Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Autonomic and Gut Microbiome Correlates of the Online Mindfulness- and Compassion-Based Intercare Program for Parental Burnout: Exploratory Secondary Analysis of a Randomized Clinical Trial

Francisco Villalón

francisco.villalon@mail.udp.cl

Diego Portales University

Maximiliano Escaffi-Schwartz

Diego Portales University

Ismael Palacios-Garcia

Diego Portales University

Daniela Moya Aros

Diego Portales University

Fernanda Rojas Vásquez

Diego Portales University

Yolanda López Del Hoyo

University of Zaragoza

Javier García Campayo

University of Zaragoza

Research Article

Keywords:

Posted Date: June 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9817403/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: Competing interest reported. FVL, YLDH, and JGC report academic activities and workshops related to mindfulness. JGC has authored books related to mindfulness. IPG is founder

of Eternal Company, which provides services related to microbiome analysis. No other disclosures were reported.

Abstract

Objectives: To examine exploratory autonomic and gut microbiome correlates associated with an online mindfulness- and compassion-based intervention for parental burnout.

Methods: This exploratory secondary analysis used data from a three-arm randomized clinical trial conducted in Chile. The parent trial included 595 women with at least one child who teleworked at least one day per week. Participants were allocated to a mindfulness- and compassion-based intervention for parental burnout (IBAP-BP), an active control (AC), or waitlist. The biological substudy included fecal microbiome (IBAP-BP, n = 15; AC, n = 17) and ECG/heart rate variability data (IBAP-BP, n = 17; AC, n = 21). Exploratory biological outcomes included lnRMSSD, gut microbial diversity/composition, and predicted microbial functional pathways inferred from 16S rRNA sequencing and PICRUSt2.

Results: Both IBAP-BP and AC reduced parental burnout relative to waitlist at 6 months (IBAP-BP vs WL: β = −0.80; 95% CI, −1.38 to −0.23; AC vs WL: β = −1.08; 95% CI, −1.63 to −0.54). In the biological substudy, IBAP-BP showed higher lnRMSSD at post-intervention (β = 0.62; 95% CI, 0.30 to 0.93) and higher phylogenetic α-diversity at 6 months (β = 0.17; 95% CI, 0.05 to 0.29) compared with AC. Three bacterial genera and one predicted tyrosine degradation pathway differed between groups after false discovery rate correction.

Conclusions: An online mindfulness- and compassion-based intervention was associated with reductions in parental burnout and preliminary autonomic and gut microbiome differences. Findings should be interpreted as exploratory and hypothesis-generating.

Trial Registration: ClinicalTrials.gov Identifier: NCT05833269.

Introduction

Parental burnout (PB) is a stress-related condition characterized by emotional exhaustion, detachment from children, reduced fulfillment in the parental role, and diminished parenting efficacy, affecting 0.3% to 8.9% of parents (Roskam et al., 2021). PB is associated with adverse physical, social, and mental health outcomes in parents, as well as increased developmental and psychological risk in children (Griffith, 2022; Mikolajczak et al., 2018). Given its chronic stress profile and intergenerational implications, identifying scalable interventions for PB is a clinical and public health priority.

Mindfulness- and compassion-based interventions (MBIs) reduce stress and burnout across diverse populations (Goldberg et al., 2022). Mindfulness practices cultivate present-moment awareness and emotional regulation (Bishop, 2004; Author, 2023), whereas compassion training is associated with reduced psychopathology and improved resilience (Tang et al., 2015; Author, 2023). Structured programs integrating mindfulness and compassion, such as the mindfulness- and compassion-based inter-care program (IBAP), typically combine meditation, reflective writing, and psychoeducation. Although

psychological benefits are well documented (Dahl et al., 2015; Goldberg et al., 2022), biological correlates remain insufficiently characterized.

Stress-related conditions have been linked to autonomic dysregulation, inflammatory activation, and alterations in gut microbial diversity and composition (Azzalini et al., 2019; Black & Slavich, 2016; Pace et al., 2009; Richter et al., 2017; Suda & Matsuda, 2022). The microbiota–gut–brain axis has therefore emerged as a candidate system linking emotional regulation, autonomic function, immune signaling, and gastrointestinal physiology (Suda & Matsuda, 2022; Tan, 2023). Preliminary studies of contemplative practices suggest possible associations with microbial diversity and composition, although findings remain heterogeneous and are frequently confounded by diet, physical activity, yoga, or retreat settings (Campaniello et al., 2022; Chao et al., 2024; Ilan et al., 2024; Jia et al., 2020; Khine et al., 2020; Lynch & Pedersen, 2016; Raman et al., 2023; Shatkin-Margolis et al., 2022; Sun et al., 2023; Swarup et al., 2025; Wang et al., 2022; Yu et al., 2024; Zeng et al., 2025).

To date, no randomized controlled trial has examined whether an online mindfulness–compassion intervention delivered independently of dietary or lifestyle prescriptions is associated with autonomic and gut microbiome correlates in parental burnout. In this exploratory biological substudy of a randomized controlled trial, we evaluated gut microbiome diversity/composition using 16S rRNA sequencing and short-term vagally mediated heart rate variability indexed by lnRMSSD following an online mindfulness- and compassion-based intervention for PB. Given the emerging nature of this field and the modest sample size, biological analyses were prespecified as exploratory and hypothesis-generating.

Methods

Design

This study reports a prespecified exploratory biological substudy of a parallel, three-arm randomized clinical trial conducted in Chile between December 22, 2022, and March 8, 2023 In the parent trial, participants were allocated to an online mindfulness- and compassion-based intervention adapted for parental burnout (IBAP-BP), an active control condition (AC), or a waitlist control group (WL). Psychological outcomes from the parent trial have been reported previously (Author & Author, 2025). The present manuscript reports previously unpublished autonomic, gut microbiome, and exploratory mechanistic analyses from a prespecified laboratory substudy conducted in the IBAP-BP and AC groups only (Supplementary Figure 1). Participants and the public were not formally involved in the design, conduct, or reporting of the trial.

Participants

The parent trial enrolled women aged ≥18 years living with at least one child and teleworking at least one day per week, criteria chosen pragmatically and based on evidence linking telework to burnout

risk (Griffith, 2022; Mendonça et al., 2022). There were no restrictions on concurrent treatments. Exclusion criteria included severe depression, substance use disorder, or psychosis. Recruitment was conducted online with electronic informed consent. The laboratory subsample was drawn from this cohort and additionally required attendance at study visits, absence of gastrointestinal disease, no recent antibiotic use or diarrhea, and no medical conditions likely to substantially affect microbiota.

