Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Check for updates

Cite this: DOI: 10.1039/d5fo01687h

Daily mango (*Mangifera indica* L.) consumption supplemented with probiotics differentially modulates inflammation and cognitive function in individuals with overweight or obesity: a placebo-controlled, double-blind, and randomized trial

Hyemee Kim,†[a] Kevin B. Kiefer,†[b] Shannon E. Schmidt-Combest,[b] Giuliana Noratto,[b] Shirley Arbizu,[b] Maria Joselyn Castellon Chicas,[b] Nicos C. Georghiades,[c] Steven E. Riechman,[c,d] Colin D. Kay,‡[e] Rodrigo San-Cristobal,[b] Stephen T. Talcott[b] and Susanne U. Mertens-Talcott*[b]

Mango, rich in gallotannins with antioxidant and anti-inflammatory properties, may enhance cognitive performance in overweight/obese individuals. However, bioavailability of mango polyphenol metabolites is reduced in this population. Probiotics may improve bioavailability by hydrolyzing parent compounds into smaller molecules. Main objective of this double-blind, randomized, controlled pilot study was to evaluate whether 8 weeks of mango and probiotic intake would enhance microbial gallotannin-metabolite levels, reduce inflammation, and improve cognitive function in overweight/obese individuals. Fifty lean participants (BMI 18–23 kg m^{-2}) and 44 overweight/obese participants (BMI 27–35 kg m^{-2}) aged 18–65 consumed 400 g of fresh mango with one placebo/probiotics capsule for 8 weeks. Cognitive function tests and blood collections occurred on days 1 and 54, with additional visual cognitive tests between days 43–54. Mango plus probiotics significantly reduced plasma Δ TNF-α in overweight/obese participants but had no effect in lean participants. Lean individuals showed improved cognitive performance (Trail Making Test A and Digit Span scores), while overweight/obese participants improved only in Digit Span Backward. Gallotannin-metabolites increased in lean participants regardless of treatment but increased only with probiotic intake in overweight/obese participants. Microbiota analysis identified enrichment of *Lactobacillus* and *Lachnospiraceae* in the mango-probiotics group, while *Clostridium* and *Streptococcus* decreased. Overweight/obese participants with higher systemic exposure to gallotannin-metabolites showed improved Δ IL-1β, Δ total ghrelin, and Δ PYY. Despite probiotic supplementation, low metabolite producers had increased Firmicutes and *Clostridiaceae*. Overall, probiotics enhanced cognitive performance in lean participants, while attenuated inflammation and improved cognitive function in overweight/obese participants, likely due to increased systemic exposure to gallotannin-metabolites.

Received 8th April 2025,
Accepted 20th October 2025

DOI: 10.1039/d5fo01687h

rsc.li/food-function

Introduction

The global prevalence of overweight and obesity is steadily increasing and is expected to affect almost 50% of the world's population by 2035.[1] Obesity not only exacerbates cardiometabolic disorders, but is also a robust correlation between obesity, cognitive impairment, and neurodegenerative diseases including dementia and Alzheimer disease.[1,2] Obesity impairs cognitive processes such as learning, memory, attention, decision-making, and executive function.[1] Additionally, obesity can reduce gut microbiome diversity, which is associated with intestinal barrier dysfunction, decreased digestion and absorption of nutrients, and cognitive impairment.[3] Low-grade inflammation and the gut-brain axis are mediators of major aspects of obesity-

[a]*Department of Food Science and Nutrition, Pusan National University, Busan, 46241, South Korea*
[b]*Department of Food Science and Technology, Texas A&M University, College Station, TX, 77843, USA. E-mail: Susanne.Talcott@ag.tamu.edu*
[c]*Department of Nutrition, Texas A&M University, College Station, TX, 77843, USA*
[d]*Department of Health and Kinesiology, Texas A&M University, College Station, TX, 77843, USA*
[e]*Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, 28081, USA*
†These authors share first authorship.
‡Present address: Arkansas Children's Nutrition Center, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

associated cognitive impairment. Investigations in animal models and some human studies have demonstrated that anti-inflammatory strategies may impact obesity-related inflammation and neurological disorders while beneficially impacting the central nervous system.[4] Recently, studies are investigating dietary strategies and bioactive food compounds as alternative preventive and complementary to therapeutic strategies to pharmacological approaches in order to restore the gut microbiota and improve cognitive impairment.[2,4]

Polyphenols are secondary plant metabolites that possess antioxidant and anti-inflammatory properties. Recently, their reciprocal interactions with gut microbiota were investigated,[5] and polymeric polyphenols demonstrated prebiotic activities to stimulate the growth of beneficial bacteria and restore gut dysbiosis.[6] While oxidative stress and inflammation also contribute to obesity and cognitive impairment,[7,8] polyphenols may have anti-obesity properties as well as beneficial effects on the gut-brain axis and cognitive function by modulating oxidative stress, inflammation, and gut microbiota.[9,10] In addition, numerous studies have demonstrated the potential of polyphenols to modulate the gut-brain axis.[11,12]

Mango is a rich source of gallic acid, gallotannins, and other polyphenolics with antioxidant, anti-inflammatory, anti-obesity, and anti-carcinogenic properties.[13,14] In addition, it has been demonstrated that mango polyphenols and their metabolites alleviate cognitive impairment.[15,16] Administration of mango leaf extract rich in mangiferin to d*b*/d*b* mice decreased tau hyperphosphorylation, a common pathology of Alzheimer's disease.[15] Given that mangiferin is also present in mango pulp, these findings support the potential cognitive benefits of fruit-derived polyphenols. Furthermore, gallic acid, the predominant phenolic acid in mango, interfered with Aβ aggregation, mitigating cognitive decline in Alzheimer's disease mouse models[17] and protecting rats from cognitive decline induced by chronic cerebral hypoperfusion and oxidative stress.[18] These findings imply that mango consumption may be beneficial for cognitive dysfunction and other chronic diseases, however, the underlying processes and their efficacy have not yet been examined in cognitive function.

Our previous data show that gallotannins abundant in mango work as prebiotics in numerous beneficial intestinal bacteria, while probiotics enzymatically hydrolyze polymeric gallotannins into smaller molecules such as gallic acid, thereby increasing their systemic exposure.[19] Previously, 6 weeks of daily mango consumption significantly increased systemic exposure to mango polyphenol metabolites in lean individuals up to 1.88-fold in plasma and 3.21-fold in urine from baseline, indicating an adaptation of metabolic activities within the intestinal microbiome.[19] In contrast, obese individuals showed no significant increase in plasma metabolite levels despite elevated urinary excretion, suggesting impaired microbial or host metabolism, likely due to obesity-associated dysbiosis.[20] The simultaneous intake of mango and probiotics may synergistically improve intestinal microbiota balance. This approach could enhance the production of gallotannin-metabolites, particularly in individuals with obesity, addressing the metabolic limitations of obesity-induced dysbiosis.[21] The combination is expected to reduce inflammation and improve cognitive function.

Therefore, the present study aimed to evaluate the effects of concomitant intake of mango and a probiotic supplement *versus* mango and a maltodextrin placebo on inflammation, gastrointestinal hormones, microbiome, and cognitive function in lean and overweight/obese individuals. We hypothesized that concomitant intake of mango and a commercially available probiotic blend will increase systemic exposure to gallotannin-metabolites to improve inflammation and gastrointestinal hormone regulation, and potentially modulate the intestinal microbiota in individuals with overweight/obesity.

Materials and methods

Study participants

Healthy adults aged 18–65 were recruited and grouped by BMI: lean (18–23 kg m^{-2}) and overweight/obese (27–35 kg m^{-2}). Participants were enrolled asynchronously *via* university-wide emails and campus flyers and pre-screened through an online survey. Exclusion criteria included recent (past 6 months) major cardiovascular events, stroke, cancer, substance or alcohol abuse, or ongoing treatment for these conditions; history of liver/kidney dysfunction, seizures, chronic infections (hepatitis B/C, HIV); current pregnancy or lactation; smoking over one pack/week; mango allergy; use of dietary supplements; or participation in other clinical/nutritional trials. Familiarization informational sessions, screening, and study trial day procedures were conducted in the Department of Food Science and Technology at Texas A&M University. This trial was authorized by the Institutional Review Board (2018-0405F) at Texas A&M University, and the protocol was registered at clinicaltrials.gov (NCT04970589). All eligible participants provided informed consent prior to the beginning of the study.

Study design and dietary intervention

This study was conducted as a placebo-controlled, double-blind, and randomized parallel clinical trial. Healthy lean (n = 50) and overweight/obese (n = 44) participants were randomly allocated to placebo or probiotics groups and instructed to consume 400 g of cubed, fresh mango along with one probiotics or placebo capsule daily for eight weeks (Fig. 1A). For reference, 400 g of mango is approximately equivalent to the pulp of 2 whole mangos excluding peel and seed or 2 cups. While this quantity is at the higher end, it is within recommended fruit intake and aligns with the dietary recommendations set by the USDA, which suggest a daily intake of 1.5 to 2 cups of fruits for women and 2 to 2.5 cups for men, making it a manageable and realistic daily consumption amount. In addition, this mango intake ensures sufficient polyphenol intake, particularly gallotannins, to observe potential physiological effects. This dose has been successfully implemented and tolerated in our previous human intervention[19] and lower doses might not elicit measurable changes.

Body weight and height were assessed on the initial screening day, and BMI was determined. Ataulfo mangoes were selected due to their higher polyphenol content than other

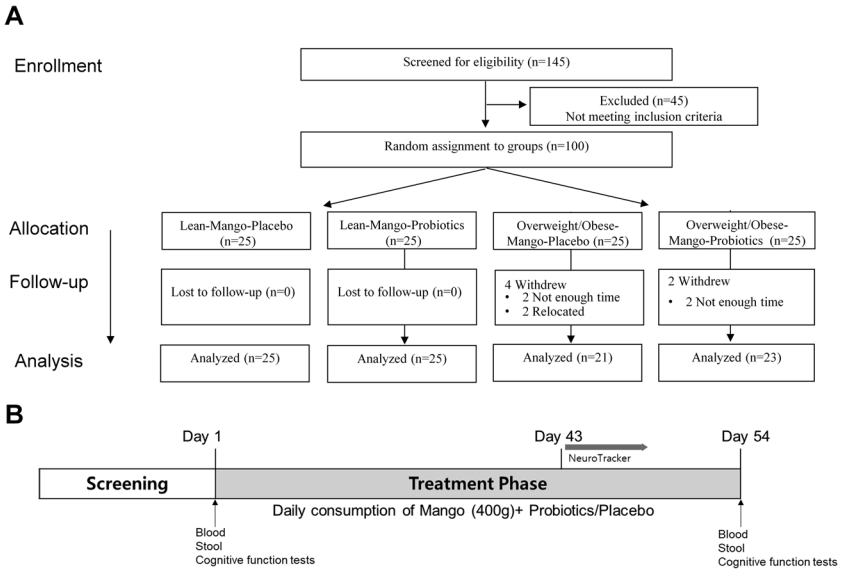

Fig. 1 Consort flow diagram for participants (A) and study design (B). This placebo-controlled, double-blind, and randomized trial included 50 lean (BMI 18–23 kg m^{-2}) and 44 overweight/obese (BMI 27–35 kg m^{-2}) participants aged 18–65 years. Participants were examined for eligibility before being randomly assigned to either the placebo or probiotics group. Participants were asked to ingest 400 g of mango supplements daily along with one placebo/probiotics tablet for eight weeks. Blood and urine samples were collected on days 1, 2, 3, 54, 55, and 56, as well as stool samples on days 1 and 54. On days 1 and 54, individuals were evaluated for cognitive function. In addition, participants engaged in 10 sessions of neurotracker training between day 43 and day 54.

