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# Association Analysis Between Gut Microbiota Characteristics and Body Weight of Maritime Workers

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## Abstract

# Background

Overweight is highly prevalent among maritime workers due to occupational characteristics such as restricted dietary supply, disrupted circadian rhythms and limited physical activity. Gut microbiota acts as a key mediator linking dietary factors to host energy metabolism and weight regulation. This study aimed to investigate the association between gut microbial characteristics and body weight in maritime workers, so as to provide scientific evidence for nutritional intervention and weight management in this population.

# Methods

Twenty-four long-term maritime workers were divided into a normal weight group (n = 12) and an overweight group (n = 12) according to body mass index. Fecal samples were collected from all participants, and metagenomic sequencing and untargeted fecal metabolomics were performed to compare gut microbial composition and metabolic profiles between the two groups.

# Results

The overweight group showed significantly lower gut microbiota alpha diversity and distinct beta diversity compared with the normal weight group (all $P < 0.05$). The overweight group had higher relative abundances of *Escherichia coli* and *Blautia spp.*, and lower relative abundances of *Clostridiales, Bacteroides faecis* and *Ruminococcus faecis*. Metabolomic analysis revealed significant alterations in lipids and lipid-like molecules in the overweight group, with notable reductions in melatonin and its precursor molecules (all $P < 0.05$).

# Conclusion

Overweight maritime workers present gut microbiota dysbiosis and impaired nutrition-related metabolic pathways, which are closely associated with unreasonable dietary patterns and decreased sleep quality in this occupational population. These findings provide a theoretical basis for improving weight status through dietary regulation and gut microbiota modulation in maritime workers.

## 1 Introduction

Overweight is associated with a wide range of adverse health outcomes, including elevated risks of metabolic disorders, cardiovascular diseases, joint damage, as well as impaired respiratory and reproductive function[1]. According to recent epidemiological surveys in China, the overweight and obesity rates among adults have reached 34.3% and 16.4% respectively, accounting for over half of the

adult population. It is well established that the gut microbiota plays a crucial role in host health, particularly in the regulation of immune homeostasis, body weight and energy metabolism[2–3]. Dysbiosis (disturbances in the gut microbial ecosystem) is widely recognized as a major contributor to overweight and its related complications. Furthermore, imbalances in gut-derived metabolites can disrupt host energy homeostasis and promote the progression of overweight.

There is strong evidence that lean and overweight populations have distinct gut microbiota profiles, typically characterized by reduced Bacteroidetes and increased Firmicutes, resulting in an elevated Firmicutes-to-Bacteroidetes ratio in overweight individuals. These findings lay a scientific foundation for exploring the pathophysiological mechanisms of overweight and developing novel prevention and treatment strategies targeting gut microbiota[4]. Moreover, decreased gut microbial diversity and depletion of specific taxa are common in overweight populations, which may further exacerbate microbial dysbiosis and impair intestinal function[5].

The gut microbiota ferments indigestible dietary substrates into vital metabolites such as short-chain fatty acids (SCFAs) and bile acids. The metabolic activities of gut microbiota are diverse, including SCFA synthesis and protein fermentation. SCFAs are bioactive molecules that participate in weight management via activation of G protein-coupled receptors (GPRs), and can be utilized to promote energy expenditure and lipid catabolism[6–7]. Protein fermentation produces metabolites including ammonia and phenolic compounds; the accumulation of these substances may lead to weight gain by disturbing the balance between energy intake and expenditure, and may further affect host signal transduction and nutrient sensing[8]. These microbial metabolic products are critical for maintaining intestinal barrier function and overall metabolic health.

Given the close association among metabolite profiles, gut microbial alterations and overweight-related metabolic disorders, this study employed metagenomic and untargeted metabolomic approaches to systematically investigate the relationship between gut microbiota composition, metabolic pathways and overweight status in maritime workers. As a special occupational group with long-term offshore operations, maritime workers have unique nutritional and lifestyle characteristics such as restricted dietary supply, high-fat dietary patterns and disrupted circadian rhythms. This study will fill the research gap in this population and provide targeted scientific evidence for nutritional intervention and weight management of occupational groups.

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## 2 Materials and Methods

# 2.1 Study Design and Subjects

Personnel engaged in long-term maritime operations ( over a year) were chosen as the study subjects. 145 participants had their body mass index (BMI) determined. Twelve participants were randomly

selected among those with a normal BMI (BMI = 21.47 ± 1.47 kg/m²), forming the normal weight group. Another twelve individuals were selected at random from the group categorized as overweight (BMI = 29.19 ± 3.72 kg/m²) and assigned to the overweight group. All participants were informed of the study's procedures and potential risks and provided written consent. This study protocol was authorized by the PLA Naval Medical Center's Human Ethics Committee under approval number AF-HEC-017.