Randomization and Blinding

Participants were randomized by an independent researcher using a computer-generated sequence stratified by parental burnout severity (PBA ≥84). Blinding of participants and instructors was not feasible; however, statistical analysts remained blinded to group allocation. Detailed randomization procedures are reported in supplementary methods and elsewhere (Author & Author, 2025).

Procedures

Intervention and instructors

The IBAP-BP intervention comprised eight weekly 2-hour online sessions with 20–30 minutes/day of mindfulness and compassion practice. The active control used daily Jacobson's progressive muscle relaxation and journaling. Both groups received psychoeducational materials, reminders, and peer-support access. Intervention and instructors details are summarized in Supplementary Methods.

Data Collection and Follow-Up

Participants completed assessments at baseline (T0), post-intervention (T1; 3 months), 6 months (T2), and 9 months (T3). In the biological substudy, fecal samples, lifestyle measures, and ECG recordings were collected at T0, T1, and T2 in the IBAP-BP and AC groups. Because microbiome and ECG availability differed across waves, sample sizes are reported separately for each outcome. To maximize retention, independent assistants sent email reminders and conducted telephone check-ins.

Stool sampling and ECG recording

Participants attended laboratory visits for fecal sample collection and resting ECG recordings using the Zephyr BioPatch HP device (250 Hz). Up to two ECG recordings per participant and wave were obtained. ECG recordings were screened using predefined quality criteria; when two valid recordings were available, the first valid recording was retained. Detailed ECG preprocessing, HRV estimation, and microbiome sequencing/bioinformatic procedures are provided in the Supplement.

Measurements

The parent trial prespecified parental burnout at 9 months (T3), assessed using the Parental Burnout Assessment (PBA), as its primary clinical outcome; clinical and safety outcomes from the parent trial have been reported elsewhere (Author & Author, 2025). The present substudy focused on exploratory biological outcomes, including gut microbiome diversity/composition and short-term vagally mediated HRV indexed by lnRMSSD

Parental burnout was assessed using the PBA (Manrique-Millones et al., 2022), mindfulness using the FFMQ-15 (Author., 2023), and quality of life using the EQ-5D (Balestroni & Bertolotti, 2012). Lower EQ-5D scores indicate better health-related quality of life.

Lifestyle measures included self-reported diet, physical activity, and sleep, from which prespecified healthy and unhealthy lifestyle indices were derived and included as covariates in microbiome analyses.

Microbiome outcomes included phylogenetic entropy (primary α-diversity metric), Shannon entropy, observed richness, Bray–Curtis β-diversity, taxonomic abundance, and predicted microbial functional pathways inferred using PICRUSt2 (Cassol et al., 2025; Richter et al., 2017; Turku, n.d.). HRV analyses focused on lnRMSSD derived from artifact-corrected 1-minute resting ECG recordings following current recommendations (Laborde et al., 2017).

Detailed descriptions of psychometric instruments, lifestyle indices, microbiome processing, ECG preprocessing, HRV estimation, and exploratory outcomes are provided in the Supplementary Methods.

Statistic

Baseline analyses, missing-data diagnostics, sensitivity analyses, and multiple-imputation procedures are described in the Supplementary Methods. Longitudinal analyses used all available observations according to randomized group assignment rather than complete-case data only. Primary longitudinal analyses were conducted using a modified intention-to-treat approach including participants with at least one post-baseline assessment (N = 324).

No formal power calculation was conducted for the biological substudy; analyses were therefore considered exploratory and hypothesis-generating.

Longitudinal outcomes were analyzed using linear mixed-effects models (LMMs) with fixed effects for group, time, and the group × time interaction, and a random intercept for participant. Models adjusted for z-standardized baseline covariates, including baseline outcome values, parental burnout, age, extraversion, and neuroticism when applicable.

Outcome ~ group + time + group × time + baseline outcome + baseline parental burnout + age + covariates + (1 | participant)

Group contrasts were estimated using estimated marginal means. Standardized mean differences (Cohen’s d) were calculated using model residual standard deviations with Wald 95% confidence

intervals.

For microbiome analyses, α-diversity was assessed using phylogenetic entropy, Shannon entropy, and observed richness. β-diversity was analyzed using Bray–Curtis dissimilarity and PERMANOVA with 9,999 permutations. Taxonomic abundance and predicted microbial functional pathways inferred using PICRUSt2 were analyzed using logit-transformed LMMs adjusted for baseline abundance and healthy/unhealthy lifestyle indices.

Holm–Bonferroni correction was applied to primary outcomes, whereas exploratory analyses used Benjamini–Hochberg false discovery rate correction within prespecified analysis families (Ranganathan et al., 2016). Detailed microbiome preprocessing, exploratory lagged models, ECG quality-control procedures, and additional statistical analyses are reported in the Supplementary Methods.

All analyses were conducted in R version 4.5.1 using *the tidyverse, lme4, lmerTest, emmeans, vegan*, and related packages (Supplementary Table 1).

Ethics

Ethical approval was granted by the Anonymous author University Research Ethics Committee (Codes 38-2022 and 06-2023). The trial was retrospectively registered at ClinicalTrials.gov after enrollment initiation (NCT-Anonymous) and followed CONSORT guidelines.

Results

Sample

A total of 595 participants were randomized in the parent trial (IBAP-BP = 161, AC = 157, WL = 277). Primary longitudinal analyses used a modified intention-to-treat (mITT) sample including all randomized participants with at least one follow-up assessment (N = 324). The biological substudy included 47 participants from the IBAP-BP and AC groups (IBAP-BP = 24; AC = 23). At baseline, fecal microbiome samples were available for 32 participants (IBAP-BP = 15; AC = 17), and usable ECG/HRV recordings for 38 participants (IBAP-BP = 17; AC = 21). Sample availability declined over follow-up and differed across biological outcomes because of participant attendance, biological sample availability, and ECG quality-control procedures (Figure 1 and Supplementary Table 2). Sociodemographic and psychological variables are reported for the full biological substudy cohort, whereas microbiome and ECG/HRV analyses used outcome-specific subsamples.

Baseline demographic, psychological, autonomic, and microbiome characteristics are presented in Table 1 and Supplementary Table 3. No significant baseline differences were observed between groups (all $p > .05$). Missingness followed a missing-completely-at-random pattern (MCAR; Hawkins test $p = 0.16$); however, analyses were conducted under a MAR framework due to the small sample size.

Outcomes

Parental burnout

Linear mixed-effects models revealed a significant group × time interaction, $F(6, 362) = 4.66$, $p < .001$. Compared with the waitlist (WL), both IBAP and AC showed significant reductions in parental burnout, with stronger effects at T2 (Holm-adjusted $p < .05$).