mango varieties. Cubed, fresh mango (400 g) contained 1.6% fiber, 353 mg L^{-1} gallic acid equivalents (GAE) of total polyphenolics (gallotannins (164 mg), gallic acid (3.64 mg), and monogalloyl glucose (91.7 mg)), and 145.6 mg of vitamin C.[19] Mango (variety Ataulfo, Mexico or Puerto Rico) was received through the National Mango Board (Orlando, FL) *via* GM Produce Sales LLC (Houston, TX). Upon arrival, the mango was processed to remove the skin and seeds, while the flesh was cubed according to good manufacturing practices (GMPs) guidelines. Cubed fresh mango flesh was divided into the daily serving size (400 g), and vacuum sealed in polyethylene/nylon food-grade bags and stored frozen (−20 °C) to preserve quality and polyphenol stability until further use. Polyphenol composition of the mango batches used in the present study was reanalysed using the same analytical protocols described in our previous study,[19] ensuring consistency while accounting for possible batch-to-batch variability. Participants were given as many frozen mango bags (400 g each) as they could store in their home freezers and were instructed to contact the study coordinators to pick up additional mango bags as needed throughout the study. They were instructed to thaw one bag in the refrigerator overnight and consume the mango at ambient temperature the following day. Participants were also instructed to consume one bag of cubed mango per day and asked to record their mango compliance daily and submit it on the last day of the study. Participants in the probiotics group received capsules containing probiotic bacteria (FDA approved as a dietary supplement) known to possess gallotannin-metabolizing enzymes.[22] These probiotic bacteria (Renew Life Ultimate Flora "Extra Care" probiotic) included *Bifidobacterium lactis* BI-04®, *Bifidobacterium lactis* HM019™, *Bifidobacterium lactis* Bi-07®, *Bifidobacterium infantis* Bi-26™, *Lactobacillus plantarum* Lp-115®, *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* NCFM®, *Lactococcus lactis* LI-23™, *Lactobacillus casei* Lc-11®, *Lactobacillus paracasei* Lpc-37, *Lactobacillus acidophilus* La-14®, *Lactobacillus brevis* Lbr-35™ (Total cultures 50 billion per day). The study protocol involved three site visits: initial screening, Day 1 and 54. On the trial days, a 12-hour fasting blood sample was collected. Participants were then instructed to ingest 400 g of cubed, fresh mango along with their experimental capsule. Two hours following the mango and capsule consumption, a second blood sample was collected. All blood samples were separated into plasma and serum aliquots *via* centrifugation. Feces samples were also collected on the study days, and all collected samples were stored at −80 °C until further analysis. To evaluate changes in cognitive performance, subjects completed two cognitive function assessments on Days 1 and 54, and 15 training sessions over 10 days of 3D Neurotracker™ (NT) spatial awareness testing between Days 43 and 54 (Fig. 1B). Participants were asked to document a detailed food and beverage record for the 72 hours prior to the first study session. Daily records of mango consumption during the study were also kept to monitor compliance. To ensure study consistency, participants were instructed to refrain from alcohol, vigorous exercise, and polyphenol-rich foods, such as coffee, tea, chocolate, and berries 72 h before each study session. In addition, participants abstained from taking any dietary supplements one week prior to the commencement of the study and for the entire duration of the study.

Analysis of inflammatory markers and hormones

Plasma samples collected during weeks 0 and 8 were analyzed to quantify the levels of inflammatory cytokines, gastrointestinal hormones, and apolipoproteins by using Milliplex Map Human Cytokine/Chemokine Magnetic Bead Panel – Immunology Multiplex Assay (HCYTOMAG-60K), Milliplex Human Metabolic Hormone Panel V3 (HMH3-34K), and Milliplex Map Human Apolipoprotein Magnetic bead panel (APOMAG-62K) bead assays (Millipore Sigma, Billerica, MA, USA), respectively. These experiments were performed on a Luminex L200 machine (Luminex, Austin, TX, USA). Data was analyzed using the Luminex xPonent 3.1 software. Human active ghrelin (EZGRA-88K) and total ghrelin (EZGRA-89K) were quantified through enzyme-linked immunosorbent assays (ELISA) (Millipore Sigma, Billerica, MA, USA).

Analysis of cognitive performance

Attention and executive function were assessed on Day 1 and Day 54 using the Trail Making test A (TMT-A) and B (TMT-B) and Wechsler Adult Scale-Revised Digit Span tests. The Trail Making tests are used as a measure of several cognitive domains with part A assessing visual search and motor speed skills, while part B evaluates higher levels of cognitive ability such as mental flexibility.[23] In the TMT-A, participants were asked to connect consecutive numbers as quickly as possible to assess speed/sequencing, but in the TMT-B, they were asked to connect consecutive numbers and letters in an alternating sequence. The duration of each task was assessed in seconds, with a lower value indicating an improvement.[24] In the Digit Span tests, participants were presented orally with a series of numbers and prompted to repeat them in the same order to assess immediate attention (forward) or in reverse order to assess memory (backward). The number of accurate responses was used as the outcome measure, with a higher value signifying better performance.[25] The matched versions of the cognitive tasks (TMTs and DST) were used throughout each session, and participants were tested with the same versions at each test point. Visual spatial awareness was assessed during the last two weeks of the intervention (between days 43 and 54) using the Neurotracker™ (NT) 3D program.[26] NeuroTracker is a cognitive training system designed to evaluate various cognitive domains, including sustained attention, short-term memory, and information processing speed. Therefore, the Neurotracker was used during the last two weeks of the study to identify differences in cognitive training performance resulting from continuous mango consumption. In this test, participants were instructed to monitor the spatial position of four target spheres from a set of four spheres identical in shape and color. When participants successfully identified the original spheres after 6 seconds of movement, the speed was increased for the next trial. If the participants were not successful, the speed for the subsequent trial decreased. Participants completed 15 total training sessions, attending 1 or 2 sessions on alternating days using the NT 3D program. The speed scores were calculated for each training session, where a higher score indicated better performance.

LC-MS analysis of plasma

Blood samples were collected prior to and 2 hours after mango consumption on Days 1 and 54. Plasma from centrifuged blood samples was acidified with formic acid. Plasma mango metabolites were quantified using UPLC-MS/MS-(ESI)-6500 + QTRAP (SCIEX, Framingham, MA, USA). Gradient separations were carried out using a Kinetex PFP UPLC column (1.7 μm, 100 Å, 100 mm, 2.1 mm ID; Phenomenex, Torrance, CA, USA). MS/MS scanning was performed using advanced scheduled multiple-reaction monitoring (ADsMRM) with positive and negative ionization mode toggling in Analyst (Version 1.6.3, SCIEX, Framingham, MA, USA), and MultiQuant (Version 3.0.2, SCIEX, Framingham, MA, USA) was used for quantitation.[27] The concentration of identified gallotannin-metabolites was calculated using 2-hour area under the curve (AUC). This 2-point (0 and 2 h) simplified AUC utilizes fewer timepoints than AUCs involving numerous blood samples at multiple timepoints and has been evaluated as sufficiently accurate for AUC estimations.[28]

16s rRNA sequencing

Bacterial DNA was extracted from stool samples using a PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany), amplified using primers 515F (5′-GTGYCAGCMGCCGCGGTAA) to 806R (5′-GGACTACNVGGGTWTCTAAT) by MR DNA laboratory (Shallowater, TX, USA), and adjusted for 16s rRNA sequencing using the Illumina MiSeq platform (San Diego, CA, USA) according to the manufacturer's instructions. After trimming, taxonomic and community composition analyses was performed using QIIME 2 (v 2021.8), as previously performed.[29]

Statistical analysis

Sample size was calculated based on the primary outcome, cognitive performance measured by the TMT results from a previous study.[30] From *a priori* statistical power analysis (p = 0.05, power = 0.8) using the G*Power program,[31] it was estimated that 25 participants in each group would give sufficient significant changes from baseline taking into account 25% dropout rate. All values are reported as mean ± SD. Tukey's fences were applied to define outliers. Data exceeding three times the interquartile range were considered outliers and were excluded from the analysis of inflammatory and hormone markers.[32] The Shapiro–Wilk and Brown–Forsythe tests were employed to evaluate the normality of the outcome variables. Due to observed differences in mean age between the lean and overweight/obese groups, an initial ANCOVA was conducted with age as a covariate. The results showed no significant differences when compared to ANOVA. If the data satisfied parametric assumptions, a 3-way ANOVA was performed, followed by the Sidak's multiple comparison test. For data that did not meet these assumptions, a 3-way ANOVA was carried out after applying a normal quantile transformation.

To investigate potential associations between changes in polyphenol metabolites, gut microbiota, and all biomarkers, Spearman correlation coefficients were calculated to assess the

relationships between the concentration of plasma polyphenol metabolite, gut microbiota, and other indicators. All tests were carried out using GraphPad Prism 9 (GraphPad Software, Lo Jolla, CA), and a p-value ≤0.05 was considered statistically significant.

Microbiota analyses were performed using QIIME 2 (v 2021.8) and included alpha diversity metrics such as the Shannon index, Chao1 index, and observed features. Beta diversity was assessed using Principal Coordinates Analysis (PCoA) based on unweighted UniFrac distances, with statistical significance evaluated by ANOSIM. Linear Discriminant Analysis Effect Size (LEfSe) was used to identify significantly enriched taxa, with an LDA score threshold of >3.

Mediation analysis, a statistical approach used to explore direct and indirect causal pathways, was conducted to investigate whether selected gut microbial taxa mediate the relationship between gallotannin-metabolites systemic exposure and significant hormone and inflammatory outcomes in obese participants. The exposure variable, gallotannin-metabolites systemic exposure (AUC_{0-2h} at week 8), was dichotomized by selecting the five participants with the highest and five with the lowest AUC values, forming "High" and "Low" exposure groups. For each outcome-mediator pair, linear models were fitted to estimate the direct, indirect (mediated), and total effects of the exposure. This analysis was performed using the R mediation package, which estimates p-values and confidence intervals using Monte Carlo stochastic simulation (quasi-Bayesian methods), providing robust inference for complex mediation pathways in small or moderate samples. Results were summarized as Average Causal Mediation Effects (ACME), Average Direct Effects (ADE), total effects, and the proportion mediated, with interpretation based on statistical significance and effect sizes.

Results

Baseline characteristics

There were 100 participants enrolled in the study with 94 participants completing due to the voluntary withdrawal of 6 dropouts (Fig. 1A). Anthropometric characteristics of participants are presented in Table 1. The average age and BMI for lean participants (n = 50) were 23 ± 6.24 years and 21.42 ± 1.97 kg m^{-2}, while participants with overweight/obesity (n = 44) were 32 ± 13.25 years and 30.69 ± 2.662 kg m^{-2}.

Table 1 Anthropometric characteristics of participants

	Lean		Overweight/obese	
Variable	Placebo (n = 25)	Probiotics (n = 25)	Placebo (n = 21)	Probiotics (n = 23)
Gender				
Male	48%	52%	48%	57%
Female	52%	48%	52%	43%
Age (years)	24.36 (8.54)	22.12 (3.93)	35.30 (14.38)	30.80 (12.12)
BMI (kg m^{-2})	21.65 (2.24)	21.18 (1.66)	30.52 (2.71)	30.87 (2.67)

All data is expressed in means (standard deviation) or percentages.

