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# Growth hormone attenuates obesity and reshapes gut microbiota in high-fat diet-fed mice

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ABSTRACT

Growth hormone (GH) and gut microbiota are key regulators of metabolism and have been linked to the development and treatment of obesity. Although variations in GH levels are associated with changes in gut microbiota composition, the specific effects of GH on gut microbiota and its role in obesity remain unclear. This study explored the effects of various GH doses (0.25, 0.75 and 1.5 IU/kg) on adipose tissue mass and gut microbiota in high-fat diet-induced obese mice. Notably, high-dose GH (1.5 IU/kg) significantly reduced the adipose tissue mass. This dose also reversed high-fat diet-induced gut microbiota dysbiosis, restoring microbial diversity and increasing the abundance of beneficial genera such as *Ruminococcaceae* and *Muribaculaceae*. Additionally, high-dose GH normalized several obesity-related gut microbiota pathways, including starch and sucrose metabolism, galactose metabolism, and secondary bile acid biosynthesis. GH therapy also improved intestinal barrier function, a key determinant of gut microbial homeostasis. These findings underscore the therapeutic potential of GH in obesity management through its effects on gut microbiota, providing new avenues for obesity interventions.

## 1. Introduction

Obesity, a pervasive global health crisis, poses significant threats to human well-being by increasing the risk of cardiovascular diseases, type 2 diabetes, certain cancers, and fatty liver [1]. These outcomes reduce both quality of life and lifespan, highlighting the urgent need for understanding the mechanisms of obesity and developing effective interventions.

Growth hormone (GH), a 191-amino acid polypeptide secreted by the pituitary gland, plays a crucial role in regulating growth, metabolism, and immunity by binding to its membrane receptor (GHR) [2,3]. GH exerts anabolic effects on muscle and lipolytic effects on white adipose tissue [4]. Dysregulated GH secretion is associated with obesity, as shown by moderate obesity in GH-deficient adults [5] and a strong inverse relationship between GH levels and visceral fat in healthy individuals [6,7]. Clinical trials have further demonstrated GH therapy's ability to reduce visceral and total adipose tissue while improving obesity-related cardiovascular and metabolic complications [8–11], positioning GH as a potential therapeutic option for obesity management.

Maintaining homeostasis between the host and its gut microbiota is crucial for preserving host health and guy dysbiosis has been linked to obesity and metabolic disorders [12]. The gut microbiota also mediates the metabolic improvements from pharmacological interventions. For

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example, specific beneficial bacterial species, such as *Bifidobacterium* spp. and *Akkermansia muciniphila*, have shown promise in ameliorating obesity [13], highlighting their potential as therapeutic targets. Moreover, the effects of metformin on thermogenesis are gut microbiota-dependent via activation of intestinal AMPK [14], further emphasizing the microbiota's role in pharmacological treatments. Although GH's influence in regulating immunity and metabolism-key factors in shaping gut microbiota is known, its role in gut microbiota modulation remains unclear. Significant changes in gut microbiota have been observed in both GH transgenic and GH gene-disrupted mice [15,16], and prior research has shown that hepatic GHR disruption alters gut microbiota via bile acid metabolism [17]. Nevertheless, the impact of GH therapy on the gut microbiota in obese individuals remains to be fully understood.

This study examined the effects of varying GH doses on adipose tissue mass and gut microbiota in high-fat diet (HFD)-induced obese mice. Our findings suggest that GH reduces fat mass by modulating the gut microbiota, offering insights into GH's therapeutical potential in managing metabolic diseases.

## 2. Materials and methods

### 2.1. Animals and experimental design

Four-week-old male C57BL/6 J mice, obtained from GemPharmatech (Nanjing, China), were housed under controlled environmental conditions (12-h light/dark cycle) with free access to regular chow (RC) or high-fat diet (HFD; 45 % fat, MD12032, Medicience, Yangzhou, China) and sterile water. After 16 weeks of HFD, mice were administered recombinant human growth hormone (rhGH, AnkeBio Co., Ltd, Anhui, China) via intraperitoneal injections once daily for four weeks at low (0.25 IU/kg, HFD_GHL), medium (0.75 IU/kg, HFD_GHM), and high (1.5 IU/kg, HFD_GHH) doses, respectively (Fig. 1A). Saline-treated high-fat diet (HFD) group was served as a negative control, while the RC group was used as a baseline control. To account for the circadian rhythm of endogenous GH secretion, all rhGH injections were administered at the same time each day. Each experimental group contained five mice. At the end of the treatment period, mice were euthanized by intraperitoneal injection of Avertin (20 μL/g). All experiments were conducted in accordance with the guidelines for the treatment of laboratory animals and approved by the Committee on the Ethics of Animal Experiments of Shandong Provincial Hospital Affiliated to Shandong First Medical University & Shandong Academy of Medical Sciences.

