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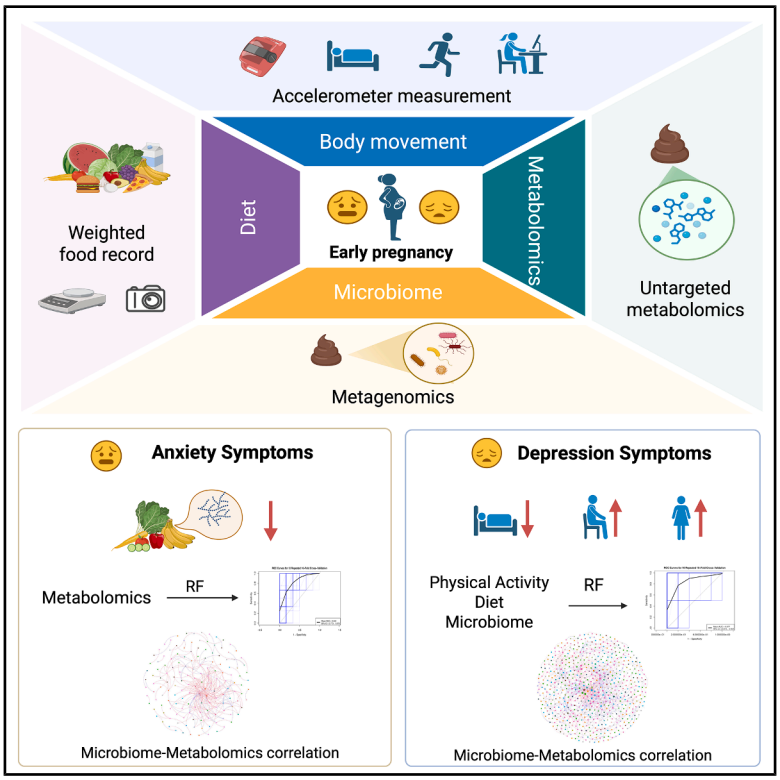
Mental health in early pregnancy: Interplay of objectively measured lifestyles, gut microbiota, and metabolomics

Graphical abstract



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In brief

Clinical finding; Metabolomics

Highlights

- Depression and anxiety in early pregnancy have distinct biological signatures
- Depression linked to sedentary behavior and gut microbiota-metabolite disruption
- Anxiety associated with fiber deficiency and metabolomic dysregulation

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Mental health in early pregnancy: Interplay of objectively measured lifestyles, gut microbiota, and metabolomics

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SUMMARY

Prenatal anxiety symptoms (AS) and depression symptoms (DS) in early pregnancy substantially impact maternal-infant health, but their pathophysiological mechanisms remain unclear. We conducted a multimodal assessment of 161 early-pregnant women. DS exhibited significantly longer standing/sitting durations and shorter nocturnal sleep and nap times ($p < 0.05$). AS was associated with lower fiber intake ($p = 0.018$). AS and DS had more complex microbial co-occurrence networks with more extensive metabolomic changes. DS was characterized by dysregulated cysteine/methionine metabolism and Bifidobacterium-mediated modulation of standing/sleep effects. Machine learning achieved best DS discrimination by combining physical activity, diet, and microbiome data (AUC = 0.877), while metabolomics alone best discriminated AS (AUC = 0.844). In conclusion, AS is associated with metabolomic dysregulation and fiber deficiency, while DS is associated with sedentary behavior and microbiota-metabolite disruption. This study establishes a precision framework prioritizing fiber interventions for AS and activity/sleep modulation targeting microbiota-metabolite pathways for DS.

INTRODUCTION

Globally, mental disorders including depression and anxiety are the leading cause of poor health and disability.[1] Pregnant and postpartum women are more susceptible to mental health issues due to increased stress and a lack of social support.[2] Although anxiety affects up to 31% of pregnant women and depression 20.7%,[3,4] both illnesses are commonly underdiagnosed and undertreated throughout the perinatal period.[5,6] Critically, the first trimester represents a vulnerable period for embryonic organ development and a phase of maternal adaptation to the physiological and psychological changes experienced by the pregnant woman. Studies demonstrate that women with elevated anxiety during early pregnancy face a 1.4-fold higher risk of preterm birth,[5] while first-trimester depression increases the risk of postpartum depression.[7] The molecular pathways underlying first-trimester prenatal anxiety and depression are still poorly understood.[8] Identifying modifiable risk factors and mechanistic biomarkers for these mental conditions may enhance risk stratification, guide interventions, and advance mechanism-driven prevention.

Promoting mental health through modifiable lifestyle behaviors such as physical activity, sleep, and diet are increasingly recognized as a cost-effective alternative to traditional psychotherapy and pharmacological treatments.[9,10] In 2020, the Canadian 24-Hour Movement Guidelines advanced previous single movement behavior recommendations by incorporating advice for physical activity, sedentary time, and sleep throughout a 24-h period.[11] Subsequent research has demonstrated the positive effects on mental well-being when all three components were met in both children and adults.[12] While 24-Hour Movement Guidelines have not been developed for pregnancy, current international guidelines recommend that pregnant women engage in at least 150 min of moderate-intensity physical activity (MVPA) each week to enhance maternal and child health, including mental well-being.[13,14] In addition to physical activity, previous studies have shown that dietary factors (e.g., omega-3 fatty acids) have a positive impact on mental health in pregnancy.[15] However, most studies focus on single behaviors (e.g., MVPA or diet), despite their known reciprocal interactions and data on integrated lifestyle patterns during pregnancy remain limited.[16] Crucially, the biological mechanisms through which these modifiable lifestyle factors influence early-pregnancy mental health are poorly understood.

Research identifies gut microbiota and their metabolites as potential regulators of mental health through the gut-brain axis.[17] According to studies on fecal transplantation, delivering depressed persons feces to germ-free mice or microbiota-depleted rats can lead the recipients to exhibit depressive-like behaviors.[18,19] However, studies in pregnant women remain limited, particularly during early pregnancy, and revealed associations between mental health problems and gut microbiota are inconsistent across studies.[20–22] Previous research on pregnant women has primarily used 16S rRNA sequencing, which offers information about the gut microbiota's makeup but cannot distinguish between specific species or microbial functions. Furthermore, these investigations have rarely included metabolomics or microbiome analysis. This disagreement is significant because fecal microbiota transplantation (FMT) involves the transfer of both microbiota and their metabolites, making it difficult to determine whether the effects observed are caused by specific microbial functions or the metabolites produced by these microorganisms. Critically, the interplay between microbiome-metabolite dynamics and lifestyle drivers (e.g., diet, physical activity) remains unaddressed in integrated studies. As lifestyle factors serve as primary drivers of gut microbiome composition and metabolic profiles, neglecting these interactions may lead to an incomplete understanding of the roles that the microbiome and its metabolites play in mental health among pregnant women.

This study integrated objective assessments of physical activity, dietary intake, fecal metagenomics, and metabolomics from 161 women in early pregnancy within a prospective cohort to systematically investigate the correlations among lifestyle choices, gut ecosystem dynamics, and maternal mental health outcomes. Using multi-omics profiling, we show how diet and daily activity patterns alter microbiome-metabolite interactions to impact prenatal anxiety and depression in early pregnancy. This foundation enables the development of precision therapeutics for prenatal mental health through targeted lifestyle modifications and microbiome modulation approaches.