# 2.2 Materials

## 2.2.1 Metagenomics

Reagents: Key reagents used included the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA) for DNA extraction, agarose (Biowest, Spain) for electrophoresis, the NovaSeq Reagent Kit (Illumina, USA) for high-throughput sequencing, and the NEXTFLEX Rapid DNA-Seq Library Prep Kit (Bioo Scientific, USA) for library preparation.

Instruments:

Instrumentation comprised a Beckman GS-15R Centrifuge for sample processing, a SMA Microvolume Spectrophotometer (Meilin Hengtong, China) and a Turner BioSystems TBS380 Fluorometer for nucleic acid quantification, a Bio-Rad 164–5050 Electrophoresis system for quality control, a Tecan Infinite F50 Plate Reader for absorbance/fluorescence measurements, a Biometra Tone 96G Cycler for PCR amplification, and an Illumina HiSeq 2500 platform for sequencing.

## 2.2.2 Untargeted Metabolomics

Reagents:

Chromatography-grade methanol and MS-grade acetonitrile (both from Beijing Vokai) served as mobile phase components, supplemented with chromatography-grade formic acid (Tianjin Kemiou) for mobile phase modification.

Instruments:

Metabolomic analysis was performed using a Thermo Fisher UHPLC-Q Exactive HF-X Mass Spectrometer coupled to a Waters HSS T3 UHPLC Column. Sample preparation utilized a Wanbo TL-48R Homogenizer for tissue disruption, a Gene Company SBL-10DT Covaris M220 Sonicator for cell lysis, and a Beckman GS-15R Centrifuge for separation. Solvent handling involved a Heal NW10LVF Water System for purification, a Jingxin JXDC-20 Nitrogen Evaporator for gentle solvent removal, and a Sartorius Electronic Balance for precise measurements. Additional equipment included a Huamei LNG-T88 Concentrator for sample volume reduction and a Haier − 70°C Freezer for sample storage.

# 2.3 Procedures

## 2.3.1 Metagenomic Analysis

The study participants' fecal samples were used to extract genomic DNA and assessed using 1% agarose gel electrophoresis. The extracted genomic DNA was fragmented and prepared for the construction of paired-end libraries. This step was followed by amplification through bridge PCR and subsequent sequencing.

### 2.3.2 Untargeted Fecal Metabolomics

After homogenization (2 min, − 10°C, 60 Hz) and centrifugation (10 min, 13,000 rpm, 4°C), 400 µL of the supernatant was dried, reconstituted in 300 µL of methanol:water (1:4, v/v), vortexed, and centrifuged (10 min, 13,000 rpm, 4°C). 150 µL of the supernatant was transferred to an autosampler vial for analysis. QC samples were prepared by pooling equal aliquots.

## 2.4 Data Analysis

### 2.4.1 Metagenomic Data Analysis

For quality control, Fastp[9] was used to process raw sequencing reads. High-quality reads were assembled via MEGAHIT[10], and Prodigal[11] was used to predict open reading frames (ORFs). Using CD-HIT[12], all predicted gene sequences were clustered (≥ 90% sequence identity and coverage). SoAPaligner[13] was used to calculate gene abundance, and Diamond[14] was used to perform functional annotation against the KEGG database.

### 2.4.2 Untargeted Metabolomics Data Analysis

Raw data were processed in MSdial to construct a data matrix, extract peaks, identify compounds, and conduct statistical analyses for differential metabolite screening. METLIN and the Human Metabolome Database (HMDB) were consulted during the qualitative analysis. Peaks with > 50% missing values, identification scores < 30, or high standard deviation were excluded. Principal component (PCA) and orthogonal partial least squares discriminant (OPLS-DA) analyses were performed on the refined dataset using SIMCA-P 14.1. Seven-fold cross-validation assessed model robustness. When mapped to KEGG for pathway enrichment, differential metabolites were those with VIP > 1 in OPLS-DA and $p < 0.05$ in univariate t-tests. Key biological pathways were identified through enrichment analyses using Fisher's exact test and Python's scipy.stats[15].

# 3 Results

## 3.1 Gut Microbiota Diversity Assessment

When comparing the overweight cohort to the normal weight group, an analysis of alpha diversity indices showed a trend toward a decline in microbial diversity. The Shannon and Simpson indices, in particular, showed statistically significant decreases *($P < 0.05$*; Fig. 1A,B), suggesting a reduced gut microbial diversity among individuals who were overweight. According to Principal Component Analysis (PCA) and Analysis of Similarities (ANOSIM) at the species level, there were no discernible variations in the gut

microbiota composition between the normal weight and overweight groups (Fig. 1C,D). The Wilcoxon rank-sum test, however, indicated significant divergences in community structure between the two cohorts ($P < 0.05$; Fig. 1E).