### 2.2. Sample collection and processing

Body composition (lean mass, fat mass and body fluids) was measured using a Bruker Minispec LF90 II TD-NMR body composition analyzer (Bruker Optics, Inc., Billerica, MA). White adipose tissues located in the epididymal, mesenteric, perinephric and inguinal regions, brown adipose tissue (BAT) from the subscapular region and intestine samples were dissected, weighed, and either fixed in 4 % paraformaldehyde for subsequent histological analysis or snap-frozen in liquid nitrogen and stored at −80 °C for further studies. Cecal contents were collected, immediately snap-frozen, and stored at −80 °C for gut microbiota analysis.

### 2.3. Measurement of serum GH level

Blood samples were collected by retro-orbital bleeding, and serum was separated from the whole blood by centrifugation at1000 g for 15 min. Serum GH concentration was measured using mouse GH ELISA kit (MEIMIAN, Yangcheng, China), according to the manufacturer's instructions.

### 2.4. Histological analysis

Fresh tissues fixed in 4 % paraformaldehyde for 24 h were sectioned (5 μm), embeded in paraffin, and then stained with H&E. The digital images were captured with a digital pathology slide scanner (KFBIO, Ningbo, China).

### 2.5. Immunohistochemistry

The sections were incubated with 0.3 % hydrogen peroxide in the dark at room temperature for 25 min to inhibit endogenous peroxidase activity, then incubated with goat serum for 30 min at room temperature, with the respective primary antibodies overnight at 4 °C, and with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody for 50 min at room temperature. DAB staining was used for visualization. The primary antibodies for immunohistochemical analysis were Occludin, Claudin-1 and ZO-1 respectively (Proteintech, Wuhan, China).

### 2.6. Western blot analysis

Total protein was extracted from colon tissue samples using RIPA lysis buffer (Seven, Beijing, China) supplemented with a protease inhibitor cocktail (Seven). Protein concentration was measured with BCA protein assay kit (Epizyme Biotechnology, Shanghai, China). Proteins were separated by 10 % SDS-PAGE and transferred onto nitrocellulose (NC) membranes (Solarbio, Beijing, China). The NC membranes were blocked with rapid blocking solution (Seven) at room temperature for 1 h, incubated with primary antibodies overnight at 4 °C, including Anti-β-Actin (ABclonal, Wuhan, China), Anti-Occludin (Proteintech), and Anti-Claudin-1 (Proteintech), and with a secondary antibody (rabbit IgG HRP, ABclonal) at room temperature for 1 h. Protein bands were visualized using an electrochemiluminescence system (ClinX, Shanghai, China).

### 2.7. Analysis of the bacterial community in cecal content

Total DNA from cecal content was extracted, and the hypervariable V3–V4 region of 16 S rRNA gene was amplified with the universal primers: 343 F (5′-TACGGRAGGCAGCAG-3′) and 798 R (5′-AGGGTATCTAATCCT-3′) and sequenced using the Illumina NextSeq 2000 platform by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). The paired-end raw sequencing data were processed in QIIME2 [18] for diversity and taxonomic composition analysis. In brief, the paired-end reads were denoised and stitched by DADA2 after demultiplexing and quality examination, resulting in an amplicon sequence variant (ASV) table and the representative sequences. To avoid the bias resulted from different sequencing depths in the diversity analysis, the ASV table was subsampled based on the minimum count observed across all samples. The α- and β-diversity analyses were conducted on the normalized ASV table. Permutation on a multivariate analysis of variance (PERMANOVA) was applied to test the statistical significance of the differences between groups. Taxonomical classification was performed to analyze the relative abundance of bacterial species in different groups. The taxonomy was initially assigned to the representative sequences using a classifier trained on the Silva 138 99 % 16 S reference, after which the taxonomic composition of the bacterial community in each sample was analyzed based on the ASV table. Taxonomic composition was visualized using stacked bar charts and a heatmap at the phylum and genus levels. These were plotted using the R software packages ggplot 2 and pheatmap.