RESULTS

Demographic characteristics of the participants

The current study included 161 participants with a median age of 30 years, comprising healthy controls (HC, $n = 99$), individuals with depression symptoms (DS, $n = 36$; EPDS >13), and those with anxiety symptoms (AS, $n = 53$; S-STAI >40). Among these, 26 participants exhibited both DS and AS. Half of participants were primiparous, and 18% of participants were classified as overweight or obese prior to pregnancy (see online supplemental document Table S1). Psychometric evaluation of the instruments revealed good internal consistency and sampling adequacy: EPDS (α = 0.860, KMO = 0.814) and STAI (α = 0.857, KMO = 0.920).

Distinct lifestyle behaviors among pregnant women with DS and AS

We first identified distinct 24-h behavioral and dietary profiles across DS, AS and HC (Figure 1). Actigraphy results demonstrated distinct 24-h activity patterns between the DS and HC groups where DS women showed fragmented rhythms characterized by prolonged sedentary time and contracted sleep periods (Figure 1A). Specifically, women in the DS group exhibited prolonged standing time (mean difference: 0.47 h/day, $p = 0.033$) and sitting time (mean difference: 0.8 h/day, $p = 0.045$), alongside increased sedentary time while experiencing diminished nocturnal sleep duration (mean difference: −0.65 h/night, $p = 0.020$; Figure 2C) and nap duration (mean difference: −0.7h/day, $p = 0.034$ Figure 1C). Furthermore, the pre-conception questionnaire indicates that the DS group exhibited lower levels of leisure-time physical activity (LTPA) prior to pregnancy compared to HC. Nonetheless, no significant differences between DS and HC were observed in dietary intake (see online supplemental document Table S2).

In contrast to DS, the AS group demonstrated distinct nutritional patterns, with a significantly reduced daily dietary fiber consumption compared to HC (mean difference: −3.1 g/d,

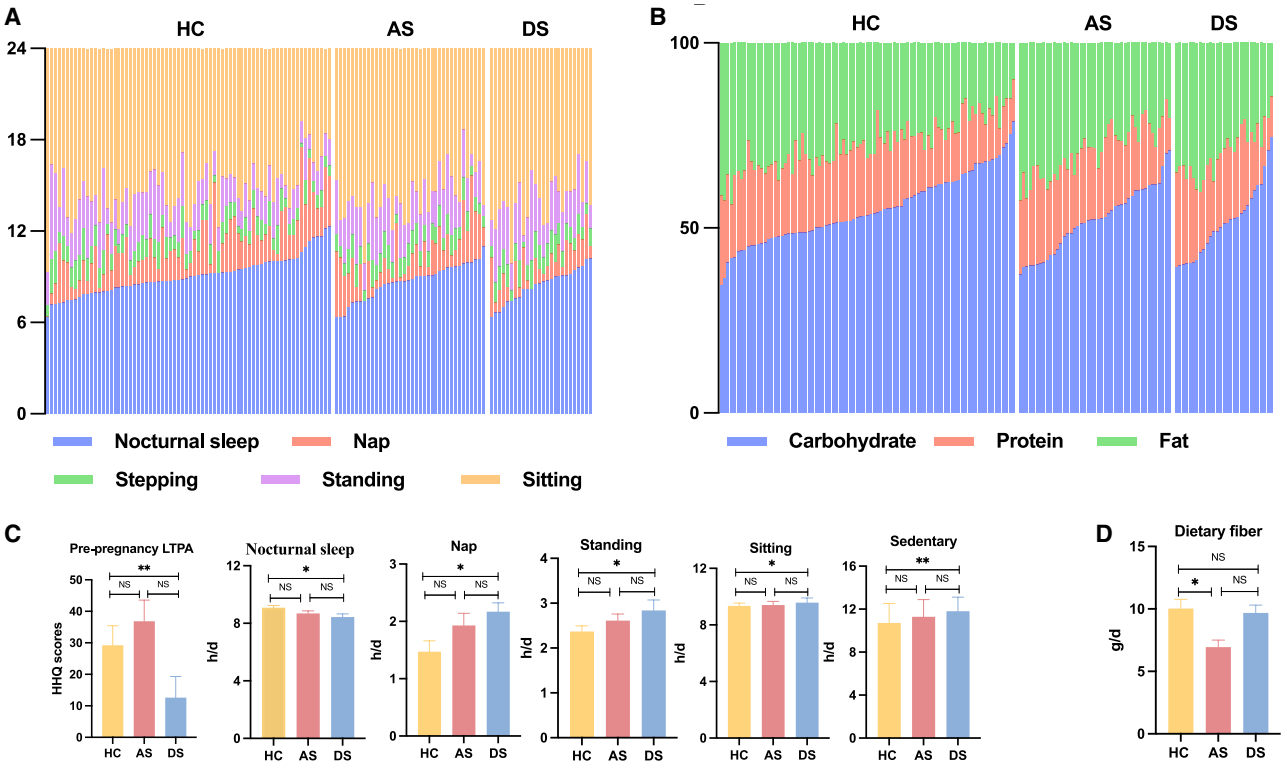

Figure 1. Comparative analysis of daily behavioral patterns between groups

(A) 24-h activity chronogram. The horizontal axis displays individual subjects arranged by group, while the vertical axis corresponds to daily time spend in the activity.

(B) Daily energy intake from macronutrients. The horizontal axis displays individual subjects arranged by group, while the vertical axis corresponds to percentage of daily energy intake from macronutrients.

(C and D) Quantitative behavioral pattern comparison. Bar graphs show group differences in key behavioral metrics ($p < 0.05$). HC: healthy control; AS: anxiety symptoms; DS: depression symptoms; LTPA: leisure time physical activity.

$p = 0.018$; Figure 1D). Physical activity parameters exhibited no statistically significant differences between the AS and HC groups (see online supplemental document Table S3).

No significant differences in fecal microbial diversity across study groups

Using metagenomic data to profile fecal microbiota diversity in pregnant women stratified by mental health status. No significant differences in alpha diversity metrics were observed between any of the groups (Figures 2A and 2B). Beta diversity analysis via principal coordinate analysis (PCoA) revealed no statistically distinct clustering of microbial community structures across mental health groups (Figures 2C and 2D), suggesting comparable overall compositional profiles.

Fecal microbial composition and functions linked to early pregnancy DS and AS

A comparative analysis of gut microbiota using metagenomic sequencing data were performed across the HC, AS, and DS groups. The DS group exhibited three significantly enriched species (*Bifidobacterium catenulatum*, *Eisenbergiella* sp. , and *Bifidobacterium pseudocatenulatum*), while AS group was characterized by the significant enrichment of two species (*Gemmiger qucibialis* and *Catenibacterium* sp.). The HC showed enrichment of *Eubacterium siraeum* (Figure 3A) (LEfSe LDA ≥ 2, $p < 0.05$). However, these differences were no longer significant after FDR correction, suggesting the initial findings may reflect lack of robust effect sizes.

Functional metagenomic profiling identified distinct functional divergences across groups. Compared to HC, both AS and DS groups showed predominant alterations in amino acid biosynthesis and secondary metabolite production pathways. Specifically, the AS group exhibited enrichment in Wnt signaling pathway and brassinosteroid biosynthesis, while the DS group showed higher abundance in cysteine and methionine metabolism as well as pantothenate and CoA biosynthesis (Figure 3). In addition, there were no significant differences in fecal concentrations of the SCFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) between the DS and HC groups or between the AS and HC groups ($p > 0.05$) (online supplemental document Figure S1).