## 3.2 Microbiota Composition and Differential Abundance

According to Venn diagram analysis at the species level, the overweight group had more unique microbial species than the normal weight group (4885 vs. 2149) (Fig. 2A). The gut microbiota profiles at the phylum and species levels were shown in detail in community bar plot visualizations. *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria* were the dominant phyla in both groups. The *Firmicutes* proportions were similar in both groups, but the overweight group had reduced *Bacteroidetes* and elevated *Proteobacteria* (Fig. 2B). At the species level, *unclassified Clostridium, Prevotella copri, Escherichia coli*, and *unclassified Lachnospiraceae* were prevalent, with the overweight group showing notably higher proportions of *E. coli* and *unclassified Lachnospiraceae* (Fig. 2C).

At the phylum level, differential abundance analysis revealed 25 phyla where the two groups varied significantly. Three phyla (*Platyhelminthes, Basidiomycota*, and the *provisional Curtisiaceae*) were more prevalent in the overweight group ($P < 0.05$), while 22—including *Verrucomicrobia, Melainabacteria, Euryarchaeota, Spirochaetes, Synergistetes, Chloroflexi*, and *Deferribacteres*—were less abundant ($P < 0.05$; Fig. 2D). At the species level, 1163 taxa revealed significant differences in abundance. The overweight group had higher abundances of *E. coli, unclassified Enterobacteriaceae, unclassified Proteobacteria*, and *Blautia spp.* ($P < 0.05$). On the contrary, *Clostridiales, Bacteroides faecis*, and *Ruminococcus faecis* were depleted ($P < 0.05$) in the overweight group when compared to the normal weight group (Fig. 2E).

## 3.3 Fecal Metabolite Profiling

Significant distinctions in the fecal metabolomic profiles of the two groups were found using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) of both cationic and anionic profiles, validated by permutation testing (Fig. 3A–D). 235 differential metabolites were found across both ionization modes when the selection criteria ($P < 0.05$, OPLS-DA VIP ≥ 1, FC = 1) were applied (Fig. 3E). 11 metabolites (9 of which were annotated) with VIP ≥ 3 and FC > 1.5 were of particular interest; all of these were downregulated in the overweight group. These included beta-Doradecin, D-urobilin, stercobilin, 14α-hydroxyhardwickiic acid, 9-pentadecenoic acid, 7,11-bisdeacetylpentyl 7-(3-methylvalerate) 11-(3-hydroxy-3-methylbutyrate), palmitoleic acid, phenylalanine, 6-hydroxyglutaric acid, glycyrrhizin A, and 5'-deoxy-5-fluorocytidine.

## 3.4 Metabolic Pathway and KEGG Enrichment Analyses

Classification of the 235 identified differential metabolites using the Human Metabolome Database (HMDB) revealed that nearly half were lipids and lipid-like molecules (Fig. 4A). 34 different metabolites were implicated in 46 metabolic pathways, according to Kyoto Encyclopedia of Genes and Genomes

(KEGG) pathway enrichment analysis. The most prevalent metabolic pathways were lipid metabolism, amino acid metabolism, and the metabolism of vitamins and cofactors (Fig. 4B). Tryptophan metabolism, steroid hormone biosynthesis, porphyrin and chlorophyll metabolism, and flavonoid biosynthesis were the four most significantly enriched pathways ($P < 0.01$; Fig. 4C). According to the KEGG topological analysis (Fig. 4D), the biosynthesis of steroid hormones and tryptophan metabolism were significantly enriched ($P < 0.01$). The metabolites melatonin, N-acetylserotonin, 4-(2-aminophenyl)-2,4-dioxobutanoic acid, and 2-formamidobenzoic acid were associated with the metabolism of tryptophan. Dehydroepiandrosterone, 11β,17α, 21-trihydroxypregnenolone, 19-oxosteroid, and 17α, 20α-dihydroxycholesterol were among the metabolites linked to steroid hormone biosynthesis; among these, dehydroepiandrosterone was a crucial node in the pathway topology (Fig. 4E). Melatonin and N-acetylserotonin were adjacent to each other in the pathway (Fig. 4F), indicating that there might be a metabolic perturbation involving these compounds may be present.