### 2.8. Prediction of the bacterial community function

The functional capabilities of bacterial community were evaluated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) pipeline [19]. Specifically, the ASV

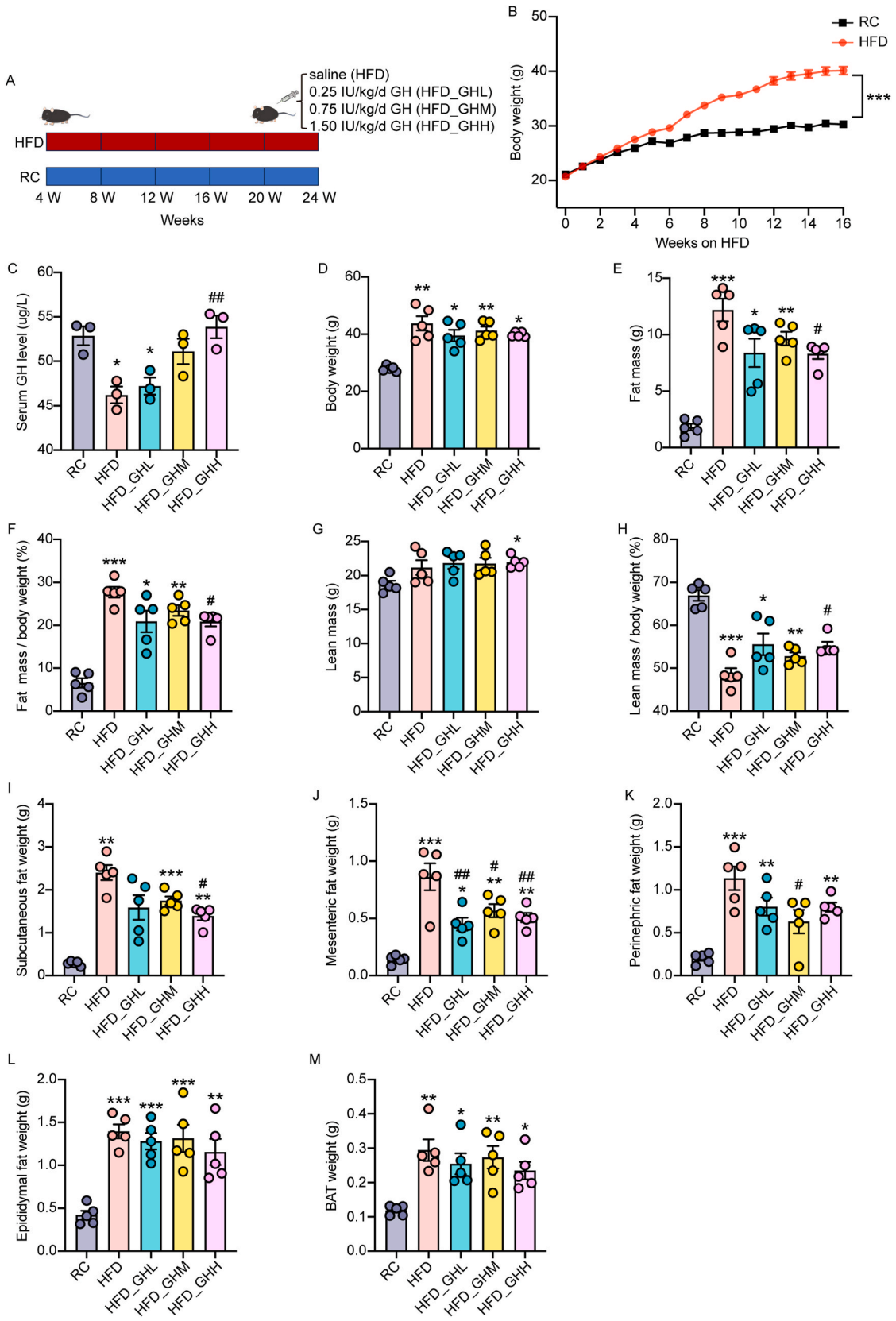


**Fig. 1.** Experimental design and effects of GH on body weight and fat mass. **A:** Scheme of the treatments of the mice. **B:** Growth curve of the RC and HFD treated mice. **C:** Change of serum GH level and HFD induction and GH treatment. **D-M:** Effects of different doses of GH on body weight (D), fat mass (E), ratio of fat mass to body weight (F), lean mass (G), ratio of lean mass to body weight (H), subcutaneous fat mass (I), mesenteric fat mass (J), perinephric fat mass (K), epididymal fat mass (L), and brown adipose tissue (BAT) mass (M). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

table generated by QIIME2 was used as input for PICRUSt2. This approach allowed for the prediction of functional profiles, which were subsequently annotated against two comprehensive databases: the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Clusters of Orthologous Groups (COG). By mapping the predicted functional gene families to KEGG Orthology (KO) identifiers and COG categories, we were able to facilitate the interpretation of microbial metabolic pathways and gene functions, thereby providing a comprehensive understanding of the functional potential of the gut microbiota.