Fecal metabolomics and related function alterations associated with DS and AS

Fecal metabolomic profiling unveiled pronounced metabolic dysregulation across study groups. An overview of the non-targeted

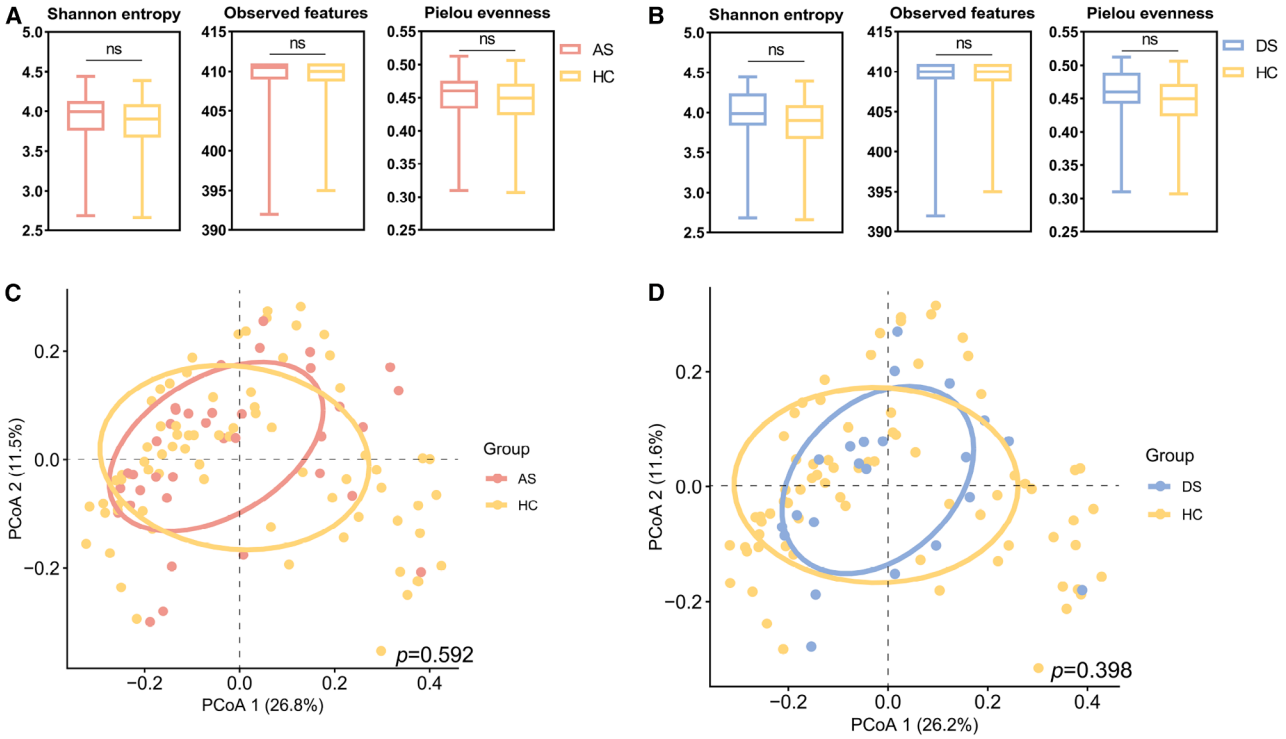

Figure 2. Gut microbiome diversity across study groups

(A) Microbial α-diversity between DS vs. HC groups.

(B) Microbial α-diversity between AS vs. HC groups.

(C and D) β-diversity analyses (weighted UniFrac distances) between DS vs. HC (C) and AS vs. HC (D) groups. HC: healthy control; AS: anxiety symptoms; DS: depression symptoms.

metabolomics data, including sample distribution and metabolite classes, is provided in online supplemental document Figure S2. Three-group comparison (HC, AS, and DS) using LEfSe revealed that three bile acid-related metabolites (7-ketodeoxycholic acid, tauroursodeoxycholic acid, and prasterone sulfate) were significantly elevated only in the HC group, with similarly lower levels in both AS and DS (Figure 3B).

Metabolite set enrichment analysis further linked AS samples to disruptions in taurine/hypotaurine metabolic processes and bile acid metabolism and DS samples to neuroactive pathway dysregulation (including glycerophospholipid metabolism and cholinergic synapse pathways (Figures 3C and 3D).

Alterations in cysteine/methionine metabolism in DS

Notably, both bacterial genomic features and metabolomic profiles exhibited convergent enrichment patterns in cysteine/methionine metabolism during DS-HC comparisons. Integration of enzyme-centric (EC) and metabolomic data revealed mechanistic links between microbial gene activity and host metabolism. The DS group showed reduced levels of 3-methylthiopropionate and 2-oxobutanoate, concomitant with downregulation of three key enzymes in cysteine/methionine metabolism: aspartate kinase [EC:2.7.2.4], methionine synthase [EC:2.1.1.13], and methylthioribose kinase [EC:2.7.1.100] (Figure 3E). This triad of gene-metabolite perturbations strongly suggests suppression of methionine-cysteine cycling in depressive states.

Integrated lifestyles and muti-omics for discriminating DS and AS from HC

Potential interactions between bacterial communities and metabolic profiles in response to mental health status were assessed using correlation network analysis. Overall network complexity, as reflected by node and edge counts, progressively increased from HC to AS to DS across all three network types (Figure 4A). For microbiome subnetworks, which allowed individual-level statistical testing, node counts increased progressively from HC to AS to DS, Edge counts were significantly higher in both AS and DS compared to HC ($p < 0.05$). For metabolomics subnetworks and microbiome-metabolomics networks, node and edge counts also increased stepwise from HC to AS to DS. However, the average degree, a measure of network connectivity density revealing different modes of structural reorganization. Microbiome networks showed dense connectivity in anxiety, whereas metabolomics networks exhibited progressively tighter integration with disease severity. Notably, microbiome-metabolomics network was strongest in healthy controls and progressively diminished in AS and DS, highlighting a loss of microbe-metabolite coordination in disease states.

We applied a multi-level random forest models (diet, physical activity, microbiome, and metabolomics) to identify key biological features underlying prenatal DS and AS, assessing mental health patterns in pregnant women (Figure 4B). Our results revealed that the model using physical activity and dietary factors