# 4 Discussion

The obese group had demonstrated a reduction in alpha diversity of gut microbial diversity and an increased abundance of some bacterial taxa. This reduced alpha diversity represents a decrease in the overall diversity of the gut microbiome, consistent with findings from Turnbaugh et al.[16]. Venn diagram comparisons revealed that the obese group contained more unique bacterial species. Furthermore, the heightened beta diversity in the overweight subjects indicates increased inter-individual heterogeneity in microbiota composition, which is indicative of lower reproducibility and greater complexity compared to normal-weight controls. This underscores the existence of a compromised gut microbial homeostasis as well as host-microbiota imbalance. Maintenance of this homeostasis is vital to host health and is intimately involved in the maintenance of health as well as disease pathogenesis[17–18]. Both animal and human data consistently link obesity with dysbiosis of the gut microbiota and overall reduced microbial diversity, implicating an imbalance of microbiota in triggering inflammatory pathways and metabolic derangement and thereby obesity's etiology and pathogenesis[19–20].

At the species level, a significant increase in the numbers of *Escherichia coli* in the obese group (P = 0.02) was observed. Woongjae Yoo et al.[21] suggests that a high-fat diet can inhibit the inhibition of respiratory electron acceptors by colonocytes, which would increase oxygen and nitrate in the gut and promote proliferation of *E. coli*. Elevated numbers of *E. coli* may then have a role in inducing adiposity, at least in part due to its Gram-negative cell wall component, lipopolysaccharide (LPS). Plasma LPS elevation has been causally linked to obesity and inflammation in animal models[22]. Cani PD et al.[23] determined that alteration of gut microbiota using antibiotics inhibited metabolic endotoxemia and reduced cecal LPS, resulting in reduced adiposity and reduced inflammatory markers in visceral adipose tissue. Collectively, these findings suggest that a high-fat diet would trigger E. coli cell growth and LPS liberation, thus playing a role in adipose tissue increase and inflammation in an obese individual. Targeting E. coli could be a promising method for preventing and treating obesity.

Relative to normal-weight subjects, overweight subjects had a lower percentage of *Bacteroidetes*, a higher percentage of *Proteobacteria*, and the same percentage of *Firmicutes*. These variations were not significant (*Bacteroidetes* P = 0.053, *Proteobacteria* P = 0.06). In concordance with Turnbaugh PJ et al.[24], *Bacteroidetes* were reduced and *Firmicutes* unchanged in obese cohorts, but the anticipated overrepresentation of *Actinobacteria* was not observed, perhaps due to constraints of sample size. Walters WA et al.[25] proposed that the *Firmicutes*/*Bacteroidetes* ratio is not an accurate biomarker due to the influence of diet, methodology, and other confounders. Of particular interest is the increased abundance of *Proteobacteria* in overweight subjects since this phylum is sensitive to environmental perturbations and its dominance can signal gut dysbiosis[26]. Safari Z et al.[27] also observed *Proteobacteria* enrichment as a feature of dysbiosis in obese subjects.

Decreased amounts of *Faecalibacterium prausnitzii* and *Roseburia faecis* were also reported among overweight subjects, as evidenced by large-scale studies that have indicated F. prausnitzii as an obesity marker[28]. Similar reductions were observed by Sinisterra-Loaiza L et al.[29], who also recorded a positive correlation between these bacteria and recommended dietary fiber.

Untargeted metabolomic analysis of fecal samples determined that nearly half of the differential metabolites were of the lipid and lipid-like molecules, indicating that overweight status is associated with gut lipid and lipid-related metabolic alterations. KEGG pathway enrichment analysis also determined significant enrichment in tryptophan metabolism, wherein four metabolites had significant differences. Of special note were melatonin and N-acetylserotonin, which are sequential in the metabolic pathway, in the obese group, indicating a change in these metabolic processes. N-acetylserotonin is an endogenous intermediate of melatonin biosynthesis from serotonin.

While melatonin is prominently recognized for its sleep-pacing mechanism, more and more evidence connects melatonin with overweight and obesity. Deng Xiaojian et al.[30] demonstrated that intraperitoneal injection with melatonin in high-sugar and high-fat diet-induced obese SD rats reduced body weight by 16% and abdominal fat mass by 50%. Melatonin oral supplementation has also been reported to reduce waist circumference, decrease plasma triglycerides, and enhance metabolic syndrome[31]. Nuttapong Yawoot et al.[32] revealed that melatonin effectively inhibits adipose tissue inflammation through inhibition of adipocyte apoptosis, immune cell invasion, and the production of pro-inflammatory adipokines.

Other studies reveal that melatonin enhances intracellular and exosomal α-ketoglutarate in adipose tissue to control macrophage invasion and NF-κB activation, preventing adipose inflammation during obesity[33]. But because this study was cross-sectional, one cannot deduce the temporal relationship between overweight, sleep disturbances, and melatonin metabolism. Further research must elucidate the interaction between sleep, overweight, and melatonin metabolism.