Plasma levels of inflammatory cytokines, gastrointestinal hormones, and apolipoproteins

To evaluate the differential effects of mango supplemented with probiotics on obesity-associated clinical outcomes, plasma levels of inflammatory cytokines, gastrointestinal hormones, and apolipoproteins were assessed at the beginning and end of the study. No significant differences of pro-inflammatory cytokine concentrations were found before or after 8 weeks of mango intake with or without probiotics in lean participants and participants with overweight/obesity (Table 2). However, Δ TNF-α was lower (−0.293 pg mL^{-1}; p = 0.0470) in participants with overweight/obesity who consumed probiotics compared to the placebo and lean participants (Fig. 2A). Obesity is associated with alterations in the secretion and regulation of gastrointestinal hormones. Leptin is overexpressed in adipose tissue resulting in leptin resistance and increased plasma concentrations in association to higher body fat percentages. Other gastrointestinal hormones including GIP, GLP-1, and PYY secretory responses are also impaired and reduced.[33] Leptin levels were higher in participants with overweight/obesity (6.13 pg mL^{-1}) compared to lean participants (2.95 pg mL^{-1}) at baseline ($p \leq 0.0001$) but leptin levels were lowered only in overweight/obese participants, while in lean participants leptin levels increased regardless probiotic intake after 8 weeks of mango consumption (Table 2). Additionally, participants with overweight/obesity had lower Δ leptin than lean participants (Fig. 2B). Changes in PYY were lower in participants with overweight/obesity after 8 weeks of mango intake compared to all lean participants, but this change was mitigated in individuals with overweight/obesity from the addition of probiotics (Fig. 2C). The mixed effects analysis showed that mango and probiotics consumption increased GLP-1 by +9.19% while mango independently of probiotics resulted in a −7.31% reduction of GLP-1 over the course of the trial (Treatment × Time interaction: p = 0.0454; Table 2; Fig. 2D). There were no changes in GLP-1 between BMI classifications after mango consumption with and without probiotics (Fig. 2E).

Cognitive function

Cognitive function was assessed using the neuropsychological tests TMT-A and TMT-B and the Digit Span test. Both TMT-A and TMT-B measure executive functions including psychomotor speed, visual search, and attention while the Wechsler Adult Scale-Revised Digit Span test measures short-term verbal and working memory.[23] Mango consumption improved cognitive function test scores in lean participants by reducing the time to complete TMT-A (−23.57%, $p \leq 0.0001$) and TMT-B (−19.99%, p = 0.0089) while the mango supplemented with probiotics improved TMT-A (−19.11%, p = 0.001), Digit Span Forward (+12.16%, p = 0.0436), and Digit Span Backward (+14.95%, p = 0.0199) in the lean group (Table 3). Mango independent of probiotics and supplemented with probiotics only

Table 2 Pro-inflammatory markers and gastrointestinal hormones before and after 8-week mango consumption with probiotics or placebo in lean and overweight/obese individuals

		Lean				Obese				*p*-Value[a]
		Placebo		Probiotics		Placebo		Probiotics		
Variable	Group	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	TM × Time
Pro-inflammatory markers										
TNF-α (pg mL^{-1})	Mean	2.227^{ns}	2.193	2.235	2.187	2.814	2.711	2.776	2.483	0.6679
	SD	0.902	0.941	0.678	0.755	1.077	1.228	1.321	1.138	
	N	25	25	25	25	20	20	23	23	
IFN-γ (pg mL^{-1})	Mean	3.493^{ns}	3.498	3.399	3.525	2.725	2.727	2.333	2.131	0.3154
	SD	2.012	2.113	2.022	2.425	2.002	1.772	1.733	1.328	
	N	20	20	25	25	19	19	21	21	
IL-1β (pg mL^{-1})	Mean	0.173^{ns}	0.165	0.196	0.205	0.203	0.186	0.145	0.166	0.1127
	SD	0.132	0.117	0.158	0.163	0.169	0.152	0.186	0.181	
	N	22	22	23	23	19	19	23	23	
IL-6 (pg mL^{-1})	Mean	1.116^{ns}	1.284	0.946	1.049	1.887	1.877	1.543	1.401	0.3922
	SD	1.337	1.332	0.864	0.932	1.533	1.766	1.514	1.237	
	N	21	21	19	19	17	17	16	16	
IL-8 (pg mL^{-1})	Mean	2.929^{ns}	3.026	2.878	2.862	3.909	4.02	3.527	3.416	0.3557
	SD	1.508	1.846	1.452	1.732	1.581	1.792	1.681	1.582	
	N	21	21	20	20	18	18	20	20	
IL-10 (pg mL^{-1})	Mean	2.459^{ns}	2.939	2.407	2.744	2.186	2.183	1.968	2.056	0.8697
	SD	1.651	1.814	1.558	1.584	1.522	1.442	1.558	1.725	
	N	23	23	23	23	18	18	22	22	
Gastrointestinal hormones										
Active ghrelin (pg mL^{-1})	Mean	218.9^{ns}	252.5	261.1	224.8	206.8	185.2	198.8	228	0.7086
	SD	89.72	95.73	122.2	90.37	82.46	87.93	107.7	99.14	
	N	19	19	20	20	18	18	22	22	
Total ghrelin (pg mL^{-1})	Mean	744.4^{ns}	747	769.3	748.8	619.9	628.3	695.6	665.9	0.564
	SD	316	228.4	307.1	326.7	214.2	182.1	234.9	192.3	
	N	19	19	20	20	18	18	22	22	
Active/total ghreline (%)	Mean	0.3538^{ns}	0.3389	0.3308	0.3271	0.3465	0.3238	0.3423	0.3581	0.5196
	SD	0.2114	0.101	0.1428	0.1341	0.0812	0.1042	0.1787	0.1368	
	N	19	19	20	20	18	18	22	22	
Leptin (ng mL^{-1})	Mean	3.06^{A}	4.20^{AB}	2.85^{A}	3.66^{AB}	6.28^{B}	5.26^{AB}	5.97^{B}	5.35^{AB}	0.7005
	SD	2.12	3.03	2.42	3.35	3.46	3.63	3.02	2.71	
	N	21	21	25	25	18	18	22	22	
GIP (pg mL^{-1})	Mean	39.91^{ns}	42.93	33.67	38.28	38.22	35.89	26.66	29.66	0.5262
	SD	21.14	21.73	27.68	23.98	23.9	19.22	22.44	21.36	
	N	20	20	24	24	17	17	23	23	
GLP-1 (pg mL^{-1})	Mean	99.22^{ns}	89.98	83.29	93.81	118.8	112.1	102.6	110.9	0.0454‡
	SD	55.39	42.5	34.8	32.4	41.58	49.15	46.82	50	
	N	24	24	25	25	21	21	23	23	
PYY (pg mL^{-1})	Mean	91.45^{ns}	110.5	97.11	110.9	121	98.41	76.14	84.18	0.0959
	SD	59.84	67.17	50.27	46.21	39.47	26.93	39.18	44.51	
	N	23	23	25	25	18	18	23	23	
Apolipoproteins										
ApoE (mg dL^{-1})	Mean	6.093^{ns}	6.298	6.345	6.015	5.391	5.339	6.697	6.395	0.2827
	SD	2.056	2.467	2.988	2.487	1.945	2.286	2.203	2.231	
	N	24	24	25	25	21	21	22	22	
ApoA-1 (mg dL^{-1})	Mean	107.7^{ns}	97.86	112	108.3	88.22	86.46	94.59	99.45	0.4068
	SD	35.74	28.57	32.75	26.94	20.51	23.79	23.18	33.43	
	N	24	24	24	24	21	21	23	23	

All data are means and standard deviation (SD). *N*: sample size. [a] Results of three-way ANOVA test, TM: Treatment (Placebo or Probiotics), Time: Week 0 or Week 8. ^{ns} stands for not statistically significant. Different capital letters indicate significantly different means ($p \leq 0.05$ based on Kruskal–Wallis test). ‡$p \leq 0.05$ based on 3-way ANOVA test after log transformation.

improved Digit Span Backward scores (+11.23%, $p = 0.0485$ and +13.56%, $p = 0.0005$, respectively) in participants with overweight/obesity. In the mixed-effects analysis, the effect of 8 weeks of mango consumption with probiotics demonstrated beneficial effects on Digit Span Test Backward scores compared to the mango placebo group. Mango and the placebo resulted in +3.49% increased accurate responses, whereas mango and probiotics led to +14.22% increased accuracy (Treatment × Time interaction: $p = 0.033$) (Table 3).

Visual spatial awareness was assessed with the perceptual-cognitive training tool Neurotracker™, which uses 3D multiple object-tracking (MOT) tasks to examine multiple cognitive functions: attention, working memory, short-term memory, and information processing speed. Mean speed threshold scores were significantly higher in the lean and placebo groups over the course of the study ($p = 0.05$ and $p = 0.018$, respectively) (Fig. 3A and C) with non-significant mean speed threshold score increases over the fifteen training sessions in

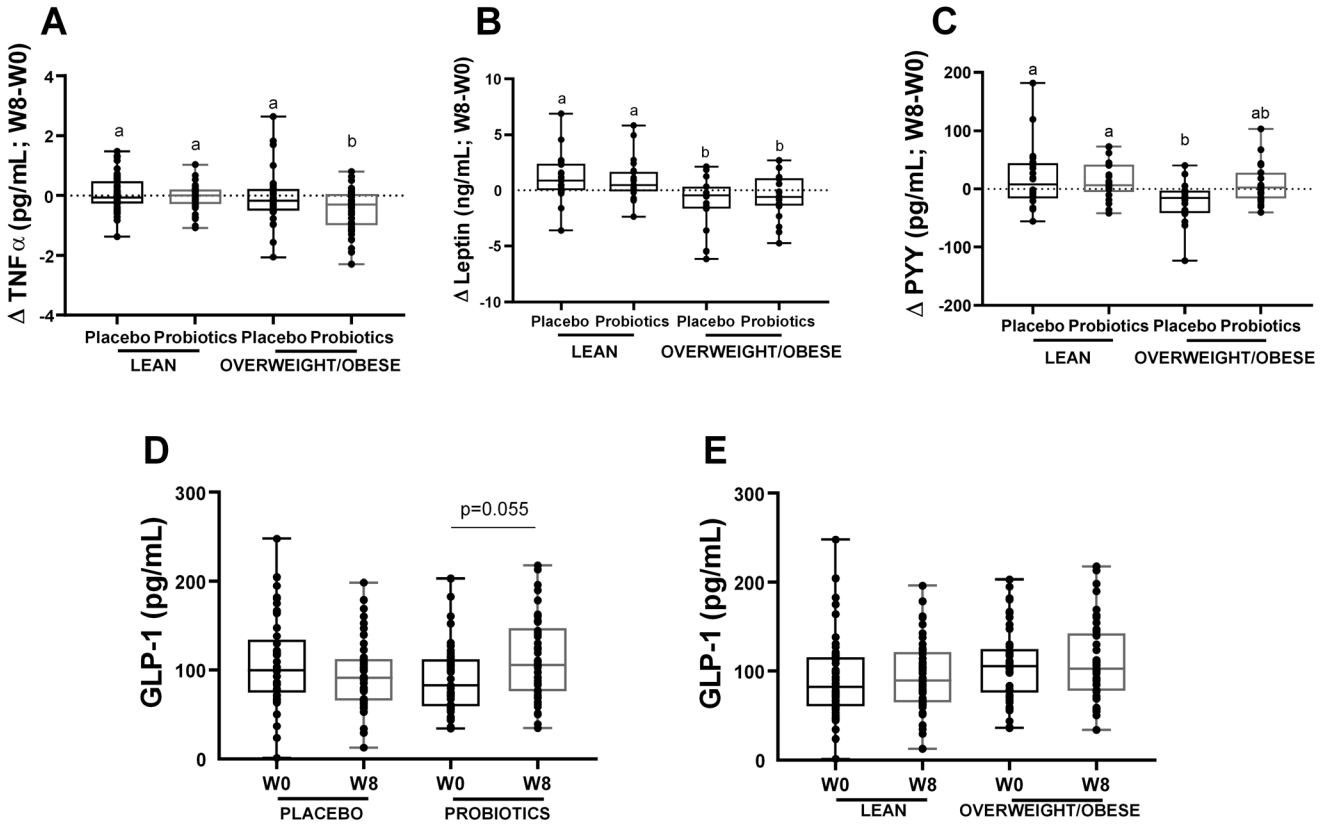

Fig. 2 Changes in inflammatory cytokines and gastrointestinal hormones in plasma after mango consumption with placebo/probiotics for 8 weeks. (A–C) Changes from baseline to 8 weeks for TNF-α, leptin, and PYY levels stratified by treatment group and BMI-classification. (d) Changes from baseline to 8 weeks for GLP-1 level in plasma stratified by treatment groups and by (e) BMI-classification. Levels of inflammatory cytokines and gut hormones were measured in plasma samples at day 1 and 54. Analysis of the differences was performed using a one-way anova followed by the Tukey test or the Kruskal–Wallis test when normality or equal variances tests failed. Different letters mean statistically significant differences at $p \leq 0.05$.

both BMI-classification and treatment groups (Fig. 3B and D). At the first training session participants with overweight/obesity received significantly lower speed scores than lean participants ($p = 0.013$; Fig. 3E). Participants within the probiotics and placebo groups significantly improved their speed scores ($p \leq 0.0001$, for both groups) from session 1 and session 15 (Fig. 3F). However, there were no score differences for any of the remaining training sessions between lean and overweight/obese BMI classifications nor between treatment groups. Overall, mango and probiotics resulted in improving TMT-A, Digit Span Forward, and Digit Span Backward in lean participants, whereas only Digit Span Backward scores were improved in participants with overweight/obesity regardless of probiotics intake. Neurotracker scores were improved in all participants regardless of treatment or BMI status. Despite implementing a study design to minimize a learning-effect for the Neurotracker activities, a possible impact should not be dismissed.