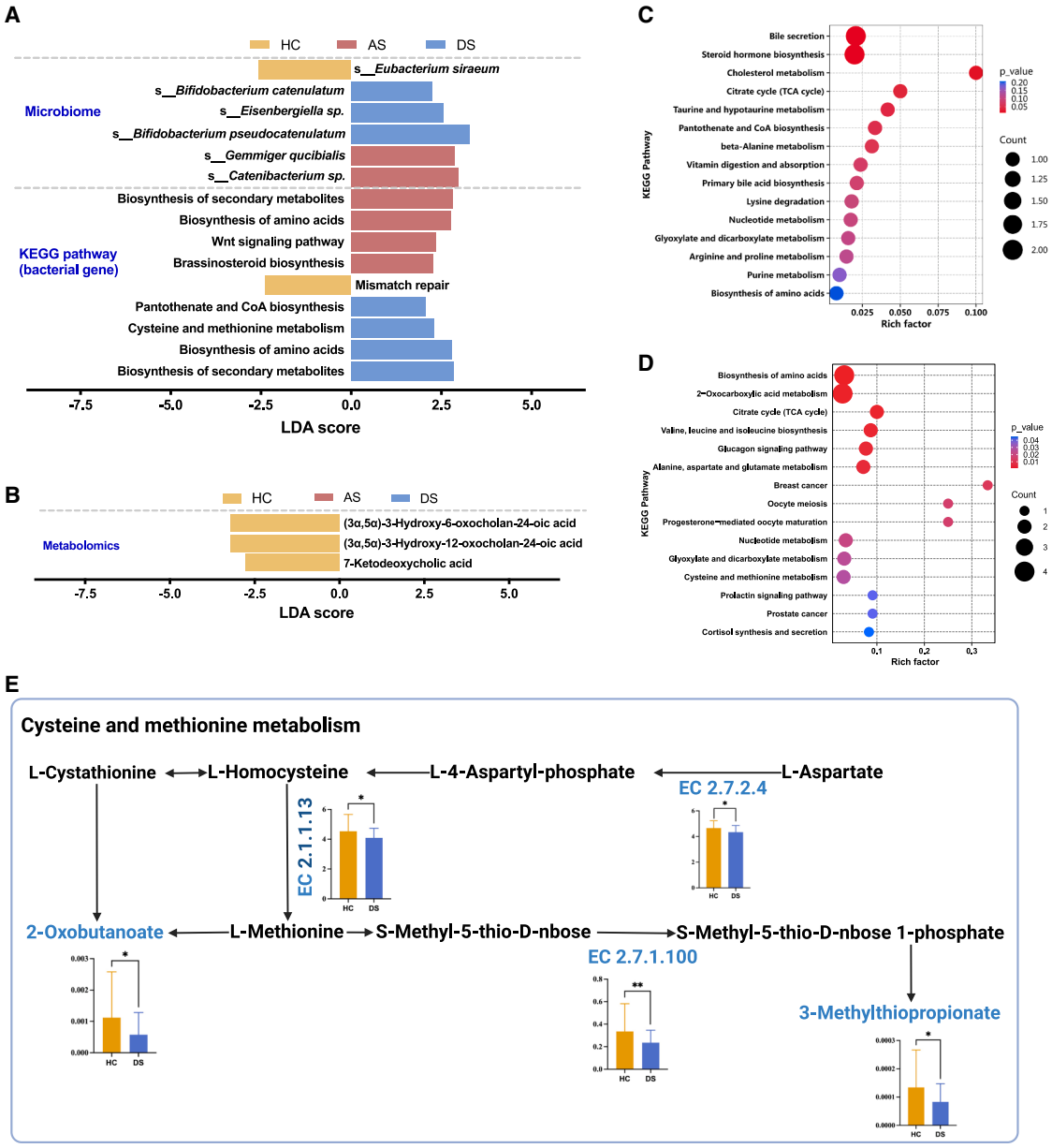

Figure 3. Differential analysis of fecal microbiota, fecal metabolites, and KEGG-enriched biological processes across study groups

(A) Comparative analysis of microbiome composition, bacterial gene-associated KEGG pathways among three groups(LEfSe LDA $\geq$2, $p < 0.05$).

(B) Comparative analysis of metabolomic profiles among three groups (LEfSe LDA $\geq$2, $p < 0.05$).

(C) KEGG enrichment analysis of metabolites (biological processes) in AS group versus HC group.

(D) KEGG enrichment analysis of metabolites (biological processes) in DS group versus HC group.

(E) Cysteine and methionine metabolism pathways mapped by microbial genes and fecal metabolism in gut ecosystem of DS. HC: healthy control; AS: anxiety symptoms; DS: depression symptoms.

alone achieved an AUC-ROC of 0.691 (95% CI: 0.601–0.782), whereas the combination of physical activity, dietary factors, and microbiota exhibited the strongest predictive performance for DS, achieving an AUC-ROC of 0.877 (95% CI: 0.813–0.941; Figure 4E). Microbiome variables such as *Clostridium scindens*, *Candidatus Copromorpha excrementavium* were the most important features associated with the risk of DS (Figure 4F). The metabolomic data alone achieved an AUROC of 0.844 (95% CI: 0.773–0.915; Figure 4C), indicating the highest discriminatory ability for AS. Microbiota related metabolomics, such as cholic acid 7-sulfate and solanidine, were the most important features associated with the risk of AS (Figure 4D).

Subsequently, a mediation analysis assessed how bacterial characteristics influence DS and AS through metabolites.

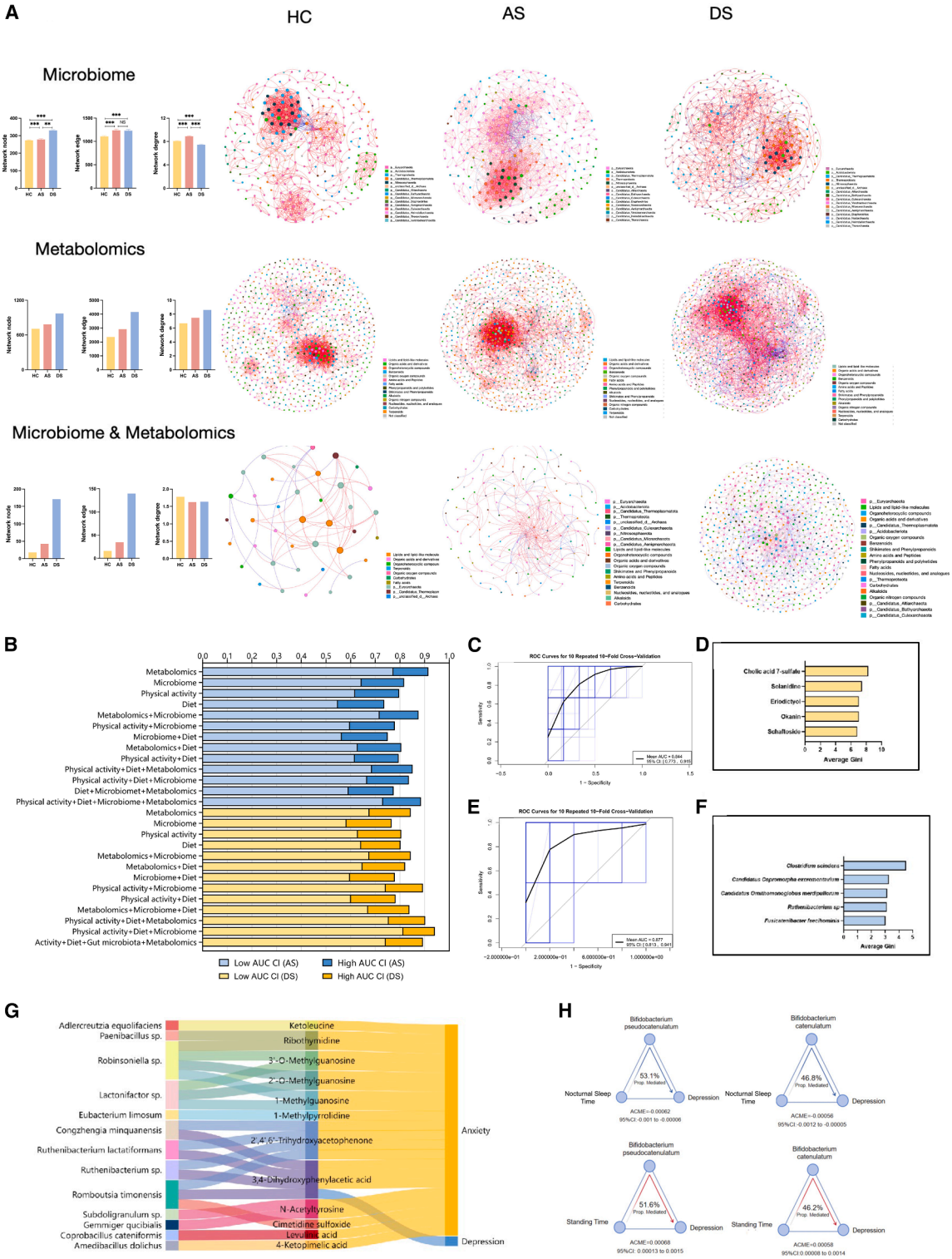

Figure 4. Interactions of lifestyle, fecal microbiome, and metabolomics in early pregnant women with anxiety symptoms or depression symptoms

(A) Significant correlations (Spearman; $p < 0.01$, $|r| > 0.07$) between microbiome, metabolomics, and microbiome-metabolomics within the HC, AS, and DS groups are shown in the network. Positive and negative correlations are shown by red and blue lines, respectively. Nodes are colored by genus or metabolomics category, while node size represents the number of correlation relationships.