KEGG pathway analysis also identified dehydroepiandrosterone (DHEA) as one of the differential metabolites with significantly reduced fecal levels in the overweight group (P = 0.02). Contrary to

previous proposals that DHEA supplementation induces fat loss, meta-analytic evidence indicates that reported reductions in fat mass are more strongly associated with elevated circulating sex hormones than with DHEA itself [34], which undermines its value as a standalone biomarker of overweight.

## 5 Conclusion

In conclusion, this study confirms that obese offshore workers possess distinct gut microbiota profiles with lower beneficial taxa, higher potentially pathogenic bacteria, and impaired lipid metabolisms, including those related to melatonin. These results highlight the importance of dietary and sleep-targeting interventions in this workforce for overweight risk reduction.

## Declarations

# Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Human Ethics Committee of PLA Naval Medical Center (Approval No. AF-HEC-017). All subjects provided written informed consent after full disclosure of the study procedures, potential risks and benefits.

# Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Generative AI

The authors declare that no Gen AI was used in the creation of this manuscript.

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# Author Contribution

CZ: Conceptualization, Funding acquisition, Validation, Writing - original draft. QS: Conceptualization, Data curation, Validation, Writing - original draft. SX: Data curation, Visualization, Writing - original draft. DW: Software, Writing - original draft. DanL: Project administration, Writing - review & editing. DongL: Project administration, Writing - review & editing. FW: Supervision, Writing - review & editing.

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# Data Availability

The datasets presented in this article are not readily available because of confidentiality regulations imposed by the Institute of Food Science and Technology. Requests to access the datasets should be directed to the corresponding authors.