At T2, effects were large (IBAP vs WL: β = −0.80, 95% CI [−1.38, −0.23], Holm-adjusted $p = .027$; $d = −1.24$; AC vs WL: β = −1.08, 95% CI [−1.63, −0.54], Holm-adjusted $p < .001$; $d = −1.68$). At T3, the effect remained significant for AC (β = −0.73, 95% CI [−1.27, −0.20], Holm-adjusted $p = .028$; $d = −1.13$) but not for IBAP. No differences emerged between IBAP and AC at any time point (Figure 2; Supplementary table 4).

Sensitivity analyses using complete-case and multiply imputed datasets yielded convergent findings, supporting the robustness of burnout reductions (Supplementary Figure 2).

Mindfulness and Quality of Life

For total FFMQ, no between-group contrasts reached statistical significance at any time point (all Benjamini–Hochberg adjusted $p \geq .388$).

For (EQ-5D, lower scores reflect better quality of life. AC showed significantly lower scores than WL at T1 and T2 after FDR correction (T1: β = −0.17, 95% CI [−0.28, −0.06], BH-adjusted $p = .012$; $d = −1.25$; T2: β = −0.16, 95% CI [−0.28, −0.04], BH-adjusted $p = .040$; $d = −1.17$). IBAP–WL contrasts were nominally significant at T1 and T3 but did not survive FDR correction (BH-adjusted $p = .056$ at both time points) (Supplementary Figure 3; Supplementary Table 4).

Heart Rate Variability

A significant group × time interaction was observed, $F(2, 49) = 7.93$, $p = .001$. IBAP showed higher lnRMSSD than AC at T1 (β = 0.62, 95% CI [0.30, 0.93], Benjamini–Hochberg adjusted $p < .001$; $d = 2.58$). The between-group difference at T2 was not significant (Benjamini–Hochberg adjusted $p = .214$). However, the between-group difference at T1 was not consistently reproduced in complete-case or imputed models, indicating limited robustness (Figure 2; Supplementary Figure 2 and table 4).

Microbiota

α-diversity

Phylogenetic entropy showed a significant group × time interaction, $F(2, 47) = 6.76$, $p = .003$. IBAP demonstrated higher phylogenetic α-diversity than AC at T2 (β = 0.17, 95% CI [0.05, 0.29], Benjamini–Hochberg adjusted $p = .019$; $d = 2.43$), with no difference at T1. The T2 between-group difference was robust in complete-case analyses but less consistent under imputation. Other α-diversity metrics

showed nominal group differences that did not survive FDR correction (Figure 2; Supplementary Figure 2 and Table 3).

β-diversity

PERMANOVA analyses revealed no significant group × time interaction (p = .269). Baseline parental burnout was associated with overall community structure (p = .024; R^2 = .041). Linear mixed-effects models of within-subject Bray–Curtis change from baseline detected a significant between-group difference in within-subject Bray–Curtis change at T2 (p < .01) (Supplementary Figures 4 and 5).

Differential Abundance

At the phylum and class levels, several taxa showed nominal time-specific differences; however, none survived global FDR correction.

At the genus level, three genera showed lower relative abundance in IBAP than AC at T2 after global Benjamini–Hochberg correction. *[Eubacterium] nodatum group* was lower in the intervention group (β = −2.47, 95% CI [−3.64, −1.29], BH-adjusted p = .047; d = −3.26). Similarly, *Lachnospiraceae FCS020 group* was reduced (β = −2.82, 95% CI [−4.21, −1.44], BH-adjusted p = .047; d = −3.29), as was *UCG-005* (β = −1.96, 95% CI [−2.94, −0.97], BH-adjusted p = .047; d = −3.07). No other genus-level contrasts remained significant after correction (Supplementary Figure 6A-D, 7A-E and table 4).

Functional pathways

Among predicted KEGG pathways, only the tyrosine degradation I pathway via fumarate (TYRFUMCAT-PWY) remained significant after FDR correction at T1 (Intervention vs Audios) (β = −8.03, 95% CI [−11.88, −4.17], BH-adjusted p = .042; d = −2.09). No other pathway differences were robust across time points (Figure 3, and supplementary table 4).

Exploratory Analyses

Baseline Associations

No significant baseline correlations were observed between parental burnout, lnRMSSD, and phylogenetic entropy after correction (Benjamini–Hochberg adjusted p > .50). EQ-5D and parental burnout were significantly correlated (ρ = .39, p < .01) (Supplementary figure 8A).

Several nominal correlations emerged between lnRMSSD and KEGG pathways and between parental burnout and specific taxa; however, none survived FDR correction. At the taxonomic level, lnRMSSD showed a strong positive correlation with Ruminococcus torques group (ρ = .64), approaching significance after correction (Benjamini–Hochberg adjusted p = .064) (Supplementary figure 8B-G).

Lagged Models

Lagged mixed-effects models showed no evidence that HRV or phylogenetic diversity at time t–1 predicted subsequent parental burnout, nor the reverse (all $p > .05$).

At the taxonomic level, two genera demonstrated significant lagged associations with subsequent parental burnout after false discovery rate correction: Fournierella ($\beta = 0.65$, 95% CI [0.39, 0.90], $p = .004$) and UCG-010 ($\beta = 0.49$, 95% CI [0.28, 0.71], $p = .004$).

No KEGG pathways remained significant after correction for multiple testing.

Discussion

Main results

In this randomized controlled trial, both the mindfulness-compassion intervention and the active control reduced parental burnout relative to waitlist, with the largest effects observed at 6 months. In the exploratory biological substudy, IBAP was associated with higher lnRMSSD at post-intervention and higher phylogenetic α-diversity at follow-up, together with differences in microbial composition and predicted functional pathways. Specifically, three genera ([Eubacterium] nodatum group, Lachnospiraceae FCS020 group, and UCG-005) showed lower relative abundance in IBAP than AC at T2 after false discovery rate correction, while predicted pathway analyses identified lower relative abundance of genes mapped to the tyrosine degradation I pathway at T1. However, these biological findings were sensitive to analytic specification and were not consistently reproduced across sensitivity analyses. Exploratory lagged analyses did not support HRV or α-diversity as mechanistic pathways underlying reductions in parental burnout.

Previous evidence

These findings extend prior evidence supporting mindfulness-based interventions for stress-related outcomes to parental burnout, a condition associated with substantial psychological and physiological burden (Goldberg et al., 2022; Griffith, 2022; Mikolajczak et al., 2018; Tang et al., 2015; Author, 2023). They also align with emerging literature suggesting that contemplative interventions may co-occur with autonomic and gut microbiome differences (Khine et al., 2020; Raman et al., 2023; Swarup et al., 2025; Wang et al., 2022). Previous observational and mixed-design studies suggest associations with vagally mediated HRV and gut microbial differences, including taxa related to short-chain fatty acid production and amino acid metabolism. In our trial, differences in α- and β-diversity were observed, although these findings were not consistently robust across sensitivity analyses. Notably, we detected increased phylogenetic diversity alongside reduced Shannon entropy, a pattern previously described in long-term meditators (Sun et al., 2023). Many prior studies combined meditation with dietary modification, yoga, or retreat settings (Jia et al., 2020; Raman et al., 2023; Sun et al., 2023; Swarup et al., 2025; Wang et al., 2022), all of which independently influence gut composition (Campaniello et al., 2022; Lynch & Pedersen, 2016; Zeng et al., 2025). By contrast, our fully online intervention isolated the psychobehavioral component.