(legend continued on next page)

Twenty-three significant pathways were identified for AS while one pathway was identified for DS (Figure 4G). Two key metabolites, 2′,4′,6′-trihydroxyacetophenone (bacterial derivatives of plant polyphenols) and 3,4-dihydroxyphenylacetic acid (metabolite of the human neurotransmitter dopamine) mediated anxiety pathways involving *Ruthenibacterium* sp., *Congzhengia minguanensis*, and *Ruthenibacterium lactatiformans*. In the meantime, 3,4-dihydroxyphenylacetic acid also mediated the effects on *Romboutsia timonensis* on DS.

Separately, a mediation analysis examined the impact of lifestyle factors on DS and AS via microbiome or metabolomics. In DS, gut microbiota emerged as the primary mediators linking lifestyle behaviors to mental health. Notably, species within the Bifidobacterium genus (*B. pseudocatenulatum* and *B. catenulatum*) mediated the effects of standing time and nocturnal sleep duration on depression symptoms (Figure 4H). The indirect effects ranged from −0.00062 to −0.00056 for nocturnal sleep duration and from 0.00058 to 0.00068 for standing time, with proportions mediated between 46% and 53% across these pathways. No significant mediation pathways were identified for AS.

DISCUSSION

Maternal mental disorders in early pregnancy have immediate and persisting adverse impacts on both mother and child.[5,7] It is essential to identify modifiable lifestyle factors and biomarkers of prenatal mental disorders that could facilitate early intervention. To our knowledge, this is the first study employing a multi-omics framework integrated with objective lifestyle measures.

We pioneer the integration of objectively measured lifestyle behaviors with multi-omics profiling (fecal metagenomics and metabolomics) in pregnancy individuals during the first trimester with DS and AS compared to HC. The individuals with DS engage in higher levels of sedentary behavior, standing, sitting and experience reduced sleep duration compared to HC. Complementing these behavioral findings, both bacterial genomic features and metabolomic profiles displayed similar patterns of enrichment in cysteine and methionine metabolism related to DS. The combination of physical activity, dietary factors, and microbiota exhibited the strongest predictive performance for DS. In contrast, those with AS exhibit low dietary fiber intake. And the best predictive performance for AS is metabolomic dataset. In addition to the direct effects of standing and sleeping, mediation analysis revealed a significant indirect effect mediated by *Bifidobacterium*, implying that physical activity may potentially have a partial influence on depressive symptoms via this microbial pathway. These findings underscore condition-specific mechanisms mediating depression and anxiety providing the opportunity to develop tailored therapies targeting gut-brain axis interactions to support mental health in early pregnancy.

Previous studies have predominantly relied on self-reported questionnaires to examine physical activity and dietary patterns in isolation, potentially overlooking significant connections between daily behaviors.[15,16] Our study addresses this limitation by employing objective, device-based monitoring to comprehensively assess behavioral patterns in early pregnancy. We identified a distinct behavioral phenotype referred to as the DS pattern, characterized by reduced preconception LTPA, coupled with increased sedentariness, prolonged standing\sitting durations, and sleep insufficiency in early pregnancy. Importantly, leveraging ActivPAL4's unique posture-discrimination capability, we identify a previously unrecognized signature in first-trimester DS. DS women exhibited significantly both prolonged sitting and standing durations versus suggesting widespread physical inactivity extending beyond conventional sedentary definitions. While our cohort did not detect associations between anxiety and physical activity, this finding aligns with objective physical activity measurements in a Spanish cohort.[23] However, the significant correlation between MVPA and depression observed in the Spanish cohort was not detected in our study. These disparities likely stem from key methodological divergences. Our larger cohort (n = 161 vs. n = 124) employed ActivPAL4 devices capturing posture-derived sedentary/activity thresholds, whereas the Spanish study used ActiGraph GT3X+ motion sensors with distinct cut-points. Importantly, the Spanish cohort was evaluated second trimester, whereas our study pioneers first trimester specific profiling, this temporal difference is biologically significant as early gestational neuroendocrine-immune reprogramming may uniquely modulate physical activity responses. Cultural and lifestyle variations may further contribute to these differential findings.

Women with DS exhibited inactivity behavior (both in standing and sitting) and reduced sleep duration, potentially attributed to neurobiological processes such as neurotrophic dysregulation,[24] including alterations in cystine and methionine metabolism and detected alteration of the gut microbiome in our cohort, as well as hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis. Such actions may contribute to a harmful cycle that encompasses symptoms of depression, sleep disturbances, and a lack of activity. Conversely, AS was linked to a lower intake of fiber, but no physical activity differed compared to HC, possibly reflecting stress-induced dietary choices or glucose metabolism/autonomic.[25] Our findings indicate that individuals with AS exhibit dysregulation in taurine and cholinergic signaling pathways. This implies customized treatments: improved physical activity and sleep duration for DS, while more dietary fiber intake for AS management.

(B) The RF classification accuracy based on physical activity, diet, microbiome and metabolomics datasets.
(C) The best predict model for AS, ROC curves are shown based on RF models, with the AUC scores.
(D) Predictors of AS ranked by their relevance.
(E) The best predict model for DS, ROC curves are shown based on RF models, with the AUC scores.
(F) Predictors of DS ranked by their relevance.
(G) Sankey diagram showing the mediation pathway from microbiome to mental health via metabolomics (only significant mediation effects are displayed).
(H) Inferred causal relationships between lifestyle, microbiome/metabolomics, and mental health (only significant mediation effects are displayed). HC: healthy control; AS: anxiety symptoms; DS: depression symptoms; RF: random forest; ROC: receiver operating characteristic; AUC: the area under the curve.

Gut dysbiosis has emerged as a potential mediator of perinatal mental health.[26] Research employing 16S rRNA sequencing has revealed microbial changes linked to depression, such as an increase in *Lachnospiraceae*, *Clostridia*,[20] and *Phascolarctobacterium*.[26] Metabolomic investigations have identified disruptions in several metabolic pathways, encompassing the metabolism of linoleic acid and phenylalanine, tyrosine, and tryptophan,[20] in addition to glycine, serine, and threonine.[27] In our study, using metagenomic sequencing, we observed no significant differences in microbial alpha- or beta-diversity between the DS, AS, and HC groups. These findings align with previous studies reporting no significant diversity changes[20] and highlight the importance of moving beyond global diversity metrics.

The lack of significant differences in fecal concentrations of six major SCFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) between the three groups indicates that altered fecal SCFA production may not characterize DS or AS in this cohort. Although it discordant with non-perinatal study,[28] this may reflect resilience in SCFAs output during pregnancy. Differences in fecal SCFA abundance seen outside of gestation may be mitigated by the substantial physiological changes brought on by pregnancy, such as increased plasma volume, changed gut motility, improved nutrient absorption efficiency, and changes in microbial metabolism.[29–31] These adaptations may alternation the fecal SCFA levels during pregnancy, reflecting enhanced SCFA absorption and utilization driven by increased metabolic demands. Alternatively, the findings underscore the primacy of host factors (absorption and receptor sensitivity) over fecal SCFA abundance itself.