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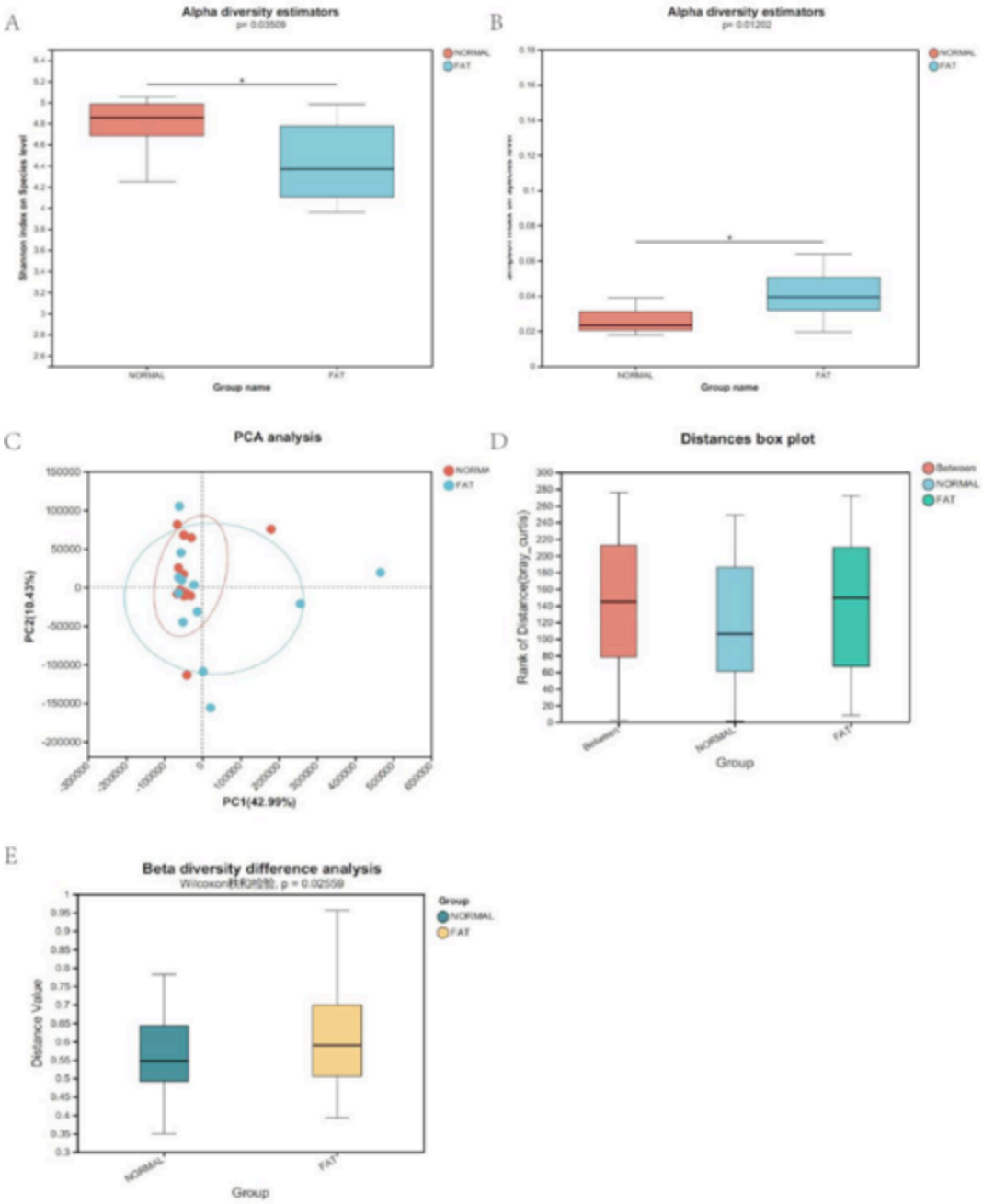
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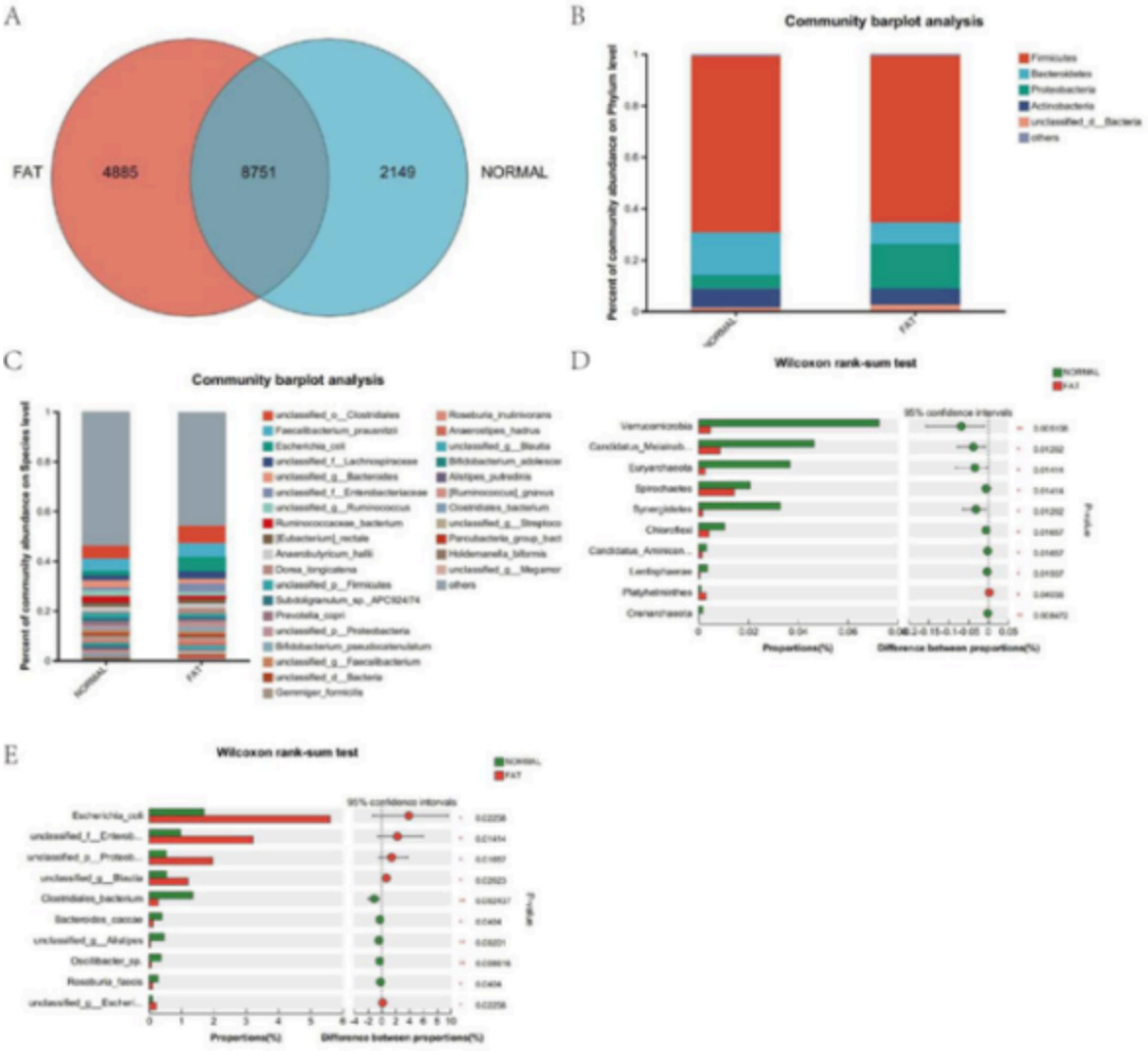
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# Figures