Correlations of plasma concentrations of polyphenolic metabolites with BMI and indicators

In the LC-MS analysis of polyphenolic metabolites, seven gallotannin-metabolites from mango were identified and quantified in plasma: pyrogallol, pyrogallol-*O*-sulfate, methylpyrogallol-*O*-sulfate, catechol, 4-methylcatechol, catechol-*O*-sulfate isomer 1 and 2. Mango consumption significantly increased the average systemic exposure to gallotannin-metabolites within lean participants by +40.01% ($p = 0.0037$). The addition of probiotics resulted in greater average systemic exposures in lean individuals by +55.15% ($p \leq 0.0001$) and individuals with overweight/obesity by +54.95% ($p \leq 0.0001$) (Table 4). In the mixed effects analysis, following 8 weeks of mango and probiotics intake, gallotannin-metabolites increased by +55.05%, while mango without probiotics only increased metabolites by +34.76% (Treatment × Time interaction: $p = 0.0032$). In addition, the mango/probiotics group exhibited significantly higher AUC_{0-2h} of gallotannin-metabolites than the mango/placebo group (Fig. 4A). The potential activity of gallotannin-metabolites was investigated by calculating the correlation between the sum of metabolites and BMI or biomarkers. There were significant correlations ($p \leq 0.05$) between changes in the sum of gallotannin-metabolites derivatives after 8 weeks of mango consumption and BMI ($p = 0.0077$, $r = -0.2809$; Fig. 4B), Δ IL-10 levels ($p = 0.0223$, $r = 0.2552$; Fig. 4C), and Δ TMT-A ($p = 0.0103$, $r = -0.2706$, Fig. 4D). These results show that after 8 weeks of mango intake there are significantly increased concentrations of mango gallotannin-metabolites in all lean par-

Table 3 Analysis of cognitive function before and after 8-week mango consumption with probiotics or placebo in lean and overweight/obese individuals

		Lean						Overweight/obese							
		Placebo			Probiotics			Placebo			Probiotics			p-Value[a]	
Variable	Group	Week 0 $N = 24$	Week 8 $N = 24$	p-Value	Week 0 $N = 25$	Week 8 $N = 25$	p-Value	Week 0 $N = 21$	Week 8 $N = 21$	p-Value	Week 0 N = 23	Week 8 N = 23	p-Value	TM × Time
Trail Making Test A	Mean	27.58	21.08	0.0001*	26.16	21.16	0.001*	25.24	22.95	0.3859	25.76	22.92	0.1274	0.7276
	SD	7.113	4.986		9.547	5.421		4.836	6.078		8.676	8.396		
Trail Making Test B	Mean	56.29	45.04	0.0089*	58.96	55.76	0.9454	55.10	53.71	0.9970	54.92	49.00	0.1514	0.6568
	SD	15.66	12.87		18.19	24.63		10.55	21.86		12.75	15.88		
Digit Span test forward score	Mean	5.792	6.083	0.6747	5.200	5.920	0.0436*	6.190	6.571	0.7545	5.480	5.640	0.8910	0.6958
	SD	1.382	1.613		1.472	1.187		1.401	1.165		1.418	1.287		
Digit Span test backward score	Mean	4.583	4.375	0.9432	3.640	4.280	0.0199*	4.143	4.667	0.0485*	4.080	4.720	0.0005*	0.033†
	SD	1.412	1.438		1.114	1.308		1.424	1.278		1.115	0.9798		

All data are means and standard deviation (SD). N: sample size. [a] Results of three-way ANOVA test, TM: Treatment (Placebo or Probiotics), Time: Week 0 or Week 8. *$p \leq 0.05$ by paired t-test. †$p \leq 0.05$ based on 3-way ANOVA test.

ticipants independent of probiotics and in participants with overweight/obesity who received probiotics (Table 4). Additionally, there were greater metabolite concentrations in the probiotic groups. Correlations revealed that as gallotannin-metabolites are increased, there is a small association with lower BMI values, lower TMT-A test scores, and increased IL-10 concentrations.

Gut microbiota composition after 8 weeks of mango consumption with probiotics

This study evaluated the impact of an 8-week intervention of mango consumption, with or without probiotics, on gut microbiota composition. In the alpha diversity analysis, which measures microbial diversity within samples, there were no significant changes in Shannon index (species evenness) after 8 weeks. However, Chao1 index (species richness) and observed features (the number of observed species) showed a dramatic increase after mango consumption, indicating enhanced microbial richness (Fig. 5A). No significant differences were observed between the probiotics and placebo groups for these metrics. Similarly, beta diversity analysis using Principal Coordinate Analysis (PCoA) based on unweighted UniFrac distances revealed distinct clustering patterns between Week 0 and Week 8, reflecting significant changes in gut microbiota composition after mango consumption (ANOSIM: R = 0.388, p = 0.001, Fig. 5B). As with alpha diversity, probiotics did not appear to influence beta diversity results. In the LEfSe analysis, several key taxa were identified as enriched in the probiotics group after 8 weeks of mango consumption. *Clostridium* levels were significantly higher in the placebo group at baseline compared to other groups. After 8 weeks of mango consumption, taxa such as *Lactobacillus* and *Lachnospiraceae* were significantly elevated in the probiotics group compared to the placebo group (Fig. 5C). While changes in the placebo group were minimal, the probiotics group exhibited significant enrichment of *Lactobacillus* and *Lachnospiraceae* after 8 weeks of mango intake, suggesting a potential synergistic effect of mango and probiotics. Interestingly, *Oscillospira* was significantly reduced after the mango consumption. On the other hand, potentially harmful bacteria, such as *Clostridium* and *Streptococcus*, showed a decreasing trend after mango consumption, indicating potential protective effects of mango on gut health (Fig. 5D). The heatmap analysis revealed correlations between specific microbial taxa and inflammatory markers, gastrointestinal hormones, and polyphenol metabolites. Taxa enriched in the probiotics group, such as *Lachnospiraceae* were negatively correlated with pro-inflammatory cytokines like TNF-α (R = −0.152, p = 0.048). In contrast, the harmful bacterium *Streptococcus* exhibited a positive correlation with IFN-γ (R = 0.203, p = 0.008), while *Clostridiaceae* showed a positive correlation with TNF-α (R = 0.225, p = 0.003) and a negative correlation with ghrelin (R = −0.224, p = 0.039). In addition, *Oscillospira*, which has been reported in previous studies to have a negative correlation with tannin acid intake,[34] showed a significant negative correlation with gallotannin metabolites

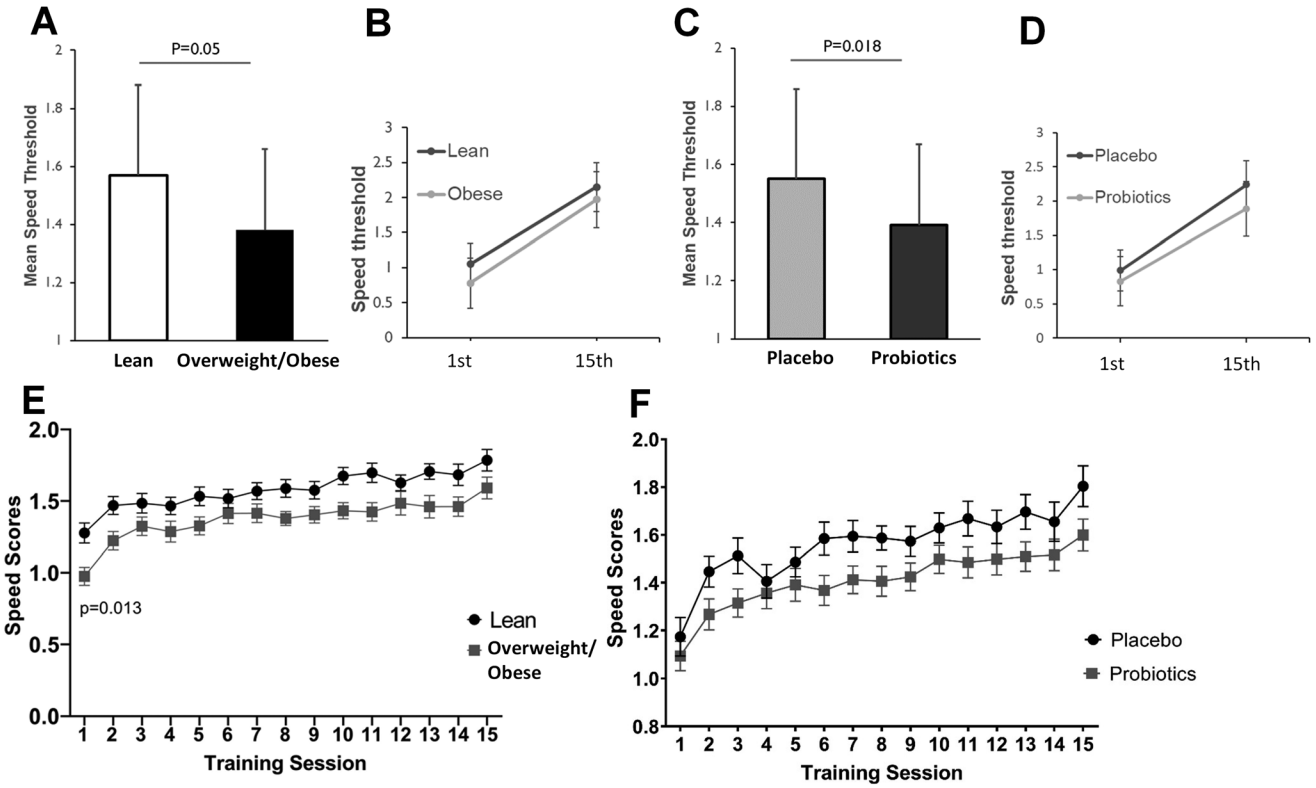

Fig. 3 Visual spatial awareness assessment using 3D Neurotracker™ 3D program (days 43–54) after mango consumption with placebo/probiotics. Spatial awareness assessment speed scores represented as mean with standard deviation bars. A higher speed threshold score indicates better performance. (A) Mean speed threshold scores between BMI-classifications (B) and the first and 15th training sessions. (C) Mean speed threshold scores for both treatment groups (D) and the first and 15th training sessions. (E) Speed threshold of the Neurotracker™ assessment over the fifteen sessions for both BMI classifications (F), and for both treatment groups. Values are expressed as mean ± SD. Analysis of the differences was performed using the Kruskal–Wallis test ($p \leq 0.05$).

in this study as well ($R = -0.301$, $p = 0.006$). These findings suggest that gut microbiota changes driven by polyphenol intake and probiotics may contribute to reduced inflammation and improved metabolic hormone regulation. Overall, the results demonstrate that mango consumption exerts a strong influence on gut microbiota composition, and the simultaneous intake of probiotics may provide a modest synergistic effect in promoting a healthier gut environment.