Using comprehensive metagenomics, we identified three specific bacterial species enriched in early prenatal DS and two bacterial species enriched in early prenatal AS. Notably, both *Bifidobacterium pseudocatenulatum* and *Bifidobacterium catenulatum* showed higher relative abundance in the DS groups. Meanwhile, these two species mediated the effects of sleep and standing on DS. This aligns with a recent Asian cohort study,[32] reporting increased *Bifidobacterium pseudocatenulatum* in depression, contrasting animal studies that suggest Bifidobacterium pseudocatenulatum CECT 7765 and Bifidobacterium pseudonumeratum W112 as a potential psychobiotic to alleviate depressive symptoms.[33,34] This divergence likely reflects species/strain-specific effects within the complex human gut ecosystem. *Bifidobacterium pseudocatenulatum* overgrowth in depression may indicate compensatory mechanisms for inflammation[35] or unintended dysbiosis from lifestyle choices (supported by its mediation of standing/sleep effects in our study). Consistent with non-pregnant populations, our study revealed that the HC group exhibited a significantly higher *Eubacterium siraeum*.[36] Similarly, the AS group displayed elevated *Catenibacterium* sp., corroborating prior non-pregnant studies,[37] which may serve as potential target for therapeutic interventions.

Mechanistic connections between gut microbial functions and host metabolism remain poorly resolved, largely due to the scarcity of paired functional genomic and metabolomic data. By integrating concurrent metagenomics and metabolomics, we direct establish gene-to-pathway mappings within depression pathophysiology. The broader "amino acid biosynthesis" pathway showed stronger enrichment in metagenomics (higher LDA) but only a low rich factor in metabolomics, reflecting its limited cross-omics support and broad coverage of multiple sub-pathways. Therefore, in DS we focused on cysteine and methionine metabolism, which was consistently enriched in both omics layers, providing a more robust basis for mechanistic interpretation. The intersection of microbial and metabolic disruptions in cysteine/methionine pathways suggests that gut microbiota significantly influence redox and epigenetic balance during the first trimester of prenatal DS. Disorders in the methionine cycle, characterized by elevated homocysteine and altered S-adenosylmethionine, likely disrupts the methylation required for neurotransmitter synthesis, such as serotonin, and the regulation of stress-related genes.[38] Additionally, impaired cysteine metabolism may reduce glutathione synthesis, exacerbating oxidative stress and neuroinflammation.[39] Pregnancy heightens vulnerability due to elevated S-adenosylmethionine requirements for fetal development.[40] Hydrogen sulphide produced by the bacteria may have a dual effect on neuroinflammation.[41] This underscores dietary or probiotic therapies aimed at these pathways as viable therapeutic methods, positioning gut bacteria as metabolic regulators of early pregnancy DS pathogenesis and linking microbial dysbiosis to host methylome and redox abnormalities. In contrast, AS was characterized by enrichment of bile acid metabolism, despite three individual bile acid metabolites being similarly reduced in both AS and DS relative to HC. This suggests a more coordinated perturbation of bile acid-related metabolism specifically associated with AS. Pregnancy involves profound physiological changes in bile acid homeostasis, making pregnant women particularly vulnerable to bile acid dysregulation. Given their established roles in neuroinflammation, stress responses, and gut-brain signaling via nuclear receptors such as FXR and TGR5,[42] bile acids may contribute to the pathophysiology of prenatal anxiety. Thus, the observed enrichment of bile acid metabolism in AS may reflect a pregnancy-specific vulnerability that warrants further investigation.

The correlation network analysis showed that microbial, metabolic, and microbiome-metabolome connections possess more complex from HC to AS and DS, as reflected by stepwise increases in node and edge counts across all three network types. This finding corroborates the stress gradient hypothesis (SGH)—an evolutionary theory in ecology stating that environmental stress promotes increased facilitation, cooperation, and mutualism, shifting ecological interactions toward more positive relationships as stress intensifies.[43] Under mild stress (AS), the gut ecosystem (including microbiota and metabolomics) may initiate facilitative modifications, strengthen correlations, and marginally increase network complexity. Stress accumulation (DS) may induce maladaptive facilitation, resulting in the breakdown of modular boundaries and the formation of inefficient, hyperconnected networks that perpetuate metabolic dysfunction and dysbiosis. However, the divergent patterns of average degree reveal that AS promotes dense microbial hyper-coupling with declining microbial-metabolic coordination, whereas DS is characterized by sparse microbial expansion and loss of microbial-metabolic communication.

Our findings indicate that behavior-microbiome interactions play an important role in the DS. The combination of physical activity, diet, and gut microbiota data provided the most accurate

predictions of DS when using random forest modeling, with gut microbiota variables identified as the most influential predictors. Crucially, mediation analysis revealed that *Bifidobacterium pseudocatenulatum* and *Bifidobacterium catenulatum* contribute to the effects of sleep and standing. According to this crosstalk, DS is induced by behavioral triggers (such as sedentary behavior and shortage sleep) that disrupt host physiology via certain gut bacteria and maybe disrupted neuroinflammation or circadian rhythms. According to these findings, physical activity and their microbiological mediators are the primary targets of early prenatal DS intervention.

In contrast, metabolomic profiling outperformed all other indicators in predicting AS through RF analysis, demonstrating the importance of systemic metabolic dysregulation. This was confirmed by mediation analysis, which discovered 23 key pathways connecting the microbiome, metabolites, and anxiety, with two metabolites produced from the microbiota acting as critical mechanistic linkages. The polyphenol derivative 2′,4',6′-trihydroxyacetophenone attenuated anxiety pathways triggered by *Ruthenibacterium* sp. and *Congzhengia minguanensis*, most likely due to its anti-inflammatory and antioxidant qualities that regulate the hyperactivity of the hypothalamic-pituitary-adrenal axis.[44] While 3,4-dihydroxyphenylacetic acid-mediated anxiety effects through three bacterial taxa while also influencing depression.[45] This dual function is aligned with literature that 3,4-dihydroxyphenylacetic acid's capacity to boost synaptic dopamine, pass the blood-brain barrier, and reduce neuroinflammation.[45] Bacterial modulation of neuroactive metabolites is highlighted as the primary treatment pathway in this metabolite-centric pathogenesis. These findings address translational gaps by implicating human-specific microbial taxa and metabolites, thereby extending preclinical models to align with them.

In conclusion, we reveal distinct gut-brain axis mechanisms that underlying the subgroups of prenatal mental health. These preliminary insights suggest potential avenues for future research, including the possibility that dietary fiber interventions for AS, or modulation of microbiota-metabolite circuits for DS, could be explored as targeted approaches to support maternal and intergenerational mental health.

Limitations of the study

First, our cross-sectional technique precludes causal inference. To establish temporal correlations, longitudinal cohorts must track microbiota-metabolite dynamics throughout the pregnant trimesters. Second, the high comorbidity between AS and DS limited our ability to disentangle the distinct effects of each condition from shared symptomatology. Third, despite our cohort's multi-center design and one of the biggest combinations of objective physical activity data, metagenomics, and metabolomics, the sample size is still insufficient to make valid results. Future research should involve more diverse groups to completely address these potential confounding effects, as socioeconomic issues may also influence multi-omics outcomes. Fourth, interventional research is needed to determine the causal association between the onset of psychological illnesses during pregnancy and behavioral changes that target certain gut microbes.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Chenxi Cai (chenxicai@xmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The metagenomics data were deposited at the NGDC Genome Sequence Archive for Human (GSA for Human: HRA012641), and the metabolomics data were stored in the Open Archive for Miscellaneous Data (OMIX: OMIX01178). Accession numbers are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

C.C. and Zhengxiao Zhang conceived and designed the study. C.C., M.D., and Zhengxiao Zhang developed the methodology. N.L., D.Q., Zhongying Zhang., C.Z., C.X., S.J., X.J., S.D., X.L., Y.W., Y.Y., L.Z., H.L., M.Y., L.L., and C.F. performed project administration. K.G., X.Z., M.Y., C.F., and D.Q. conducted the statistical analysis. L.Z., M.H.D., Zhongying Zhang, N.L., C.Z., C.X., S.J., X.J., S.D., X.L., Y.W., H.L., L.L. and Y.Y. contributed to clinical interpretation and discussion. K.G., Zhengxiao Zhang, X.Z., N.L., and C.C. drafted the manuscript. All authors reviewed and edited the manuscript. C.C. and Zhengxiao Zhang acquired funding and provided supervision.