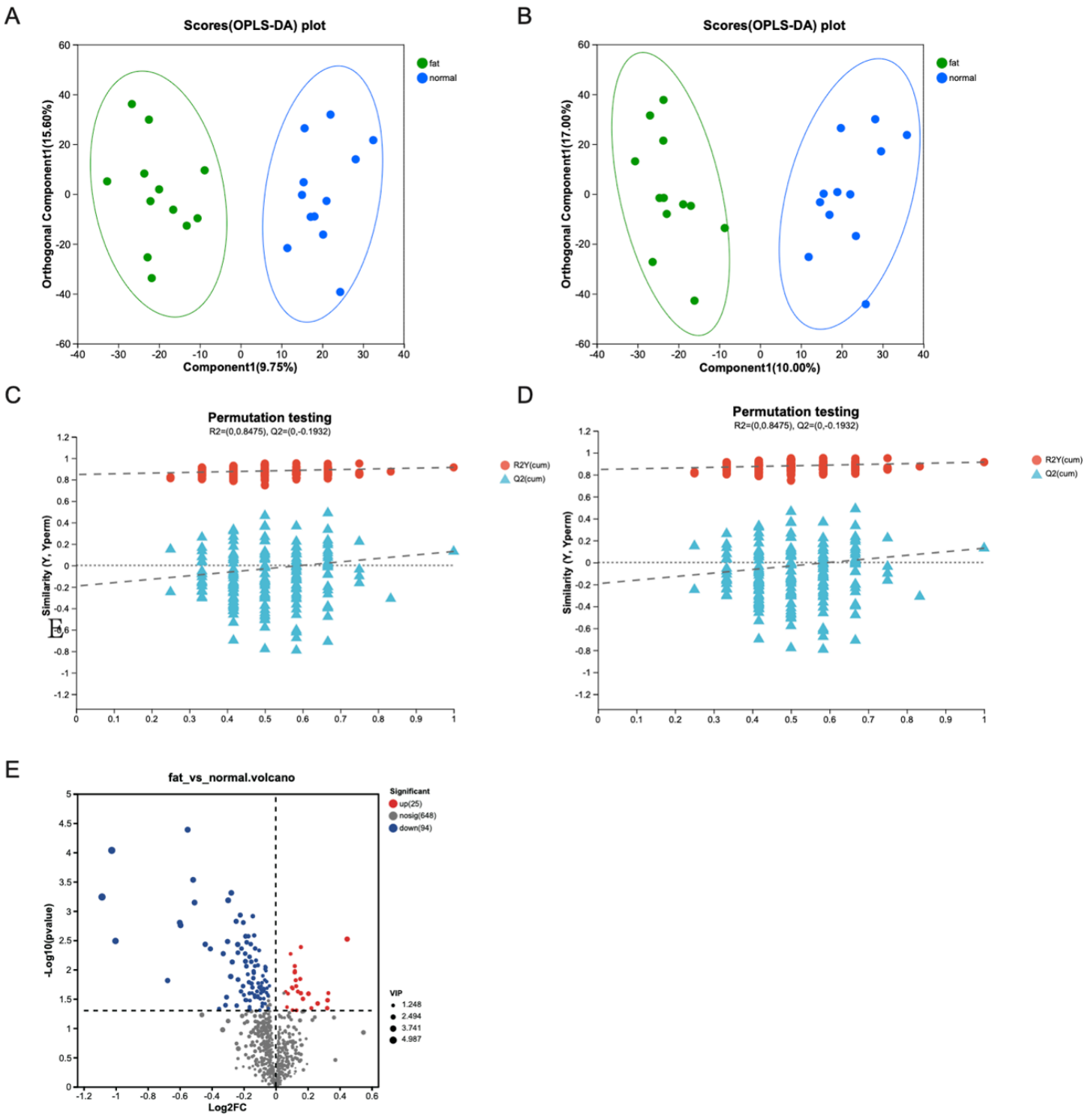
## Figure 1

Analysis of Gut Microbiota Diversity. A. Shannon diversity index; B. Simpson diversity index; C. Principal Component Analysis (PCA) plot; D. Analysis of Similarities (ANOSIM) boxplot; E. Boxplot of beta diversity differences.




## Figure 2

Analysis of Gut Microbiota Composition and Differences. A. Venn diagram; B. Bar plot of community composition at the genus level; C. Bar plot of community composition at the species level; D. Bar plot of differential analysis at the phylum level; E. Bar plot of differential analysis at the species level.