Characterization of Individuals with highest and lowest systemic gallotannin-metabolite exposure

Overall, results from this study do not convey any overwhelming response associated with the intake of probiotics in addition to mango gallotannins. For this reason, the study population was further stratified into overweight/obese individuals with the highest ($n = 5$) and lowest ($n = 5$) systemic exposure to gallotannin-metabolites at 8 weeks, which was based on the average plasma AUC_{0-2h} of total gallotannin-metabolites. The levels of their metabolite production after 8 weeks of mango consumption with probiotics are depicted in Fig. 6A. Mango consumption with probiotic intake significantly decreased Δ IL-1β ($p = 0.0159$; Fig. 6B) and Δ total ghrelin ($p = 0.0317$; Fig. 6C) and increased Δ PYY ($p = 0.0317$; Fig. 6D) in individuals with the highest systemic exposure compared to those with lowest exposure. The composition of the gut microbiota was analysed to determine the cause of this disparity. For individuals with the lowest systemic exposure to gallotannin metabolites, the level of *Firmicutes* (Phylum; Fig. 6E) increased significantly after 8 weeks of mango and probiotics consumption, whereas the level of *Bacteroidetes* (Phylum) tended to decrease ($p = 0.088$; Fig. 6F). After 8 weeks of mango and probiotics consumption, the level of unknown bacteria in the family *Clostridiaceae* was considerably greater in the lowest responders than in the highest at the genus level (Fig. 6G). The lack of gallotannin-metabolite production in the lowest responders may have increased the *Firmicutes*/*Bacteroides* (F/B) ratio, which is associated with reduced bacterial diversity and gut dysbiosis found among individuals with overweight/obesity. These findings indicate increased systemic exposure of mango gallotannin-metabolites may be associated with anti-inflammatory activities and regulation of gastrointestinal hormones by reducing IL-1β and ghrelin. Increased relative abundance of *Firmicutes* and *Clostridiaceae* suggest that reduced gallotannin exposure may be related to gut dysbiosis-associated microbiota shifts among individuals with overweight/obesity.

Table 4 Average systemic exposure (AUC_{0-2h}) of the sum of gallotannin-metabolites before and after 8-week mango consumption with probiotics or placebo in lean and overweight/obese individuals

		Lean						Overweight/obese						p-Value[a]	
		Placebo			Probiotics			Placebo			Probiotics				
Variable	Group	Week 0 $N = 25$	Week 8 $N = 25$	p-Value	Week 0 $N = 25$	Week 8 $N = 25$	p-Value	Week 0 $N = 21$	Week 8 $N = 21$	p-Value	Week 0 $N = 21$	Week 8 $N = 21$	p-Value		TM × Time
AUC_{0-2h} (μM h) gallotannin-metabolites	Mean	18.56	30.94	0.0037*	14.06	31.35	≤0.0001*	14.29	19.41	0.9694	16.41	36.43	≤0.0001*	0.0032†	
	SD	14.53	18.34		7.537	14.58		8.391	14.85		11.51	17.91			

All data are means and standard deviation (SD). N: sample size. [a] Results of three-way ANOVA test, TM: Treatment (Placebo or Probiotics), Time: Week 0 or Week 8. *$p \leq 0.05$ by Kruskal–Wallis test. †$p \leq 0.05$ based on 3-way ANOVA test after log transformation.

Furthermore, significant mediation and direct effects were observed for multiple gastrointestinal hormone/inflammation marker-microbe pairs in overweight/obese probiotic group dichotomized by gallotannin-metabolites systemic exposure (SI Table 2). *Clostridiaceae* and *Lactobacillus* genus mediated the relationship between exposure and IL-1β, with strong direct (ADE) effects and statistically significant total effects (ADE p-value = 0.004 and 0.002; total p-value = 0.056 and 0.038, respectively). Active ghrelin and total ghrelin showed highly significant mediation and direct effects for *Streptococcus* (ACME p-value = 0.002, ADE p-value < 0.001), with large effect sizes. Similarly, *Streptococcus* showed robust mediation and direct effects in models with GLP-1 (ACME p-value < 0.001, ADE p-value = 0.026), and ACME with PYY (p-value = 0.014, total p-value = 0.038), and *Lactobacillus* with PYY (ADE p-value = 0.008, total p-value = 0.046).

This analysis was exploratory, aiming to characterize the extreme ends of metabolite production within the overweight/obese cohort and generate hypotheses for future, larger studies, thus the statistical findings from these small subgroups should be interpreted with caution.

Discussion

This study was conducted to examine the effects of 8 weeks of mango and probiotics supplementation on inflammatory markers and cognitive function in lean individuals and individuals with overweight/obesity. We hypothesized that the addition of a commercially available probiotic blend to 400 g of gallotannin-rich mango per day would improve systemic exposure to gallotannin-metabolites, inflammatory markers, gastrointestinal hormones, and potentially enhance cognitive function based on gallotannin-metabolizing abilities of the probiotic strains, specifically in overweight/obese individuals. Notably, consumption of a complex food matrix may influence the absorption and efficacy of specific components. However, this holistic approach takes advantage of potential combined effects between the various food components present, which distinguishes it from interventions using single compounds.

Gallotannins are polymeric polyphenols that may be converted to gallic acid and pyrogallol by certain intestinal microbes, such as *Lactobacillus plantarum*, and possess anti-inflammatory and anti-obesity qualities.[19] Results show that 8 weeks mango consumption significantly increased the average systemic exposure to gallotannin-metabolites within lean participants by +40.01% ($p = 0.0037$). The addition of probiotics resulted in greater average systemic exposures in lean individuals by +55.15% ($p \leq 0.0001$) and individuals with overweight/obesity by +54.95% ($p \leq 0.0001$).

Obesity-associated inflammation can lead to impaired adipose tissue and increased levels of pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-1.[35,36] Obesity can alter gastrointestinal hormone secretion and regulation. Specifically, GIP, PYY, and GLP-1 secretory responses are impaired resulting in reduced levels. Several *in vitro* and *in vivo* experiments show that mango polyphenols have anti-inflammatory properties.[37–39] In individuals with overweight/obesity, 6 weeks

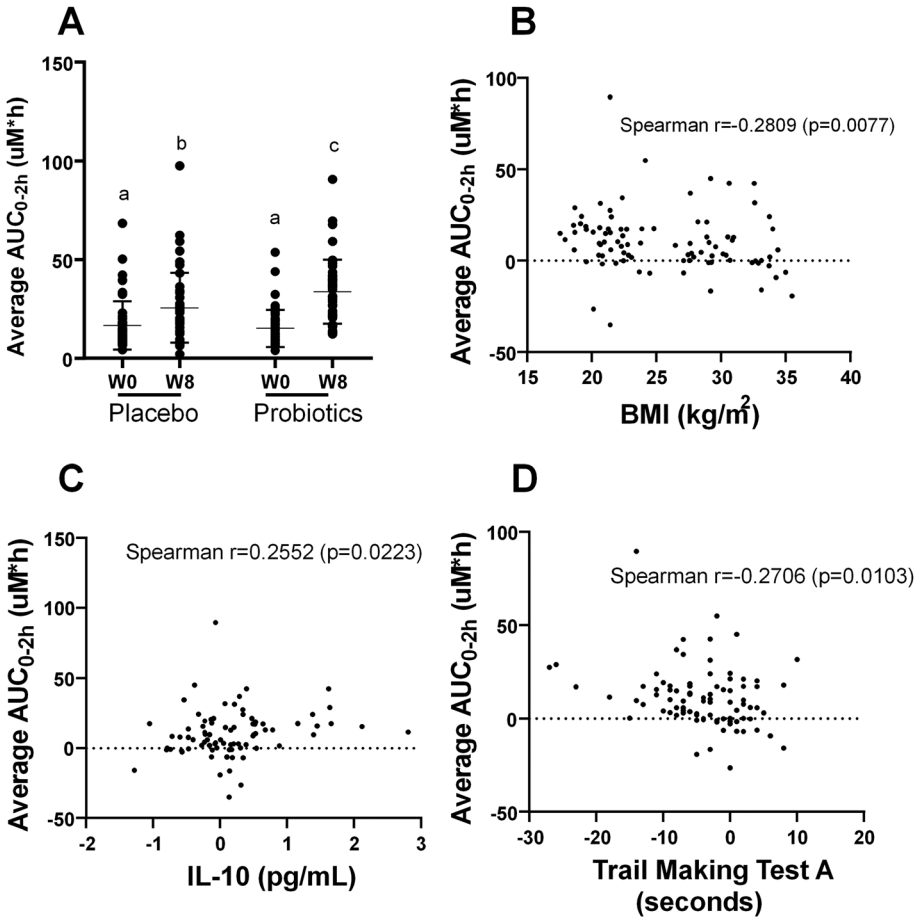

Fig. 4 Systemic exposure of the sum of gallotannin-metabolites and its correlation with BMI and IL-10. (A) Average systemic exposure (AUC_{0-2h}) to the sum of gallotannin-metabolites for W0 and W8 study days in plasma for treatment groups, and (B) BMI classifications. Analysis of significant differences was performed using the Kruskal–Wallis test ($p \leq 0.05$). (C) Spearman correlation between auc (0–2 h) of gallotannin-metabolites and IL-10 plasma concentrations, and (D) the Trail Making Test A at W8. Different letters mean statistically significant differences at $p \leq 0.05$.

of mango consumption resulted in decreases of IL-8 (−46%), MCP1 (−33%), HbA1C (−18%), and the adipokine plasminogen activator inhibitor-1 (PAI-1) (−20%), suggesting that mango-derived polyphenols may have a beneficial effect on obesity-associated inflammation.[40] In a previous study, when gallotannin and *Lactobacillus plantarum* were administered together to gnotobiotic mice, genes related to inflammation decreased while genes related to insulin sensitivity showed an increasing trend compared to the mice only administered gallotannin.[40] Based on these previous findings, we hypothesized that the addition of gallotannin-metabolizing probiotic strains to mango administration would enhance anti-inflammatory activities and modulate gastrointestinal hormones associated with obesity more significantly compared to our previous studies. However, in this study only GLP-1 was significantly altered by mango consumption with probiotics. While GLP-1 levels showed a decreasing trend with mango intake alone, they increased when mango and probiotics were consumed together. This suggests a potential synergistic effect between mango and probiotics in enhancing GLP-1 production.

The absence of a no-mango control prevents definitive attribution of the observed changes to mango gallotannins. However, previous studies demonstrating mango-specific effects—distinct from those of other polyphenol-rich fruits,[38,39] support the role of gallotannin-derived metabolites, especially when produced with the help of the administered probiotic strains, which may also contribute additional functional benefits.

Intestinal hormones are a major link within the gut-brain axis.[41] Obesity is associated with changes in the secretion and regulation of gastrointestinal hormones. Leptin is an adipokine that influences metabolic processes and is advantageous for weight management.[42] Studies demonstrate that leptin is increased during obesity due to leptin resistance[43] and is reported to be proportional to adiposity.[44] This was confirmed in the current study with elevated concentrations present in the group with overweight/obesity at the onset of the trial. After 8 weeks of mango consumption, leptin decreased by 13.48% within participants with overweight/obesity, and there was no longer a significant difference to lean participants.