Taxonomic findings were broadly consistent with prior exploratory MBI studies reporting alterations in Clostridiales-related taxa (Swarup et al., 2025; Wang et al., 2022). We also identified a novel exploratory association between Fournierella and parental burnout. Functionally, lower predicted abundance of genes mapped to tyrosine degradation pathways was tentatively consistent with prior reports implicating aromatic amino acid metabolism in stress-related phenotypes (Jia et al., 2020; Raman et al., 2023), although PICRUSt2 provides inferred rather than directly measured functional profiles (Ningthoujam et al., 2021; Tan, 2023). A novel finding in our study was the consistent association of *Fournierella* with parental burnout, not previously reported.

We observed an post-intervention between-group difference in HRV. Within the neurovisceral integration framework, HRV reflects dynamic brain–heart coupling and regulatory flexibility rather than isolated parasympathetic tone (Thayer et al., 2012; Thayer & Lane, 2000). Mindfulness training has been associated with enhanced interoceptive awareness and autonomic regulation (Tang et al., 2015), providing a plausible basis for early lnRMSSD differences. In our study, autonomic and predicted functional differences were observed before later microbial diversity/composition differences. However, exploratory lagged analyses did not support HRV or tyrosine metabolism as mechanistic pathways linking the intervention with reductions in parental burnout, and the modest size and attrition of the biological subsample substantially limit mechanistic inference.

Strengths and limitations

Strengths of this study include its randomized controlled design with an active comparator, preregistered primary outcomes, blinded analyses, and integration of psychological, autonomic, and microbial measures. Importantly, the intervention isolated the psychobehavioral component of mindfulness-compassion training without concurrent dietary, exercise, yoga, or retreat interventions that frequently confound prior microbiome studies.

Several limitations should be acknowledged. First, microbiome and HRV analyses were conducted in a small biological subsample with attrition, limiting statistical power and estimate stability. Second, most microbiome findings were exploratory, some did not survive correction for multiple testing, and exploratory lagged analyses did not support temporal pathways linking HRV or α-diversity with parental burnout. Third, although healthy and unhealthy lifestyle indices were included as covariates, diet, sleep, and physical activity were self-reported and not experimentally controlled. Fourth, 16S rRNA sequencing and PICRUSt2 provide taxonomic and inferred functional profiles rather than direct metagenomic, transcriptomic, metabolomic, or host physiological measurements. Fifth, HRV was estimated from brief 1-minute resting ECG recordings, which may reduce reliability. Sixth, exploratory lagged analyses were underpowered and should be interpreted as hypothesis-generating only. Finally, taxonomic abundance analyses were based on relative abundance data rather than dedicated compositional analytical frameworks, and microbiome analyses were not individually prespecified despite preregistration of ECG and microbiome sampling.

Implications

Taken together, exploratory autonomic and predicted functional differences were observed before later microbial diversity/composition differences, reflected in α-diversity and genus-level composition (Fig. 4). While exploratory lagged analyses did not support direct mechanistic pathways, the findings suggest preliminary psychophysiological and gut microbiome correlates associated with intervention response. Clinically, the sustained reduction in parental burnout supports scalable MBIs for caregivers, although larger preregistered multi-omic studies are required to clarify temporal and mechanistic relationships.

Future research

Larger, adequately powered randomized studies should preregister microbiome and HRV outcomes as co-primary or key secondary endpoints including specific indices and statistical plan; incorporate longitudinal sampling with tighter temporal resolution to map the ordering of psychological, autonomic, and microbial change; integrate metagenomics, metatranscriptomics, targeted metabolomics, and inflammatory markers to move beyond taxonomic inference; explicitly control or stratify by diet, sleep, and physical activity; and test mechanistic mediation using prespecified, directionally constrained models. Finally, implementation studies should evaluate scalability, adherence, and equity, ensuring that caregiver interventions are accessible in routine community settings.

Conclusion

An online mindfulness- and compassion-based intervention reduced parental burnout and showed exploratory autonomic and microbial changes, suggesting exploratory autonomic signals were observed before later differences in microbial diversity/composition. These findings nominate candidate gut–brain pathways but require confirmation in larger, multi-omic trials.

Declarations

Competing Interests

FVL, YLDH, and JGC report academic activities and workshops related to mindfulness. JGC has authored books related to mindfulness. IPG is founder of Eternal Company, which provides services related to microbiome analysis. No other disclosures were reported.

Author Contribution

FVL, IPG, and MES contributed to the conception and design of the study. Data acquisition was performed by DMA, FRV, and FVL. FVL conducted the data analysis and prepared the figures. Data interpretation was undertaken by FVL, IPG, MES, YLDH, and JGC. FVL, DMA, and FRV drafted the manuscript. IPG, MES, JGC, and YLDH critically revised the manuscript for important intellectual content.

JGC and YLDH provided supervision. FVL acquired funding.All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability

Deidentified participant data, the data dictionary, and statistical analysis code will be made available upon reasonable request to the corresponding author following publication, subject to institutional ethics approval and data-sharing agreements consistent with participant consent and privacy regulations.