### 2.9. Statistical analyses

Pairwise Spearman's correlation coefficients between metabolic phenotypes and bacterial community diversity indices or genus abundance were computed using the "psych" package in R software. The resulting correlation matrices were then visualized with the "pheatmap" package.

All values are shown as mean ± SEM. Statistical analyses were conducted using SPSS version 26.0. The Shapiro-Wilk normality test was employed to assess the normal distribution of the data. For comparisons between two groups, Student's t-test was used for normally distributed variables, while the Mann-Whitney *U* test was applied to non-normally distributed data. In the case of multiple group comparisons, one-way ANOVA with Tukey's post-hoc test (for equal variances) or Dunnett's T3 test (for unequal variances) was utilized for normally distributed variables, and the Kruskal-Wallis test was employed for non-normally distributed data. A significance level of $P < 0.05$ was set for all tests.

## 3. Results

### 3.1. GH reduces the adipose tissue mass in HFD-treated mice

HFD-induced obesity was employed to assess the impact of GH on body weight and adipose tissue mass in mice. As shown in Fig. 1B, the body weight of HFD-fed mice significantly increased compared to the control group fed regular chow (RC), and the serum GH concentration was significantly reduced (Fig. 1C). Following GH administration, serum GH levels increased in a dose-dependent manner and were restored to levels comparable to the RC group in the HFD_GHM and HFD_GHH groups. Despite this, no notable changes in body weight were observed across the HFD-treated groups and the GH-treated groups after four weeks of GH treatment (Fig. 1D). However, GH therapy resulted in a significant reduction in fat mass and the fat mass-to-body weight ratio, particularly in the HFD_GHH group, where reductions of 31.8 % and 25.3 % were observed compared to the HFD group (Fig. 1E and F). Lean mass remained unaffected across all groups, with no significant difference between RC and HFD groups or among the GH-treated HFD groups (Fig. 1G). However, the ratio of lean mass to body weight, which was lower in the HFD group compared to the RC group, was restored to the normal levels in the HFD_GHH group (Fig. 1G). Additionally, the influence of GH on adipose tissue in distinct anatomical locations was assessed. The weights of subcutaneous, mesenteric, perinephric, and epididymal adipose tissues, as well as brown adipose tissue (BAT), were found to be 2.33, 5.14, 4.70, 7.28, and 1.42 times greater, respectively, in the high-fat diet (HFD) group compared to the regular chow (RC) group (Fig. 1I–M). While adipose tissue weights remained higher in all treatment groups compared to the RC group, GH therapy, particularly at higher doses, significantly reduced subcutaneous fat weight by 42.1 % in the HFD_GHH group compared to the HFD group. Similarly, mesenteric fat weight was reduced by 47.9 %, 34.3 %, and 41.2 % in the HFD_GHL, HFD_GHM, and HFD_GHH groups, respectively, compared to the HFD group (Fig. 1I and J). However, no significant reduction in the weight of epididymal fat or BAT was observed (Fig. 1L–M). Histological analysis using H&E staining further confirmed that the adipocyte size in the subcutaneous fat was markedly enlarged in HFD-fed mice compared to RC-fed controls. Importantly, GH therapy significantly decreased adipocyte size in all treatment, demonstrating its impact on adipocyte morphology (Fig. 2).

### 3.2. GH improves the intestinal barrier impaired by HFD

The intestinal barrier plays a key role in protecting epithelial cells against microbial invasion, particularly in the large intestine, where microbial density is highest. In HFD-fed mice, the crypt depth of the colon was significantly increased compared to the RC group. GH administration at all three doses reduced the crypt depth induced by HFD (Fig. 3A and B). This suggests that GH may help restore intestinal architecture disrupted by HFD. Next, the expression of tight junction proteins, such as Occludin and Claudin-1, was evaluated via Western blot analysis (Fig. 3C). The protein levels of both Occludin and Claudin-1 were diminished in the HFD group. Notably, GH therapy, particularly at the middle (0.75 IU/kg) and high doses (1.5 IU/kg), restored these protein levels. Immunohistochemical analysis further supported these findings, showing that Occludin, Claudin-1, and ZO-1 protein levels, which were diminished by the HFD, were significantly elevated in the middle- and high-doses of GH-treated groups (Fig. 3D).