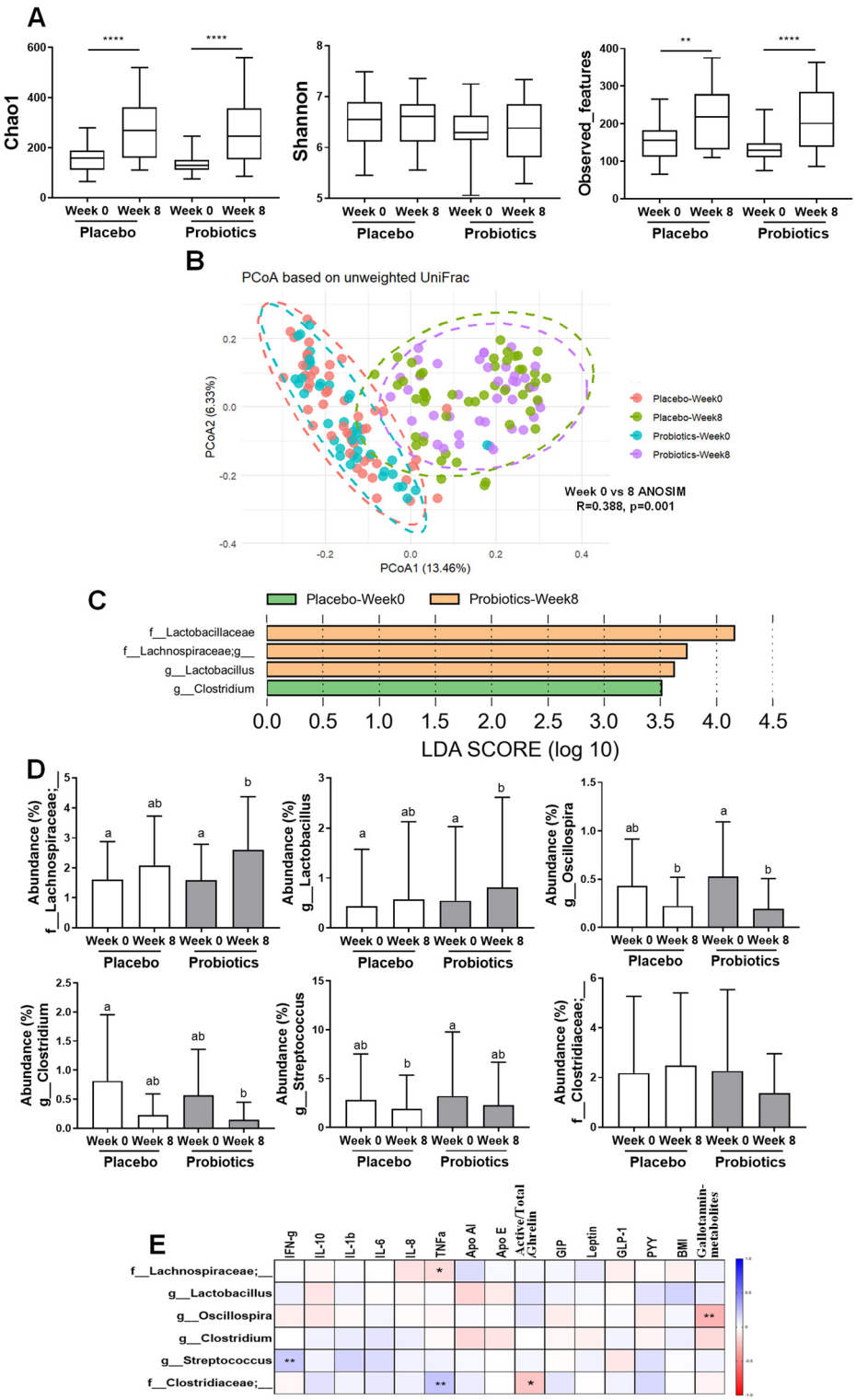

Fig. 5 Gut microbiota analysis after mango consumption with placebo/probiotics for 8 weeks. (A) Alpha diversity analysis (Chao1, Shannon, and observed features indices). (B) Beta diversity analysis using PCoA based on unweighted unifrac between placebo and probiotic groups over time. (C) LEfSe analysis identified significant taxa enriched in the probiotic group at week 8 compared to baseline. (D) The abundance (%) of significantly altered gut microbiota. (E) Spearman correlation between gut microbiota composition and inflammatory and hormonal markers. Analysis of the differences was performed using the Kruskal–Wallis test. Different letters mean statistically significant differences at $p \leq 0.05$. Asterisks indicate levels of statistical significance: $^{**}p < 0.01$, and $^{****}p < 0.001$.

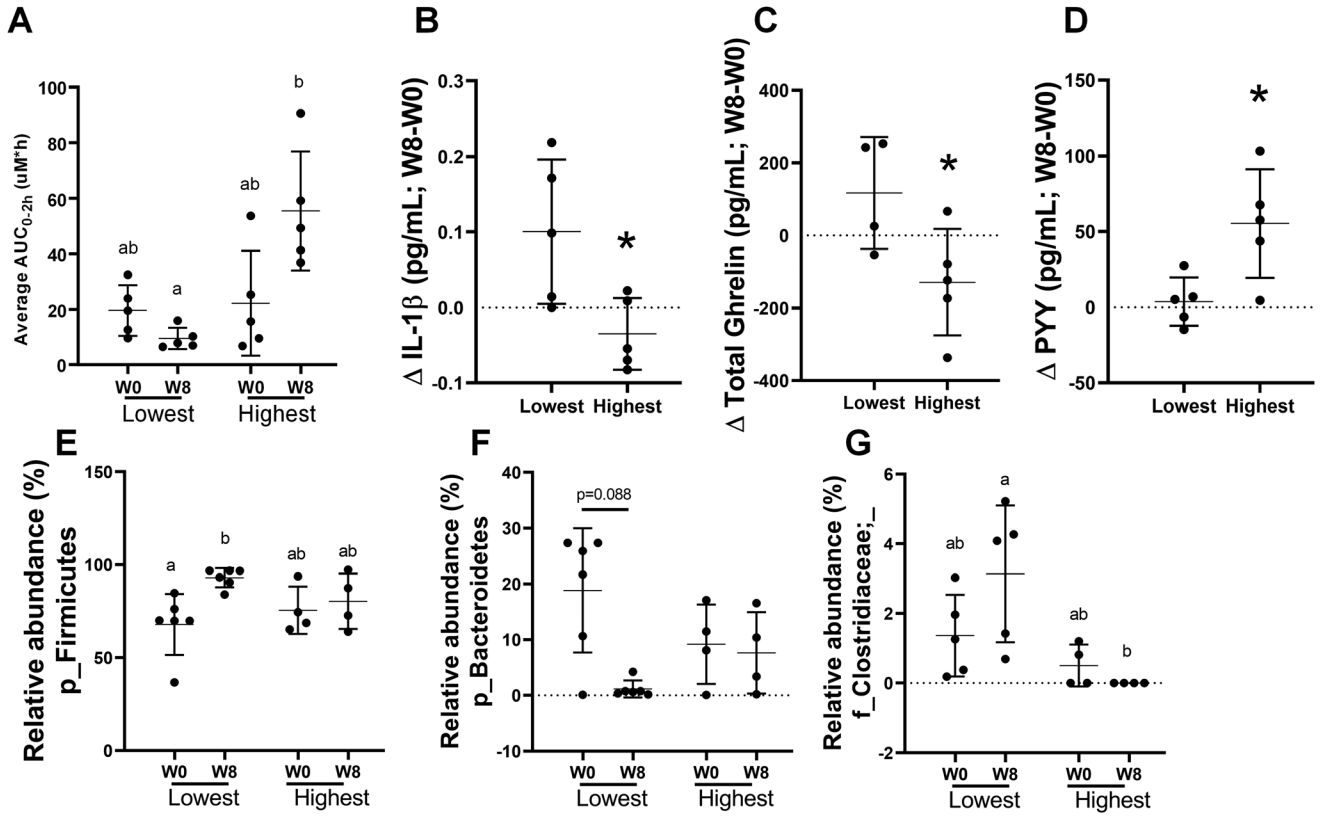

Fig. 6 Comparison of biomarker responses between overweight/obese participants with the highest (n = 5) and lowest (n = 5) AUC_{0-2h} in gallotannin-metabolites systemic exposure at W8. (A) Average systemic exposure (AUC_{0-2h}) to gallotannin-metabolites in plasma in highest and lowest responders in overweight/obese-probiotics group. (B) Changes in Δ IL-1β, (C) Δ total ghrelin, and (D) Δ PYY (E) relative abundance of *Firmicutes* (Phylum), (F) *Bacteroidetes* (Phylum), and (G) f_*Clostridiaceae*. Analysis of the differences was performed using the Kruskal–Wallis test. Different letters mean statistically significant differences at $p \leq 0.05$.

This finding indicates a potential beneficial effect of mango on elevated leptin.

Findings from the mediation analysis support a model in which *Streptococcus* acts as a key microbial mediator of the intervention's effect on important gastrointestinal hormones. The significant mediation effects for ghrelin, GLP-1, and PYY suggest that modulation of *Streptococcus* abundance could causally influence the secretion or regulation of these biomarkers. Given the known roles of ghrelin, GLP-1, and PYY in appetite signaling and metabolic regulation, the results provide preliminary evidence for a gut microbiome–hormone axis in response to the studied intervention. Further mechanistic studies and validation in larger cohorts are warranted to corroborate whether *Streptococcus* changes can drive improvements in metabolic health *via* these hormonal pathways.

Previous animal and human clinical studies investigating polyphenols and cognitive function, suggest that polyphenols can enhance specific cognitive abilities.[45] This study demonstrated that daily mango consumption for 8 weeks may improve executive attention and working memory, specifically in lean individuals. Mango intake enhanced cognitive performance in both individuals with and without overweight/obesity. Lean individuals experienced improvement in multiple cognitive function test scores (TMT-A, Digit Span Forward, and Digit Span Backward), while individuals with overweight/obesity only improved Digit Span Backward test scores. This differential response may reflect the more pronounced physiological challenges, such as chronic low-grade inflammation and gut dysbiosis, inherent to overweight and obesity, which might necessitate a more targeted and potent intervention to elicit broad cognitive benefits.

The cognitive enhancing benefits of mangoes may be attributed to polyphenolic compounds that possess antioxidant and anti-inflammatory properties.[46]

Additional research is necessary to explore the efficacy of probiotics in the lean group.

Our findings demonstrate potential anti-inflammatory activity of mango and probiotics intake. Furthermore, probiotics can modify the gut-brain axis to improve cognitive function by altering the abundance and diversity of the gut microbiota, which leads to changes in microbiota-derived metabolite production, inflammation reduction, and strengthening the intestinal barrier.[47] This evidence suggests that the cognitive performance improvements observed in this study may be due to the anti-inflammatory properties exerted by mango polyphenols and modulation of the gut microbiota *via* multi-strain probiotics.

Visual spatial awareness measured *via* the Neurotracker™ 3D program, showed differences in baseline session 1 scores between the lean and overweight/obese groups but there were no differences for the remaining training sessions between these groups. While individuals receiving probiotics had lower scores than those consuming mango alone, the between-group analysis showed no statistical differences for any training sessions. Previous studies indicated that the intake of probiotics may not improve visual spatial orientation.[48,49] Speed scores non-significantly improved over time regardless of BMI-classification or intake of probiotics. The lack of effect detected in this cognitive test might be attributed to the cognitive health of study participants who did not display cognitive deficiencies or impairments. The beneficial effects of probiotics appear to be dependent on the cognitive health of study populations, and recent evidence suggests that probiotics may enhance cognitive function or attenuate cognitive decline in populations who are at risk of cognitive dysfunction and/or for whom cognitive dysfunction may already be present.[47] However, a limitation in this study was the lack of a non-intervention control group, so it was impossible to objectively evaluate and compare the effect of mango intake on cognitive function.