References

1. Azzalini, D., Rebollo, I., & Tallon-Baudry, C. (2019). Visceral Signals Shape Brain Dynamics and Cognition. *Trends in Cognitive Sciences*, *23*(6), 488–509. https://doi.org/10.1016/j.tics.2019.03.007
2. Balestroni, G., & Bertolotti, G. (2012). [EuroQol-5D (EQ-5D): An instrument for measuring quality of life]. *Monaldi Archives for Chest Disease = Archivio Monaldi Per Le Malattie Del Torace*, *78*(3), 155–159. https://doi.org/10.4081/monaldi.2012.121
3. Bishop, S. R. (2004). Mindfulness: A Proposed Operational Definition. *Clinical Psychology: Science and Practice*, *11*(3), 230–241. https://doi.org/10.1093/clipsy/bph077
4. Black, D. S., & Slavich, G. M. (2016). Mindfulness meditation and the immune system: A systematic review of randomized controlled trials. *Annals of the New York Academy of Sciences*, *1373*(1), 13–24. https://doi.org/10.1111/nyas.12998
5. Campaniello, D., Corbo, M. R., Sinigaglia, M., Speranza, B., Racioppo, A., Altieri, C., & Bevilacqua, A. (2022). How Diet and Physical Activity Modulate Gut Microbiota: Evidence, and Perspectives. *Nutrients*, *14*(12), 2456. https://doi.org/10.3390/nu14122456
6. Cassol, I., Ibañez, M., & Bustamante, J. P. (2025). Key features and guidelines for the application of microbial alpha diversity metrics. *Scientific Reports*, *15*(1), 622. https://doi.org/10.1038/s41598-024-77864-y
7. Chao, W.-C., Huang, J.-C., Young, S.-L., Wu, C.-L., Shih, J.-C., Liao, L.-D., & Cheng, B. (2024). Interplay of yoga, physical activity, and probiotics in irritable bowel syndrome management: A double-blind randomized study. *Complementary Therapies in Clinical Practice*, *57*, 101892. https://doi.org/10.1016/j.ctcp.2024.101892
8. Dahl, C. J., Lutz, A., & Davidson, R. J. (2015). Reconstructing and deconstructing the self: Cognitive mechanisms in meditation practice. *Trends in Cognitive Sciences*, *19*(9), 515–523. https://doi.org/10.1016/j.tics.2015.07.001
9. Goldberg, S. B., Riordan, K. M., Sun, S., & Davidson, R. J. (2022). The Empirical Status of Mindfulness-Based Interventions: A Systematic Review of 44 Meta-Analyses of Randomized Controlled Trials. *Perspectives on Psychological Science*, *17*(1), 108–130. https://doi.org/10.1177/1745691620968771

10. Griffith, A. K. (2022). Parental Burnout and Child Maltreatment During the COVID-19 Pandemic. *Journal of Family Violence*, *37*(5), 725–731. https://doi.org/10.1007/s10896-020-00172-2
11. Ilan, K., Y, M., A, N., D, S., G, G., R, S., D, G., V, S.-N., O, S., M, F., S, R., S, O., J, M.-G., & A, M. (2024). Cognitive behavioral and mindfulness with daily exercise intervention is associated with changes in intestinal microbial taxa and systemic inflammation in patients with Crohn's disease. *Gut Microbes*, *16*(1), 2337269. https://doi.org/10.1080/19490976.2024.2337269
12. Jia, W., Zhen, J., Liu, A., Yuan, J., Wu, X., Zhao, P., Zhao, L., Li, X., Liu, Q., Huang, G., & Xu, A. (2020). Long-Term Vegan Meditation Improved Human Gut Microbiota. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2020*, 9517897. https://doi.org/10.1155/2020/9517897
13. Khine, W. W. T., Voong, M. L., Ng, T. K. S., Feng, L., Rane, G. A., Kumar, A. P., Kua, E. H., Mahendran, R., Mahendran, R., & Lee, Y.-K. (2020). Mental awareness improved mild cognitive impairment and modulated gut microbiome. *Aging*, *12*(23), 24371–24393. https://doi.org/10.18632/aging.202277
14. Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting. *Frontiers in Psychology*, *8*. https://doi.org/10.3389/fpsyg.2017.00213
15. Lynch, S. V., & Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. *New England Journal of Medicine*, *375*(24), 2369–2379. https://doi.org/10.1056/NEJMra1600266
16. Manrique-Millones, D., Vasin, G. M., Dominguez-Lara, S., Millones-Rivalles, R., Ricci, R. T., Abregu Rey, M., Escobar, M. J., Oyarce, D., Pérez-Díaz, P., Santelices, M. P., Pineda-Marín, C., Tapia, J., Artavia, M., Valdés Pacheco, M., Miranda, M. I., Sánchez Rodríguez, R., Morgades-Bamba, C. I., Peña-Sarrionandia, A., Salinas-Quiroz, F., … Roskam, I. (2022). Parental Burnout Assessment (PBA) in Different Hispanic Countries: An Exploratory Structural Equation Modeling Approach. *Frontiers in Psychology*, *13*. https://www.frontiersin.org/articles/10.3389/fpsyg.2022.827014
17. Mendonça, I., Coelho, F., Ferrajão, P., & Abreu, A. M. (2022). Telework and Mental Health during COVID-19. *International Journal of Environmental Research and Public Health*, *19*(5), 2602. https://doi.org/10.3390/ijerph19052602
18. Mikolajczak, M., Brianda, M. E., Avalosse, H., & Roskam, I. (2018). Consequences of parental burnout: Its specific effect on child neglect and violence. *Child Abuse & Neglect*, *80*, 134–145. https://doi.org/10.1016/j.chiabu.2018.03.025
19. Ningthoujam, D. S., Singh, N., & Mukherjee, S. (2021). Possible Roles of Cyclic Meditation in Regulation of the Gut-Brain Axis. *Frontiers in Psychology*, *12*. https://doi.org/10.3389/fpsyg.2021.768031
20. Pace, T. W. W., Negi, L. T., Adame, D. D., Cole, S. P., Sivilli, T. I., Brown, T. D., Issa, M. J., & Raison, C. L. (2009). Effect of compassion meditation on neuroendocrine, innate immune and behavioral responses to psychosocial stress. *Psychoneuroendocrinology*, *34*(1), 87–98. https://doi.org/10.1016/j.psyneuen.2008.08.011
21. Raman, M., Vishnubhotla, R., Ramay, H. R., Gonçalves, M. C. B., Shin, A. S., Pawale, D., Subramaniam, B., & Sadhasivam, S. (2023). Isha yoga practices, vegan diet, and participation in Samyama

meditation retreat: Impact on the gut microbiome & metabolome - a non-randomized trial. *BMC Complementary Medicine and Therapies*, *23*(1), 107. https://doi.org/10.1186/s12906-023-03935-8

