:** Investigation, Formal analysis. **Borja Bandera:** Resources. **José Ignacio Martínez-Montoro:** Resources. **Ana María Gómez-Pérez:** Resources. **Isabel Moreno-Indias:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **Francisco J. Tinahones:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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.

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Appendix A. Supplementary data

Brain and serum metabolism correlations with recipient mice behaviour (A) and microbiota abundance (B). Heatmap represents positive (red) or negative (blue) correlation between brain and serum metabolites with animal behaviour parameters. Spearman correlation test was run for statistical analysis. *p<0.05, **p<0.01. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.106167.

Data availability

Data will be made available on request.

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