## Figure 3

Characteristics of Fecal Metabolites. A. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) of cationic metabolites; B. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) of anionic metabolites; C. Permutation test for cationic metabolites; D. Permutation test for anionic metabolites; E. Volcano plot of differential metabolites.

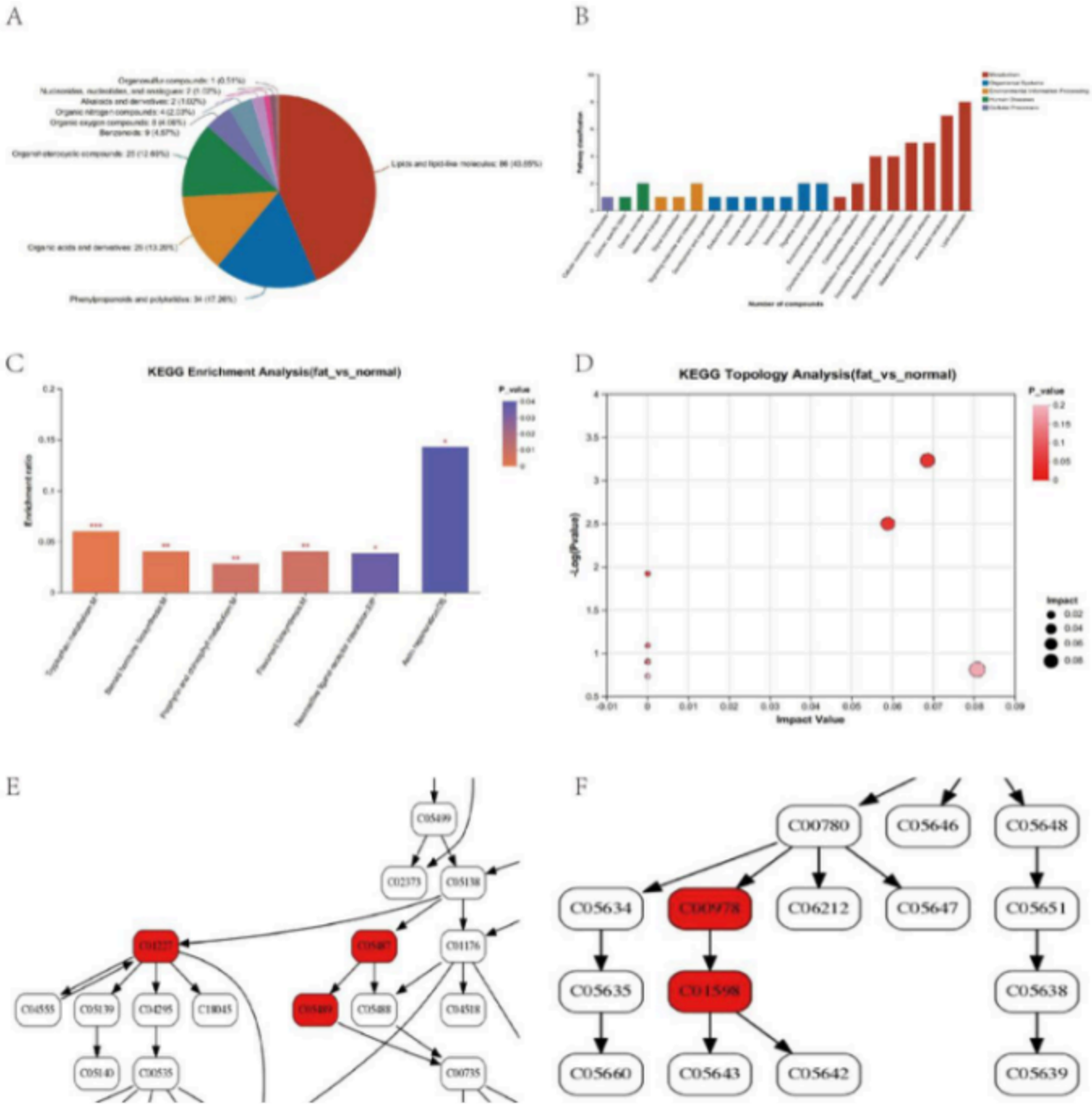



**Figure 4**

Analysis of Differential Metabolites and Metabolic Pathways. A. HMDB classification of differential metabolites; B. KEGG pathway statistics plot; C. KEGG enrichment analysis plot; D. KEGG topological analysis bubble plot; E. Partial KEGG topological pathway map of steroid hormone biosynthesis; F. Partial KEGG topological pathway map of tryptophan metabolism.