Thus, GH therapy improved the integrity of the intestinal barrier by restoring tight junction proteins.

### 3.3. GH reverses α- and β-diversity of gut microbiota impaired by HFD

The diversity of gut microbiota, an essential factor in maintaining intestinal and metabolic health, was also assessed in terms of α- and β-diversity. α-Diversity, which reflects how rich and diverse the bacterial population is within a single sample, was measured using multiple indices. The Chao1 index shows the total richness (or number) of different bacterial species, and the Faith_PD index measures the genetic variety within the microbial community. Both the Chao1 index and the Faith_PD index were significantly reduced in the HFD group, indicating a loss of microbial richness and phylogenetic diversity due to HFD (Fig. 4A and B). GH treatment led to a dose-dependent increase in these mice, with the highest dose (1.5 IU/kg) significantly restoring the Chao 1 and Faith PD indices to levels comparable to those in the RC group (Fig. 4A and B). This suggests that GH treatment can help recover the diversity of the gut microbiota, which is important because diverse microbial communities are generally linked to better gut health and metabolic function. The other diversity indices we used (Pielou_e, Shannon, and Simpson), which measure the evenness and general diversity of bacterial populations, did not show significant changes with GH treatment (Fig. 4C–E). Correlation analysis showed that the Chao1 and Faith_PD indices negatively correlated with subcutaneous and perinephric fat mass and the overall fat ratio, while they were positively correlated with the lean mass ratio (Fig. 4F). This suggests that increased microbial diversity is associated with healthier body composition.

β-Diversity, which compares microbial differences between samples, was analyzed using partial least squares discriminant analysis (PLS-DA). The results revealed distinct clustering of the bacterial communities among the samples, with samples from the HFD group being separated from the RC and HFD_GHH groups but not from the HFD_GHL and HFD_GHM groups (Fig. 4G), indicating significant shifts in the gut microbial community due to the high-fat diet. The PERMANOVA test confirmed these differences, showing significant differences in the bacterial community structure between the RC group and the HFD, HFD_GHL, and HFD_GHM groups ($P < 0.05$) but not between the RC and HFD_GHH group (Table 1). Moreover, the bacterial community in the HFD group was significantly different from that in the HFD_GHH group, but not from those in the HFD_GHL and HFD_GHM groups. The F value, indicating the dissimilarity between groups, was highest between the RC and HFD groups and decreased progressively with increasing GH doses, indicating that high-dose GH treatment was able to restore the gut microbiota composition to a level similar to that of the RC group.

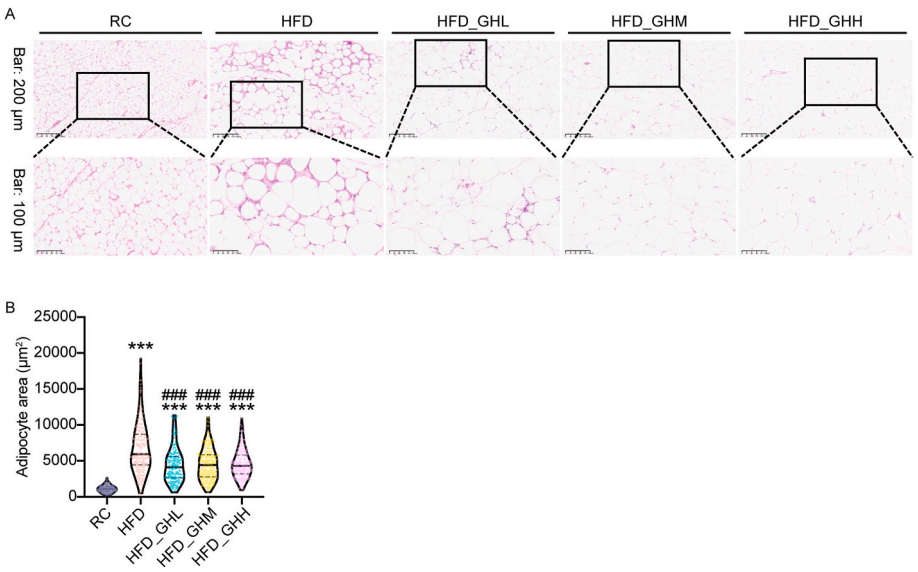


**Fig. 2.** Change of subcutaneous adipocyte under treatment of different doses of GH. **A:** H&E staining of the subcutaneous adipose tissue. **B:** Quantification of adipocyte area of subcutaneous adipose tissue. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group.