DECLARATION OF INTERESTS

The authors have no conflicts of interest to report. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human fecal samples (for DNA and metabolite extraction)	Chenxi Cai Lab, Xiamen University	N/A
Chemicals, peptides, and recombinant proteins		
FastPure Stool DNA Isolation Kit (YH-Feces)	MJYH	T10-100
TIANMicrobe Magnetic Envir-DNA Kit 4	Tiangen	DP713-T8
REPLI-g Single Cell Kit (96)	QIAGEN	150345
Critical commercial assays		
NEXTFLEX Rapid DNA-Seq Kit	Bioo Scientific	NOVA-5144
FastDNA™ Spin Kit for Soil	MP Biomedicals	6560–200
NovaSeq™ X Series 25B Reagent Kit (300 Cycle)	Illumina	20104706
Deposited data		
The raw data of metagenomics generated	This paper	NGDC Genome Sequence Archive for Human (GSA for Human): HRA012641
The raw data of metabolomics generated	This paper	Open Archive for Miscellaneous Data (OMIX): OMIX01178
Software and algorithms		
GraphPad Prism 10.1.2	GraphPad	https://www.graphpad.com
SPSS 4.4.1	IBM	https://www.ibm.com/products/spss-statistics
R 27.0	R	https://www.r-project.org
Gephi 10.1	Gephi	https://gephi.org

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The data for this study were obtained from 161 first-trimester pregnant women (under 14 gestational weeks) who participated in the Maternal & Young Child Health Outcome Study in Everyday Life (MyChose) cohort, a prospective, multi-center longitudinal study conducted in Xiamen and Zhangzhou, China. MyChose cohort investigates the behavioral effects on maternal-child health from early pregnancy to five years postpartum by assessing multiple-omics data and objectively measured lifestyle factors at critical developmental milestones. Inclusion criteria for the present study were enrolled in during first trimester (2022–2024), the availability of depression and anxiety assessment with dietary record, accelerometry data and fecal sample collected in first trimester (<14 weeks of gestation). Exclusion criteria included (i) having a diagnosis of psychotic illnesses (e.g., schizophrenia, bipolar or borderline personality disorder) prior to pregnancy; (ii) having diagnostic diseases (e.g., chronic inflammatory disorders, diabetes, cardiovascular disease, thyroid disease, or cancer) before this pregnancy; (iii) less than 18 years old; (iv) treated with antibiotics within 4 weeks prior to fecal sample collection.

METHOD DETAILS

Participants engaged in a face-to-face interview with research staff during gestational weeks 8 to 14 in the first visit to obtain information on pre-conception health history and lifestyle (i.e., physical activity, sedentary time, sleep, and diet). After that, the participants were asked to fill out early pregnancy assessments and provide samples, which included stool samples, a 3-day food diary with photos, 7 days of activity tracking, and mental health surveys, all done within the same week before they reached 14 weeks of pregnancy.

Depression and anxiety symptom assessment

Pregnant women completed the 10-item Edinburgh Postnatal Depression Scale (EPDS) and the 20-item State-Trait Anxiety Inventory (STAI). Both the EPDS and STAI measure current symptom levels, with higher scores indicating greater severity. A total score above

13 on the EPDS is typically considered to indicate depressive symptoms,[46] while a score above 40 on the state anxiety scale indicates high maternal state-anxiety.[47] Sampling adequacy for the EPDS and STAI questionnaires were assessed using the Kaiser-Meyer-Olkin (KMO). The reliability was assessed using the Cronbach's alpha coefficient.

Accelerometry measurements

The 24-hour movement behaviors were objectively assessed via the ActivPAL4 accelerometer (PAL Technologies Ltd., Glasgow, UK), which features an inclinometer to document free-living activities (sitting/lying, standing, stepping) throughout consecutive 24-hour periods. Participants received video instructions and comprehensive written guidelines. The devices were waterproofed by wrapping in a finger cot, then adhered to the right thigh using a Tegaderm patch (3M Company, Saint Paul, MN, USA) for 24 hours a day over a duration of 7 days. Participants were instructed to concurrently wear the device and maintain a diary documenting time spent in bed, wake and sleep durations, and periods of non-wear. Upon completion of the wear period, participants returned the monitor and diary via a pre-paid mailing envelope. Following our laboratory's implementation of standard activPAL processing protocols, data were downloaded using PALtechnologies software (v.7.2.38) and exported as event files, followed by diary-informed cleaning. Nonwear times were removed by trained researcher who followed standardized processes that combined participant diary logs. The outputs documented the duration spent in various body positions, including sitting time, standing time, and stepping time, quantified in 15-second increments. The total duration of sedentary behavior, light and moderate-vigorous intensity physical activity was determined based on the metabolic equivalents (METs) of the recorded movements. A minimum of four days with 24 hours of wear time was required to be deemed valid wear.

Dietary assessment

Participants documented all foods, beverages, and dietary supplements consumed over two weekdays and one weekend day using written notes, photographs, and weights with kitchen scales. They also recorded the nutritional labels of commercial products and cooking methods of meals. Researchers calculated daily nutrient and energy intakes for comparison and assessment by systematically categorizing reported consumption data and matching it with items in the Chinese Food Composition II (2019) database and USDA Foods Database.

Stool sample collection

Pregnant participants were provided with sterile fecal collection kits accompanied with detailed, illustrated instructions. Samples were either self-collected at home or collected in hospital by the researcher. All specimens were transported to the laboratory in insulated containers filled with ice within two hours of collection. Upon arrival, they were immediately aliquoted and stored at -80°C until subsequent analysis.

Fecal short chain fatty acids analysis

Six SCFAs (acetic, propionic, isobutyric, butyric, isovaleric and valeric acid) were determined by gas chromatography. Fecal samples, previously mixed with a 5% phosphoric acid solution at a 1:4 ratio and stored at -80°C, were thawed on ice. Samples were well mixed and centrifuged at 10,000 × g for five minutes to yield a clear supernatant following the addition of 1 mm zirconia beads. Subsequently, 200 μL of the supernatant was transferred to a new tube, mixed with 50 μL of internal standard solution (2-ethylbutyric acid, Sigma-109959), and subjected to three extractions with anhydrous diethyl ether. A 200 μL aliquot of the mixed ether extract was transferred into a chromatography vial for analysis. A gas chromatograph, Agilent 7890B-GC, equipped with a flame ionization detector (FID) and an HP-INNOWax capillary column (30 m × 0.32 mm internal diameter × 0.25 μm film thickness; Agilent), was utilized for quantitative analysis.