22. Ranganathan, P., Pramesh, C. S., & Buyse, M. (2016). Common pitfalls in statistical analysis: The perils of multiple testing. *Perspectives in Clinical Research*, *7*(2), 106–107. https://doi.org/10.4103/2229-3485.179436
23. Richter, C. G., Babo-Rebelo, M., Schwartz, D., & Tallon-Baudry, C. (2017). Phase-amplitude coupling at the organism level: The amplitude of spontaneous alpha rhythm fluctuations varies with the phase of the infra-slow gastric basal rhythm. *NeuroImage*, *146*, 951–958. https://doi.org/10.1016/j.neuroimage.2016.08.043
24. Roskam, I., Aguiar, J., Akgun, E., Arikan, G., Artavia, M., Avalosse, H., Aunola, K., Bader, M., Bahati, C., Barham, E. J., Besson, E., Beyers, W., Boujut, E., Brianda, M. E., Brytek-Matera, A., Carbonneau, N., César, F., Chen, B.-B., Dorard, G., … Mikolajczak, M. (2021). Parental Burnout Around the Globe: A 42-Country Study. *Affective Science*, *2*(1), 58–79. https://doi.org/10.1007/s42761-020-00028-4
25. Shatkin-Margolis, A., White, J., Jedlicka, A. E., Tam, T., Hill, A., Yeung, J., Crisp, C. C., & Pauls, R. N. (2022). The effect of mindfulness-based stress reduction on the urinary microbiome in interstitial cystitis. *International Urogynecology Journal*, *33*(3), 665–671. https://doi.org/10.1007/s00192-021-04812-z
26. Suda, K., & Matsuda, K. (2022). How Microbes Affect Depression: Underlying Mechanisms via the Gut–Brain Axis and the Modulating Role of Probiotics. *International Journal of Molecular Sciences*, *23*(3), 1172. https://doi.org/10.3390/ijms23031172
27. Sun, Y., Ju, P., Xue, T., Ali, U., Cui, D., & Chen, J. (2023). Alteration of faecal microbiota balance related to long-term deep meditation. *General Psychiatry*, *36*(1), e100893. https://doi.org/10.1136/gpsych-2022-100893
28. Swarup, S., Gupta, A., Chung, M., Radhakrishnan, V., Davis, V., Lynch, M. D. J., Charles, T. C., Cheng, J., & Mendoza, G. (2025). Rapid shift of gut microbiome and enrichment of beneficial microbes during arhatic yoga meditation retreat in a single-arm pilot study. *BMC Complementary Medicine and Therapies*, *25*(1), 51. https://doi.org/10.1186/s12906-025-04783-4
29. Tan, H.-E. (2023). The microbiota-gut-brain axis in stress and depression. *Frontiers in Neuroscience*, *17*. https://doi.org/10.3389/fnins.2023.1151478
30. Tang, Y.-Y., Hölzel, B. K., & Posner, M. I. (2015). The neuroscience of mindfulness meditation. *Nature Reviews Neuroscience*, *16*(4), 213–225. https://doi.org/10.1038/nrn3916
31. Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience & Biobehavioral Reviews*, *36*(2), 747–756. https://doi.org/10.1016/j.neubiorev.2011.11.009
32. Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, Arousal in Anxiety*, *61*(3), 201–216. https://doi.org/10.1016/S0165-0327(00)00338-4

33. Turku, U. of. (n.d.). *Chapter 8 Beta diversity | Introduction to microbiome data science*. Retrieved 11 September 2025, from https://microbiome.github.io/course_2021_radboud/beta-diversity.html
34. Author
35. Author
36. Author
37. Wang, Z., Liu, S., Xu, X., Xiao, Y., Yang, M., Zhao, X., Jin, C., Hu, F., Yang, S., Tang, B., Song, C., & Wang, T. (2022). Gut Microbiota Associated With Effectiveness And Responsiveness to Mindfulness-Based Cognitive Therapy in Improving Trait Anxiety. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.719829
38. Yu, J., Zhang, Y., Wells, J. C. K., Wei, Z., Bajaj-Elliott, M., Nielsen, D. S., & Fewtrell, M. S. (2024). A Stress Reduction Intervention for Lactating Mothers Alters Maternal Gut, Breast Milk, and Infant Gut Microbiomes: Data from a Randomized Controlled Trial. *Nutrients*, *16*(7), 1074. https://doi.org/10.3390/nu16071074
39. Zeng, Q., Feng, X., Hu, Y., & Su, S. (2025). The human gut microbiota is associated with host lifestyle: A comprehensive narrative review. *Frontiers in Microbiology*, *16*, 1549160. https://doi.org/10.3389/fmicb.2025.1549160

Figures

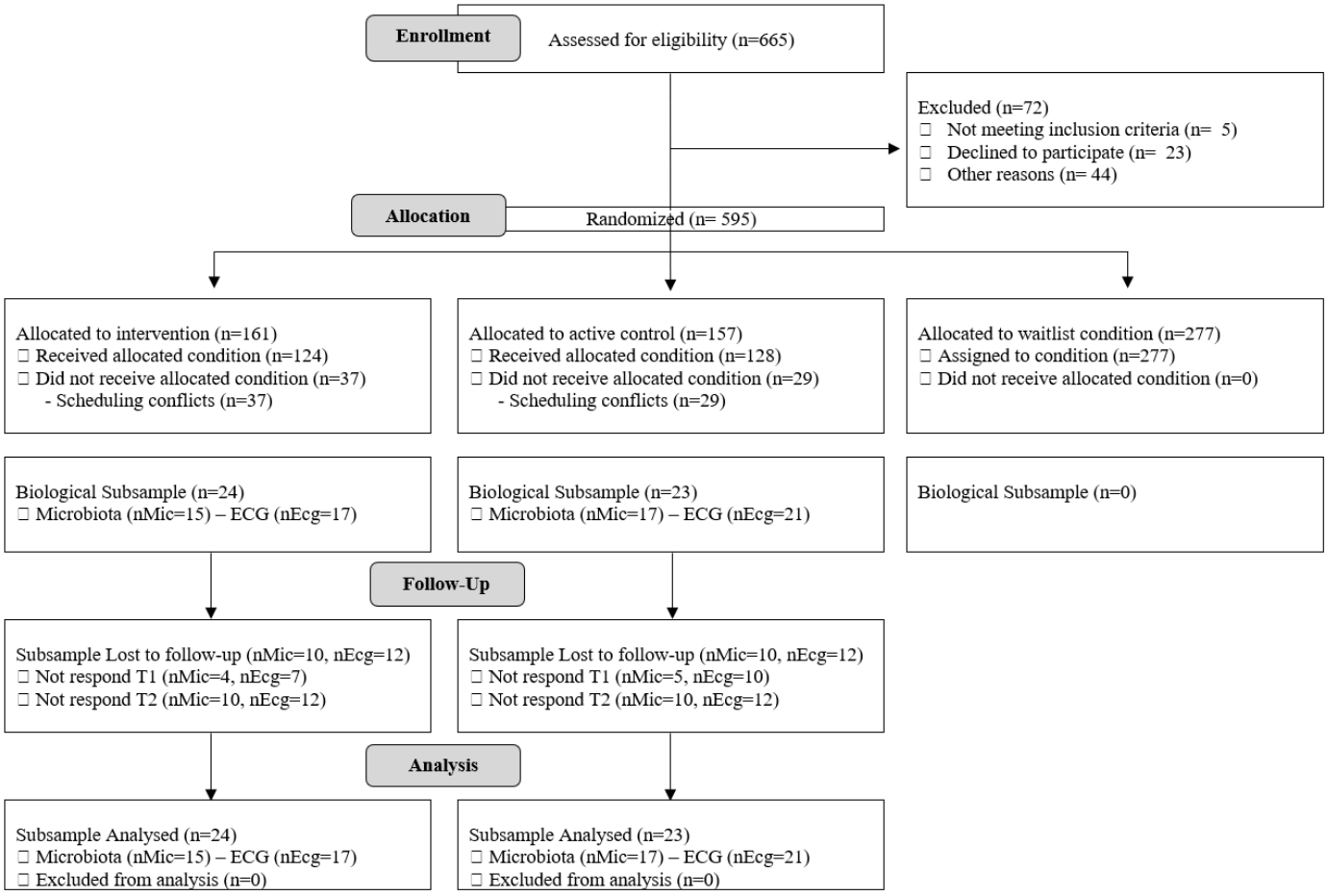


Figure 1

CONSORT flow diagram of trial.