This study demonstrated a significant impact of mango consumption on gut microbiota composition over an 8-week intervention period, regardless of the addition of probiotics. Enhanced microbial richness highlights mango's potential to promote a diverse and healthy gut environment. Beta diversity analysis further confirmed notable shifts in microbiota composition after mango intake, as evidenced by distinct clustering patterns, which were independent of probiotics supplementation. However, when probiotics were consumed alongside mango, a synergistic effect was observed, leading to an increased abundance of beneficial taxa such as *Lactobacillus* and *Lachnospiraceae*.[50,51] This finding suggests that the synergy between mango polyphenols and probiotics may contribute to improved gut health. This is further supported by the inverse correlation observed between *Lachnospiraceae* and inflammatory markers such as TNF-α. Additionally, reductions in harmful bacteria such as *Clostridium* and *Streptococcus* were observed following mango consumption, aligning with previous evidence of polyphenol-driven anti-inflammatory effects.[52,53] Notably, *Streptococc*us exhibited a positive correlation with IFN-γ, linking it to pro-inflammatory responses. Interestingly, although *Oscillospira* is commonly regarded as a beneficial taxon,[54] its decrease in this study highlights its negative correlation with gallotannin metabolites (Fig. 5E),[34] suggesting a nuanced interaction between mango polyphenols and microbial pathways. We recognize the limitation of not analyzing short-chain fatty acids (SCFAs), as these microbial metabolites could offer a more direct connection between the observed alterations in gut microbiota and the resulting systemic metabolic and anti-inflammatory effects.

In general, results indicate that mango polyphenols not only reshape microbial composition but also influence metabolic functions of the microbiota, potentially contributing to reduced inflammation and improved regulation of metabolic hormones, as evidenced by correlations with inflammatory cytokines. While probiotics did not significantly alter overall diversity metrics, their role in selectively enriching beneficial taxa such as *Lactobacillus* and *Lachnospiraceae* underscores their additive benefits when combined with mango intake. This synergistic interaction highlights the value of dietary and supplemental interventions in shaping a gut microbiota conducive to better health.

Future studies should investigate the long-term effects of these microbial changes on host health and elucidate the mechanisms underlying the interplay between polyphenols and probiotics in modulating gut microbiota and associated health outcomes.

To elucidate the impact of inter-individual variability in the production of microbial metabolites within the same BMI-classification and treatment groups, participants with overweight/obesity were stratified by their systemic exposure to gallotannin-metabolites and $n = 5$ of those individuals with the highest and lowest systemic exposure were selected. Individuals with the lowest systemic exposure had more *Firmicutes* and protein-metabolizing *Clostridiaceae* compared to the highest exposure. Increases in the abundance of specific *Firmicutes* species in the gut is used as a marker of gut dysbiosis.[55] The function of *Clostridiaceae* in protein metabolism, and its induction may indicate that these individuals have consumed an excessive amount of meat (rich in protein) or a low ratio of carbohydrates to protein.[56] In this context, the absence of repeated, comprehensive dietary analyses throughout the 8-week intervention presents a limitation of this study, precluding a detailed assessment of how ongoing dietary habits, such as protein intake, might have contributed to these observed microbial shifts. In addition, individuals with the lowest exposure exhibited reduced levels of the satiety hormone PYY, as well as elevated levels of the proinflammatory cytokine IL-1β and total ghrelin compared to those with the highest systemic exposure, despite being assigned the same amount of mango with probiotics. The inter-individual variability in response, particularly among overweight/obese participants, suggests that unaccounted-for dietary and physiological factors, along with individual differences in compliance, may significantly influence the efficacy of anti-inflammatory food interventions.

Another limitation of this study is the lack of a control group that did not receive mango, as all groups received mango administration. The absence of such control complicates the comparative analysis of mango-specific effects. Further investigations should incorporate an appropriate control group to enhance the robustness of the findings and delineate the unique benefits of mango *versus* a general fruit or no-intervention effect. Additionally, the study is limited by the impact of the treatment on inflammation-associated cytokines. In a previous study,[19] the AUC of inflammation biomarkers was assessed over 8 hours while in this study inflammation markers were only evaluated 0–2 h. We also acknowledge that inter-individual differences in blood volume—particularly between lean and obese participants—could theoreti-

cally influence the total systemic burden of circulating biomarkers, even if plasma concentrations (expressed in pg mL^{-1}) appear similar. While our analysis followed standard clinical protocols based on plasma concentrations, future studies may consider incorporating blood volume normalization or pharmacokinetic modelling to more accurately estimate systemic exposure. Another important limitation relates to the relatively small sample size, which may be underpowered to detect subtle or heterogeneous changes in gut microbiota composition. Therefore, the microbiota-related outcomes should be interpreted with caution as exploratory findings, requiring validation in larger cohorts. As with any nutrition intervention study of free-living individuals, aspects of treatment-adherence are an additional concern when dealing with great inter-individual variability. Yet, similar to the previous experiment, the number of metabolites produced had a negative correlation with BMI and a positive correlation with Δ IL-10. This study confirmed that individuals with overweight/obesity produced fewer metabolites than lean individuals. Furthermore, individuals with greater concentrations of metabolites have higher levels of the anti-inflammatory cytokine IL-10.

Conclusions

Mango intake in general improved cognitive performance (Trail Making Test A and Digit Span scores) in lean participants regardless of probiotics intake while in overweight/obese participants improved in Digit Span Backward only when supplemented with probiotics.

The mango intake plus probiotics decreased ΔTNF-α in overweight/obese participants compared to the placebo and lean participants.

Mango intake enhanced microbial richness and promoted shifts in microbiota composition, indicating its potential to support a diverse and healthy gut, independent of probiotic use. However, combined intake of mango and probiotics had a significant effect, increasing beneficial taxa like Lactobacillus and Lachnospiraceae. Findings from this pilot study partially support the initial hypothesis that 8 weeks of daily concomitant intake of mango and probiotics increased systemic exposure to gallotannin-metabolites in all participants regardless of BMI classification. Systemic exposure to mango-derived gallotannin metabolites may help promote immune tolerance and cognitive function, as indicated by a significant positive correlation with IL-10 and improvement in Trail Making Test A scores. Overweight/obese individuals with the highest levels of these metabolites also showed reduced plasma IL-1β and total ghrelin, suggesting potential anti-inflammatory and appetite-suppressing effects, alongside increased PYY levels, which may enhance satiety and reduce food intake.

Overall, these findings suggest that mango supplemented with probiotics may be a preventive approach in obesity-associated inflammation and cognitive function decline. Designs of follow-up studies should consider a non-intervention control group, more stringent monitoring of compliance, the analysis of SCFAs, and overall dietary intake of an increased number of participants.

Author contributions

SER and SUMT designed the study. KBK, SSC, SA, MJCC, NCG, SER, and SUMT conducted the research and collected the data. CK, performed chemical analysis. HK, KBK, SSC, SA, SER, and SUMT analyzed the data. HK, KBK, SSC, SA, MJCC, SER, NCG, STT, and SUMT drafted the manuscript. GN and SUMT critically reviewed the manuscript, GN edited manuscript, RS-C performed statistical analyses. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Thus, there are no conflicts to declare.

Ethics statement

The studies involving humans were approved by Texas A&M University Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Data availability

Raw data from microbiota and plasma metabolites can be accessed at the Texas Data Repository (TDR) database: Microbiota: https://doi.org/10.18738/T8/C8BPZA; Metabolites: https://doi.org/10.18738/T8/NBKJQ1. Raw data from cytokines, gastrointestinal hormones, and cognitive function are presented in supplementary materials.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5fo01687h.

Acknowledgements

The study was partially supported by the USDA–AMS–National Mango Board. The authors thank the study participants, research assistants, and nurses including Cathy Craig in the Department of Food Science and Technology at Texas A&M University for their support with this study. The authors also thank Stephen Balsis in the Department of Psychology at University of Massachusetts Lowell for assisting with the design of the Trail Making Tests A and B and Wechsler Adult Scale-Revised Digit Span tests.