DNA extraction, fecal metagenomic sequencing analysis

DNA was isolated from fecal samples using the FastPure Stool DNA Isolation Kit (MJYH, Shanghai, China), following the manufacturer's guidelines specific to bacterial DNA. The DNA was fragmented into approximately 400 bp segments using the Covaris M220 instrument (Gene Company Limited, China). Paired-end libraries were constructed with the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA). Metagenomic sequencing was carried out on the Illumina NovaSeq platform (Illumina Inc., San Diego, CA, USA) following the NovaSeq Reagent Kits protocol provided by the manufacturer. The primary sequences underwent quality filtering using Fastp (version 0.20.0, https://github.com/OpenGene/fastp), preserving high-quality paired-end and single-end reads. The DNA sequences were then assembled using MEGAHIT (version 1.1.2, https://github.com/voutcn/megahit), leveraging the succinct de Bruijn graph approach. Contigs of at least 300 bp in length were selected as the final assembly results. Open reading frames (ORFs) in the contigs were predicted using Prodigal or MetaGene tools (http://metagene.cb.k.u-tokyo.ac.jp/). The predicted gene sequences were clustered for redundancy using CD-HIT (version 4.6.1, http://www.bioinformatics.org/cd-hit/). All high-quality reads were mapped to the non-redundant gene set using SOAPaligner (version 2.21, http://soap.genomics.org.cn/), and the abundance of genes in each sample was quantified. Taxonomic annotation of non-redundant genes was determined by aligning sequences against the NCBI NR database using DIAMOND (version 2.0.13, http://ab.inf.uni-tuebingen.de/software/diamond/). Functional annotations of KEGG were obtained. The raw sequencing data is deposited into the Sequence Read Archive (SRA) of NGDC (https://ngdc.cncb.ac.cn/gsub/submit/bioproject/subPRO063598/overview) under BioProject PRJCA043266.

Untargeted metabolomics analysis

Fecal samples weighing approximately 100 mg (±1 mg) were collected and combined with beads and 500 μL of extraction solvent composed of methanol, acetonitrile, and water in a 2:2:1 (v/v) ratio. The extraction solution also contained deuterated internal standards. The mixture was vortexed for 30 seconds before being homogenized at 35 Hz for 4 minutes and sonicated for 5 minutes in a 4 °C water bath, with this step repeated three times. To precipitate proteins, samples were incubated for 1 hour at -40°C, followed by centrifugation at 12,000 rpm (RCF = 13,800 × g; R = 8.6 cm) for 15 minutes at 4 °C. The supernatant was transferred to a new glass vial for analysis, and quality control (QC) samples were prepared by mixing equal aliquots of the supernatants from the samples. For the analysis of polar metabolites, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed using an ultra-high-performance liquid chromatography (UHPLC) system (Vanquish, Thermo Fisher Scientific) equipped with a Waters ACQUITY UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) and coupled to an Orbitrap Exploris 120 mass spectrometer. The mobile phase comprised 25 mmol/L ammonium acetate and 25% ammonia hydroxide in water (pH = 9.75) (solvent A) and acetonitrile (solvent B). The auto-sampler was maintained at 4 °C, and the injection volume was set at 2 μL. For non-polar metabolites, LC-MS/MS analysis was conducted using a similar UHPLC system (Vanquish, Thermo Fisher Scientific) with a Phenomenex Kinetex C18 column (2.1 mm × 100 mm, 2.6 μm), also connected to the Orbitrap Exploris 120 mass spectrometer. The mobile phase consisted of 0.01% acetic acid in water (eluent A) and a mixture of isopropanol and acetonitrile (1:1, v/v) (eluent B). The auto-sampler temperature was maintained at 4 °C, and an injection volume of 2 μL was used. Electrospray ionization (ESI) source conditions were adjusted as follows: sheath gas flow rate at 50 Arb, auxiliary gas flow rate at 15 Arb, capillary temperature at 320 °C, full MS resolution set at 60,000, and MS/MS resolution at 15,000. Collision energies were set to 20/30/40, with the spray voltage at 3.8 kV (positive mode) or -3.4 kV (negative mode). Raw data were subsequently processed with R based on the XCMS package for peak detection, extraction, alignment, and integration. Metabolite identification utilized the R package and BiotreeDB (V3.0).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were conducted using R and/or GraphPad Prism, unless stated otherwise. We employed the Mann-Whitney U test or t-test to assess the significance of the difference between two groups, with a p-value of <0.05 being significant. For multiple comparisons in LEfSe and KEGG pathway enrichment analyses, the False Discovery Rate (FDR) was applied to adjust for repeated testing.

For microbial diversity, metagenomic sequencing data were used to profile the gut microbiome. The 'vegan' package in R was used to compute α-diversity metrics from species abundance data. α-diversity indices were compared between the HC, AS, and DS groups using the Mann-Whitney U test. For β-diversity, Bray-Curtis distances were calculated and visualized using principal coordinates analysis (PCoA) with the 'ggplot2' package.[48] Differences in microbial community composition across groups were assessed using permutational multivariate analysis of variance (PERMANOVA; 9,999 permutations) implemented in the 'vegan' package.[48] Differential analysis of the microbiome and metabolomics datasets was performed using LEfSe across the three groups (HC, AS, and DS) with a non-parametric approach, focusing on features with a linear discriminant analysis (LDA) score greater than 2.[49] A KEGG pathway enrichment analysis of metabolomics (n=622 for AS and n=859 for DS) was conducted to identify significantly enriched pathways with a Fisher's test p-value threshold set at < 0.05.

Correlation analysis methods were employed using metagenomic sequencing data to study the relationship between the microbiome and metabolome, and their interconnections within different groups. The ''Hmisc'' package in R was used to analyze the connections between microbiome and metabolome data, focusing on details like nodes, edges, and weights.[50] Correlations with $p < 0.01$ and Spearman correlation coefficients with absolute values greater than 0.7 were screened and left behind. The network layout was visualized using ''Fruchterman Reingold'' in Gephi. Microbial nodes were visualized at the genus level, while metabolites were grouped into compound classes to improve readability. We subsequently extracted the subnetworks of microbiome of individual samples and calculated their topological features using the ''igraph'' and ''ggNetView'' packages. To assess between-group differences in network complexity, this analysis was performed only for microbiome data, as the method is specifically applicable to microbiota networks.

The efficacy of first-trimester multi-omics datasets in distinguishing maternal anxiety and depression was assessed by random forest classification models.[51] Recursive feature elimination, utilizing the R package Boruta, identified the optimal feature sets for each model, considering the large dimensionality and encompassing hundreds of features across datasets.[52] Essential attributes were identified by analyzing Gini impurity reduction, and model efficacy was evaluated using average receiver operating characteristic (ROC) curves and area under the curve (AUC), accuracy, and R2 across iterations.

We employed a straightforward mediation method to investigate the interplay between lifestyle, microbiota, and metabolomics. The ''Hmisc'' package in R was employed to determine pairwise correlations initially across lifestyles, gut microbiome, metabolome, and psychological state variables. Only variable pairs with significant correlations (Spearman, $p < 0.01$, $|r| > 0.7$) were retained for further mediation analysis. The ''mediation'' package was employed to examine mediation effects in significantly correlated data pairings.[53] Mediation analysis was performed using sequential regression models were needed to assess the relationship among lifestyle factors, microbiome, metabolomics and psychological outcomes. Two separate mediation models were constructed: (1) microbiome-metabolomics-psychological state, and (2) lifestyles-microbiome/metabolomics-psychological state. The importance of

the indirect (mediation) effect was assessed at $\alpha = 0.05$ (two-tailed) to ascertain the existence of the pathway, and only significant mediation pathways are reported.

ADDITIONAL RESOURCES

The study protocol was approved by the Human Research and Ethics Committee of Xiamen University in accordance with the Declaration of Helsinki (Ethic number: XDYX202211K39). All participants were provided with written informed consent.