Loss to follow-up is shown separately for fecal microbiota samples (*nMic*) and ECG samples (*nEcg*). IBAP = mindfulness- and compassion-based inter-care program; AC = active control; WL = waiting list. The biological substudy included partially overlapping fecal microbiome and ECG/HRV samples from the intervention and active control groups. The waitlist group did not undergo biological sampling. Sample sizes varied by outcome and time point because of participant attendance, sample availability, and ECG quality-control criteria. Detailed sample availability by wave and outcome is reported in Supplementary Table 2.

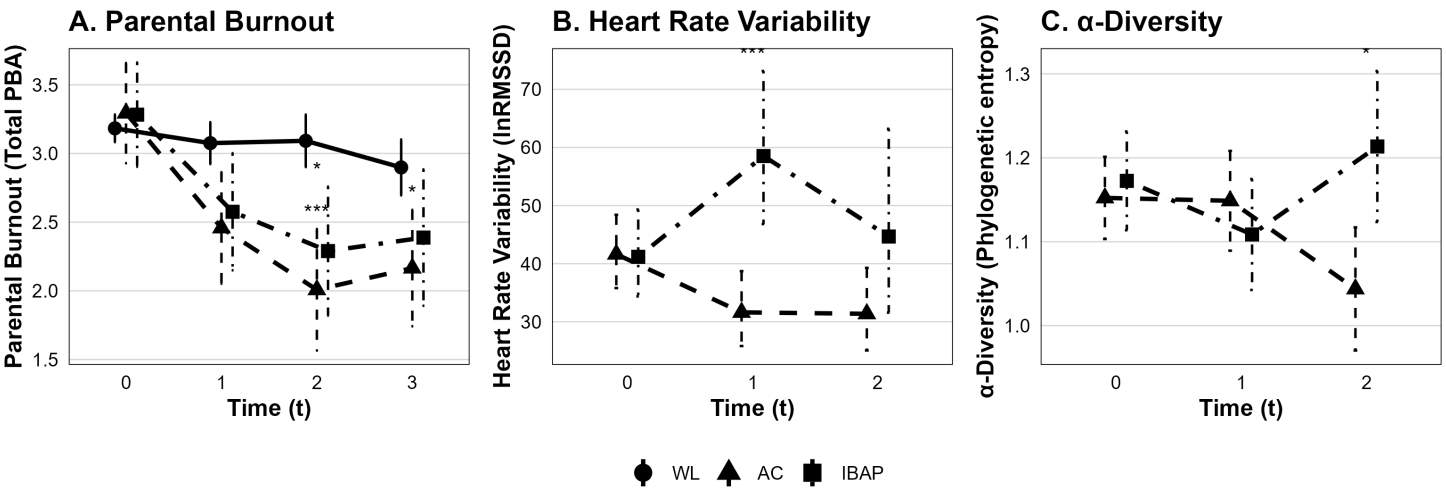


Figure 2

Estimated marginal means of parental burnout, heart rate variability, and α-diversity over time by group.

(A) Parental Burnout, (B) Heart Rate Variability (lnRMSSD), and (C) α-Diversity (phylogenetic entropy) at baseline and follow-up. Lines show estimated marginal means with 95% CIs; symbols denote groups (WL, AC, IBAP). Asterisks indicate contrasts significant after Holm–Bonferroni correction (*p<0·05; **p<0·01; ***p<0·001). WL = waiting list; AC = active control; IBAP = mindfulness- and compassion-based inter-care program.