References

1 P. D. O'Brien, L. M. Hinder, B. C. Callaghan and E. L. Feldman, Neurological consequences of obesity, *Lancet Neurol.*, 2017, **16**, 465–477.
2 A. Agustí, M. P. García-Pardo, I. López-Almela, I. Campillo, M. Maes, M. Romaní-Pérez and Y. Sanz, Interplay between the gut-brain axis, obesity and cognitive function, *Front. Neurosci.*, 2018, **12**, 155.
3 E. E. Noble, T. M. Hsu and S. E. Kanoski, Gut to brain dysbiosis: mechanisms linking western diet consumption, the microbiome, and cognitive impairment, *Front. Behav. Neurosci.*, 2017, **11**, 9.
4 M. Solas, F. I. Milagro, M. J. Ramírez and J. A. Martínez, Inflammation and gut-brain axis link obesity to cognitive dysfunction: plausible pharmacological interventions, *Curr. Opin. Pharmacol.*, 2017, **37**, 87–92.
5 T. Ozdal, D. A. Sela, J. Xiao, D. Boyacioglu, F. Chen and E. Capanoglu, The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility, *Nutrients*, 2016, **8**, 78.
6 G. Ma and Y. Chen, Polyphenol supplementation benefits human health via gut microbiota: A systematic review via meta-analysis, *J. Funct. Foods*, 2020, **66**, 103829.
7 C. A. Massaad and E. Klann, Reactive oxygen species in the regulation of synaptic plasticity and memory, *Antioxid. Redox Signaling*, 2011, **14**, 2013–2054.
8 J. Zhao, W. Bi, S. Xiao, X. Lan, X. Cheng, J. Zhang, D. Lu, W. Wei, Y. Wang and H. Li, Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice, *Sci. Rep.*, 2019, **9**, 1–12.
9 I. Bakoyiannis, A. Daskalopoulou, V. Pergialiotis and D. Perrea, Phytochemicals and cognitive health: Are flavonoids doing the trick?, *Biomed. Pharmacother.*, 2019, **109**, 1488–1497.
10 M. J. R. Howes, N. S. Perry, C. Vásquez-Londoño and E. K. Perry, Role of phytochemicals as nutraceuticals for cognitive functions affected in ageing, *Br. J. Pharmacol.*, 2020, **177**, 1294–1315.
11 D. Serra, L. M. Almeida and T. C. Dinis, Dietary polyphenols: A novel strategy to modulate microbiota-gut-brain axis, *Trends Food Sci. Technol.*, 2018, **78**, 224–233.
12 S. Filosa, F. Di Meo and S. Crispi, Polyphenols-gut microbiota interplay and brain neuromodulation, *Neural Regener. Res.*, 2018, **13**, 2055.
13 M. Masibo and Q. He, Major mango polyphenols and their potential significance to human health, *Compr. Rev. Food Sci. Food Saf.*, 2008, **7**, 309–319.
14 M. Kumar, V. Saurabh, M. Tomar, M. Hasan, S. Changan, M. Sasi, C. Maheshwari, U. Prajapati, S. Singh and R. K. Prajapat, Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities, *Antioxidants*, 2021, **10**, 299.
15 C. Infante-Garcia, J. J. Ramos-Rodriguez, I. Delgado-Olmos, C. Gamero-Carrasco, M. T. Fernandez-Ponce, L. Casas, C. Mantell and M. Garcia-Alloza, Long-term mangiferin extract treatment improves central pathology and cognitive deficits in APP/PS1 mice, *Mol. Neurobiol.*, 2017, **54**, 4696–4704.
16 E. L. Wightman, P. A. Jackson, J. Forster, J. Khan, J. C. Wiebe, N. Gericke and D. O. Kennedy, Acute effects of a polyphenol-rich leaf extract of Mangifera indica L.(Zynamite) on cognitive function in healthy adults: A double-blind, placebo-controlled crossover study, *Nutrients*, 2020, **12**, 2194.
17 M. Yu, X. Chen, J. Liu, Q. Ma, Z. Zhuo, H. Chen, L. Zhou, S. Yang, L. Zheng and C. Ning, Gallic acid disruption of Aβ1–42 aggregation rescues cognitive decline of APP/PS1 double transgenic mouse, *Neurobiol. Dis.*, 2019, **124**, 67–80.
18 M. S. Korani, Y. Farbood, A. Sarkaki, H. F. Moghaddam and M. T. Mansouri, Protective effects of gallic acid against chronic cerebral hypoperfusion-induced cognitive deficit and brain oxidative damage in rats, *Eur. J. Pharmacol.*, 2014, **733**, 62–67.
19 R. C. Barnes, H. Kim, C. Fang, W. Bennett, M. Nemec, M. A. Sirven, J. S. Suchodolski, N. Deutz, R. A. Britton, S. U. Mertens-Talcott and S. T. Talcott, Body Mass Index as a Determinant of Systemic Exposure to Gallotannin Metabolites during 6-Week Consumption of Mango (Mangifera indica L.) and Modulation of Intestinal Microbiota in Lean and Obese Individuals, *Mol. Nutr. Food Res.*, 2019, **63**, 1800512.
20 H.-S. Ejtahed, P. Angoorani, A.-R. Soroush, S. Hasani-Ranjbar, S.-D. Siadat and B. Larijani, Gut microbiota-derived metabolites in obesity: a systematic review, *Biosci. Microbiota, Food Health*, 2020, **39**, 65–76.
21 H. F. Zahid, A. Ali, A. R. Legione, C. S. Ranadheera, Z. Fang, F. R. Dunshea and S. Ajlouni, Probiotic yoghurt enriched with mango peel powder: Biotransformation of phenolics and modulation of metabolomic outputs after in vitro digestion and colonic fermentation, *Int. J. Mol. Sci.*, 2023, **24**, 8560.
22 N. Jiménez, J. A. Curiel, I. Reverón, B. de las Rivas and R. Muñoz, Uncovering the Lactobacillus plantarum WCFS1 gallate decarboxylase involved in tannin degradation, *Appl. Environ. Microbiol.*, 2013, **79**, 4253–4263.
23 C. R. Bowie and P. D. Harvey, Administration and interpretation of the Trail Making Test, *Nat. Protoc.*, 2006, **1**, 2277–2281.
24 T. A. Salthouse, What cognitive abilities are involved in trail-making performance?, *Intelligence*, 2011, **39**, 222–232.
25 B. N. Axelrod, N. L. Fichtenberg, S. R. Millis and J. C. Wertheimer, Detecting incomplete effort with digit span from the Wechsler Adult Intelligence Scale—Third Edition, *Clin. Neuropsychologist*, 2006, **20**, 513–523.
26 C. Vater, R. Gray and A. O. Holcombe, A critical systematic review of the Neurotracker perceptual-cognitive training tool, *Psychon. Bull. Rev.*, 2021, **28**, 1458–1483.
27 D. C. Nieman, N. D. Gillitt, G.-Y. Chen, Q. Zhang, W. Sha, C. D. Kay, P. Chandra, K. L. Kay and M. A. Lila, Blueberry and/or banana consumption mitigate arachidonic, cytochrome P450 oxylipin generation during recovery from 75 km cycling: a randomized trial, *Front. Nutr.*, 2020, **7**, 121.
28 P. Suchartlikitwong, S. Anugulruengkitt, N. Wacharachaisurapol, W. Jantarabenjakul, J. Sophonphan, T. Theerawit, T. Chatsuwan, T. Wattanavijitkul and

T. Puthanakit, Optimizing Vancomycin Use Through 2–Point AUC–Based Therapeutic Drug Monitoring in Pediatric Patients, *J. Clin. Pharmacol.*, 2019, **59**, 1597–1605.

29 R. Pilla, B. C. Guard, A. B. Blake, M. Ackermann, C. Webb, S. Hill, J. A. Lidbury, J. M. Steiner, A. E. Jergens and J. S. Suchodolski, Long-term recovery of the fecal microbiome and metabolome of dogs with steroid-responsive enteropathy, *Animals*, 2021, **11**, 2498.

30 S. D. Anton, N. Ebner, J. M. Dzierzewski, Z. Z. Zlatar, M. J. Gurka, V. M. Dotson, J. Kirton, R. T. Mankowski, M. Marsiske and T. M. Manini, Effects of 90 days of resveratrol supplementation on cognitive function in elders: a pilot study, *J. Altern. Complementary Med.*, 2018, **24**, 725–732.

31 H. Kang, Sample size determination and power analysis using the G* Power software, *J. Educ. Eval. Health Prof.*, 2021, **18**, 17.

32 S. Karthikeyan, M. K. Dimick, L. Fiksenbaum, H. Jeong, B. Birmaher, J. L. Kennedy, K. Lanctôt, A. J. Levitt, G. E. Miller and A. Schaffer, Inflammatory markers, brain-derived neurotrophic factor, and the symptomatic course of adolescent bipolar disorder: A prospective repeated-measures study, *Brain, Behav., Immun.*, 2022, **100**, 278–286.

33 M. Farhadipour and I. Depoortere, The function of gastrointestinal hormones in obesity—implications for the regulation of energy intake, *Nutrients*, 2021, **13**, 1839.

34 H. Xu, X. Zhang, P. Li, Y. Luo, J. Fu, L. Gong, Z. Lv and Y. Guo, Effects of tannic acid supplementation on the intestinal health, immunity, and antioxidant function of broilers challenged with necrotic enteritis, *Antioxidants*, 2023, **12**, 1476.

35 F. M. Schmidt, J. Weschenfelder, C. Sander, J. Minkwitz, J. Thormann, T. Chittka, R. Mergl, K. C. Kirkby, M. Faßhauer, M. Stumvoll, L. M. Holdt, D. Teupser, U. Hegerl and H. Himmerich, Inflammatory cytokines in general and central obesity and modulating effects of physical activity, *PLoS One*, 2015, **10**, e0121971.

36 M. F. Landecho, C. Tuero, V. Valentí, I. Bilbao, M. de la Higuera and G. Frühbeck, Relevance of Leptin and Other Adipokines in Obesity-Associated Cardiovascular Risk, *Nutrients*, 2019, **11**, 11.

37 H. Kim, N. Banerjee, R. C. Barnes, C. M. Pfent, S. T. Talcott, R. H. Dashwood and S. U. Mertens-Talcott, Mango polyphenolics reduce inflammation in intestinal colitis—involvement of the miR–126/PI3K/AKT/mTOR axis in vitro and in vivo, *Mol. Carcinog.*, 2017, **56**, 197–207.

38 H. Kim, M. J. Castellon-Chicas, S. Arbizu, S. T. Talcott, N. L. Drury, S. Smith and S. U. Mertens-Talcott, Mango (Mangifera indica L.) polyphenols: Anti-inflammatory intestinal microbial health benefits, and associated mechanisms of actions, *Molecules*, 2021, **26**, 2732.

39 C. Fang, H. Kim, R. C. Barnes, S. T. Talcott and S. U. Mertens-Talcott, Obesity-Associated Diseases Biomarkers Are Differently Modulated in Lean and Obese Individuals and Inversely Correlated to Plasma Polyphenolic Metabolites After 6 Weeks of Mango (Mangifera indica L.) Consumption, *Mol. Nutr. Food Res.*, 2018, **62**, e1800129.

40 C. Fang, H. Kim, L. Yanagisawa, W. Bennett, M. A. Sirven, R. C. Alaniz, S. T. Talcott and S. U. Mertens-Talcott, Gallotannins and Lactobacillus plantarum WCFS1 mitigate high–fat diet–induced inflammation and induce biomarkers for thermogenesis in adipose tissue in gnotobiotic mice, *Mol. Nutr. Food Res.*, 2019, **63**, 1800937.

41 M. Carabotti, A. Scirocco, M. A. Maselli and C. Severi, The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems, *Ann. Gastroenterol.*, 2015, **28**, 203–209.

42 G. Dessie, B. Ayelign, Y. Akalu, T. Shibabaw and M. D. Molla, Effect of Leptin on Chronic Inflammatory Disorders: Insights to Therapeutic Target to Prevent Further Cardiovascular Complication, *Diabetes, Metab. Syndr. Obes.*, 2021, **14**, 3307–3322.

43 N. Perakakis, O. M. Farr and C. S. Mantzoros, Leptin in leanness and obesity: JACC state-of-the-art review, *J. Am. Coll. Cardiol.*, 2021, **77**, 745–760.

44 R. B. Harris, Direct and indirect effects of leptin on adipocyte metabolism, *Biochim. Biophys. Acta*, 2014, **1842**, 414–423.

45 D. Vauzour, Dietary polyphenols as modulators of brain functions: biological actions and molecular mechanisms underpinning their beneficial effects, *Oxid. Med. Cell. Longevity*, 2012, **2012**, 914273.

46 W. Yang, K. Cui, X. Li, J. Zhao, Z. Zeng, R. Song, X. Qi and W. Xu, Effect of polyphenols on cognitive function: evidence from population-based studies and clinical trials, *J. Nutr. Health Aging*, 2021, **25**, 1190–1204.

47 J. Eastwood, G. Walton, S. Van Hemert, C. Williams and D. Lamport, The effect of probiotics on cognitive function across the human lifespan: A systematic review, *Neurosci. Biobehav. Rev.*, 2021, **128**, 311–327.

48 G. M. Moloney, C. M. Long-Smith, A. Murphy, D. Dorland, S. F. Hojabri, L. O. Ramirez, D. C. Marin, T. F. Bastiaanssen, A.-M. Cusack and K. Berding, Improvements in sleep indices during exam stress due to consumption of a Bifidobacterium longum, *Brain, Behav., Immun.: Health*, 2021, **10**, 100174.

49 J. R. Kelly, A. P. Allen, A. Temko, W. Hutch, P. J. Kennedy, N. Farid, E. Murphy, G. Boylan, J. Bienenstock and J. F. Cryan, Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects, *Brain, Behav., Immun.*, 2017, **61**, 50–59.

50 R. Huang, F. Wu, Q. Zhou, W. Wei, J. Yue, B. Xiao and Z. Luo, Lactobacillus and intestinal diseases: Mechanisms of action and clinical applications, *Microbiol. Res.*, 2022, **260**, 127019.

51 M. Vacca, G. Celano, F. M. Calabrese, P. Portincasa, M. Gobbetti and M. De Angelis, The controversial role of human gut lachnospiraceae, *Microorganisms*, 2020, **8**, 573.

52 D. Samul, P. Worsztynowicz, K. Leja and W. Grajek, Beneficial and harmful roles of bacteria from the Clostridium genus, *Acta Biochim. Pol.*, 2013, **60**, 515–521.

53 F. Cardona, C. Andrés-Lacueva, S. Tulipani, F. J. Tinahones and M. I. Queipo-Ortuño, Benefits of polyphenols on gut

microbiota and implications in human health, *J. Nutr. Biochem.*, 2013, **24**, 1415–1422.

54 J. Yang, Y. Li, Z. Wen, W. Liu, L. Meng and H. Huang, Oscillospira-a candidate for the next-generation probiotics, *Gut Microbes*, 2021, **13**, 1987783.

55 S. Stojanov, A. Berlec and B. Štrukelj, The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease, *Microorganisms*, 2020, **8**, 1715.

56 E. N. Bermingham, P. Maclean, D. G. Thomas, N. J. Cave and W. Young, Key bacterial families (Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae) are related to the digestion of protein and energy in dogs, *PeerJ*, 2017, **5**, e3019.