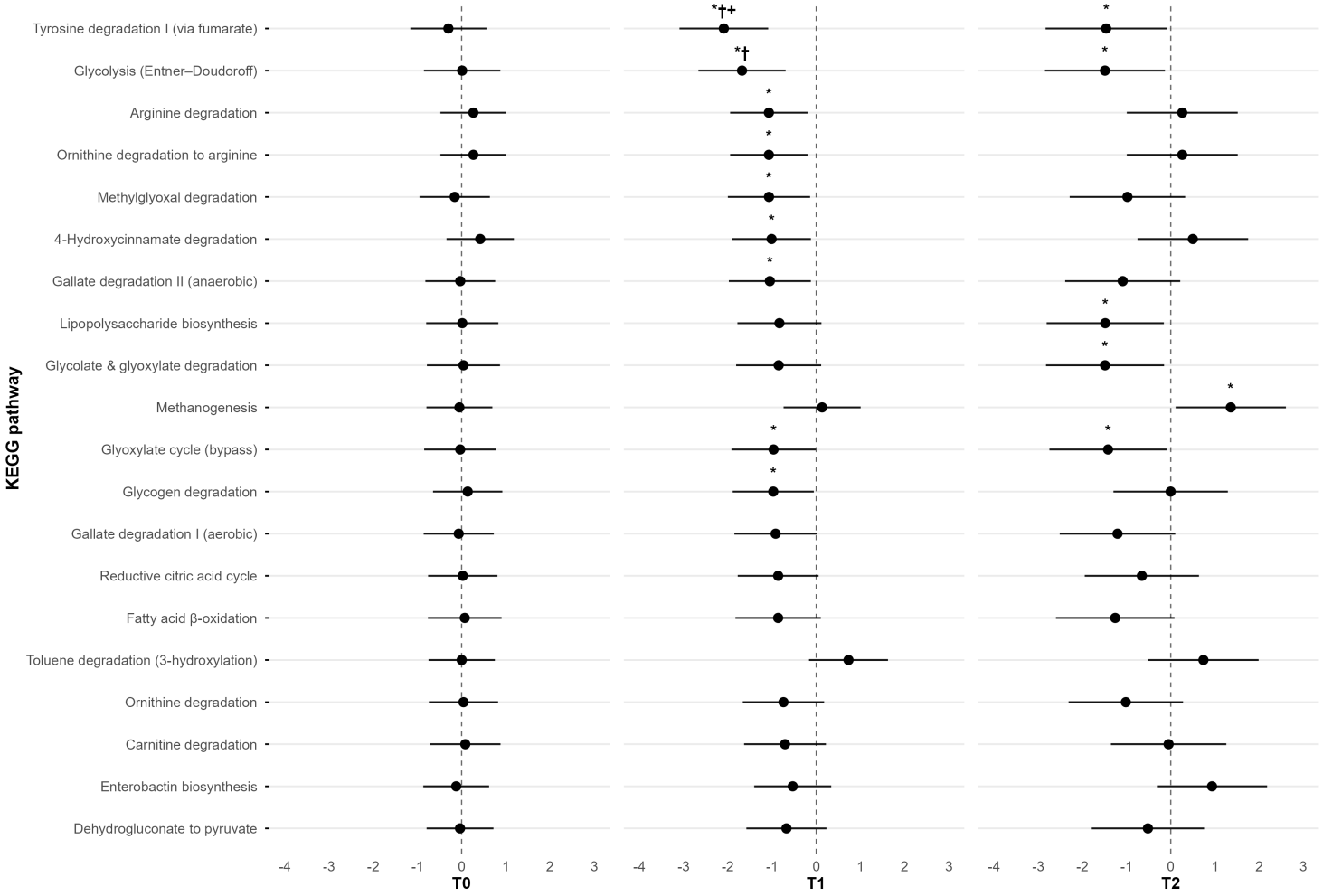


Figure 3

Differential abundance of predicted KEGG pathways by group and time

Forest plots show estimated contrasts (IBAP − AC) with 95% CIs for selected metabolic pathways at baseline (T0), 3 months (T1), and 6 months (T2). Effect sizes are presented on the logit scale; positive values indicate higher relative abundance in IBAP. AC = active control; IBAP = mindfulness- and compassion-based inter-care program. * unadjusted p<0·05; † Withing time Holm-adjusted p<0·05; + Benjamini–Hochberg–adjusted p<0·05.

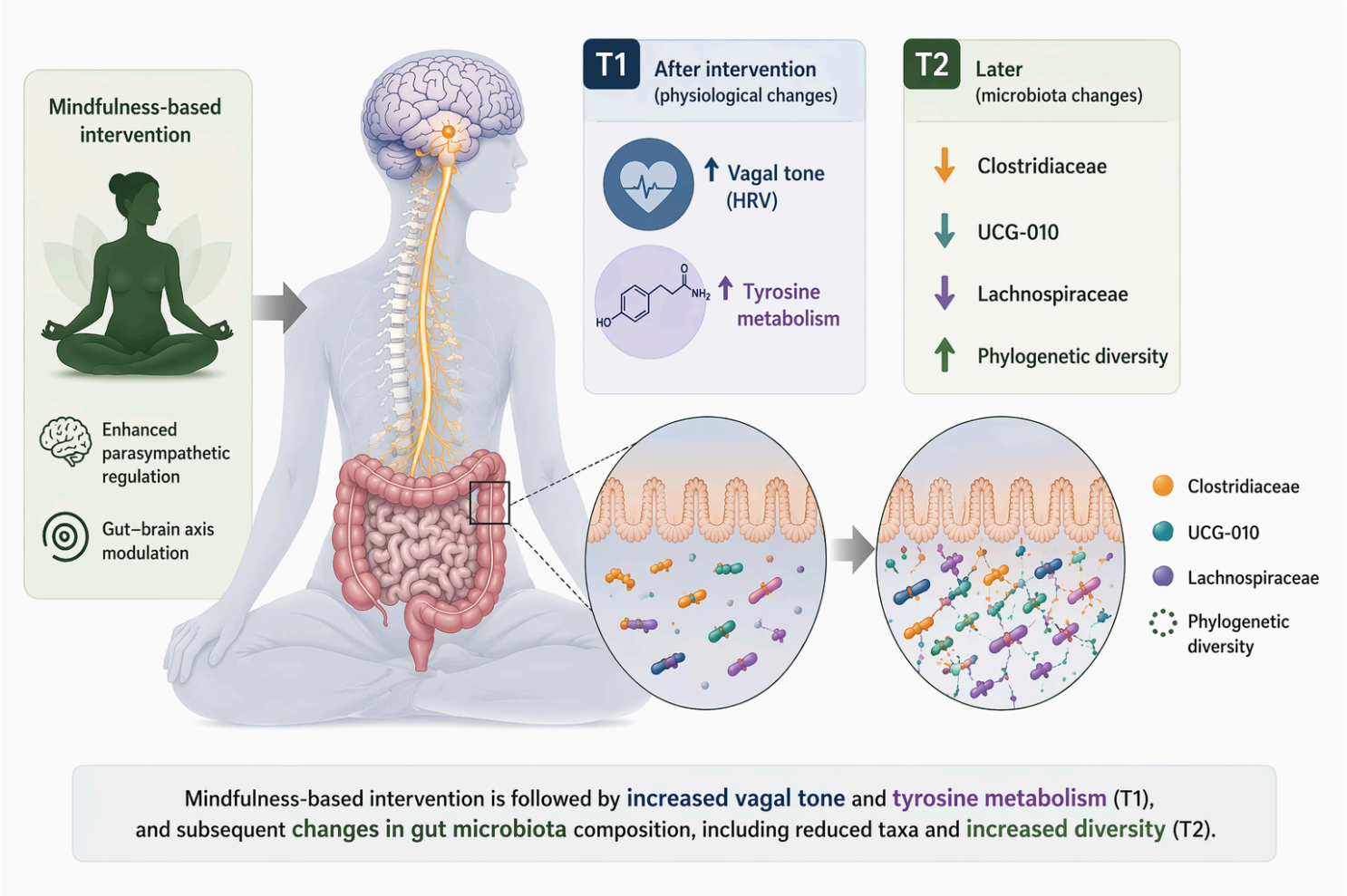


Figure 4

Temporal pattern of autonomic and gut-microbiome changes.

The schematic summarises the trial's main signals. Across the study, parental burnout decreased in the intervention arms. At T1 (early post-intervention) the intervention was associated with higher heart rate variability (HRV) and an exploratory reduction in microbial tyrosine-metabolism functions. At T2 (later follow-up) the microbiome showed greater α-diversity (phylogenetic entropy) together with lower relative abundance of Clostridiaceae, Lachnospiraceae and the UCG-010 group in the intervention versus control. This pattern suggests early autonomic and predicted functional differences followed by later microbial diversity/compositional differences.

(This is a preliminary AI-generated graphic. The content reflects the intended final figure; however, before publication, a final version designed by a professional graphic designer and adapted to the journal's visual style and color scheme will be provided.)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 00CONSORT2025checklist.docx
- 00Supplement3000.docx