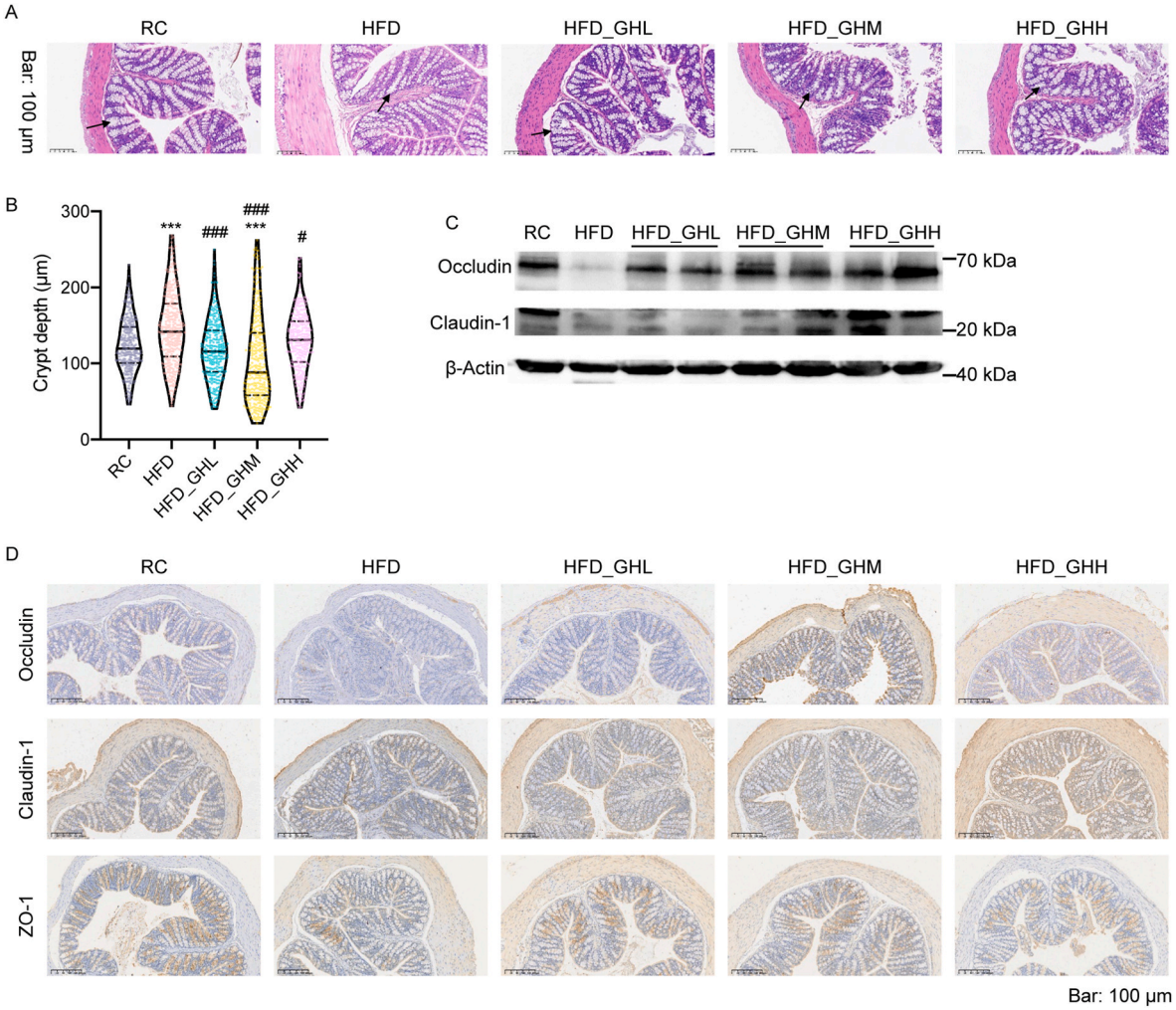


**Fig. 3.** Effects of GH on gut barrier. **A:** H&E staining of the colon. The arrows indicate the crypts. **B:** Quantification of crypt depth. **C:** Analysis of Occludin and Claudin-1 protein levels by Western blot. **D:** Analysis of the express level of Occluding, Claudin-1 and ZO-1 by immunohistochemistry. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group.

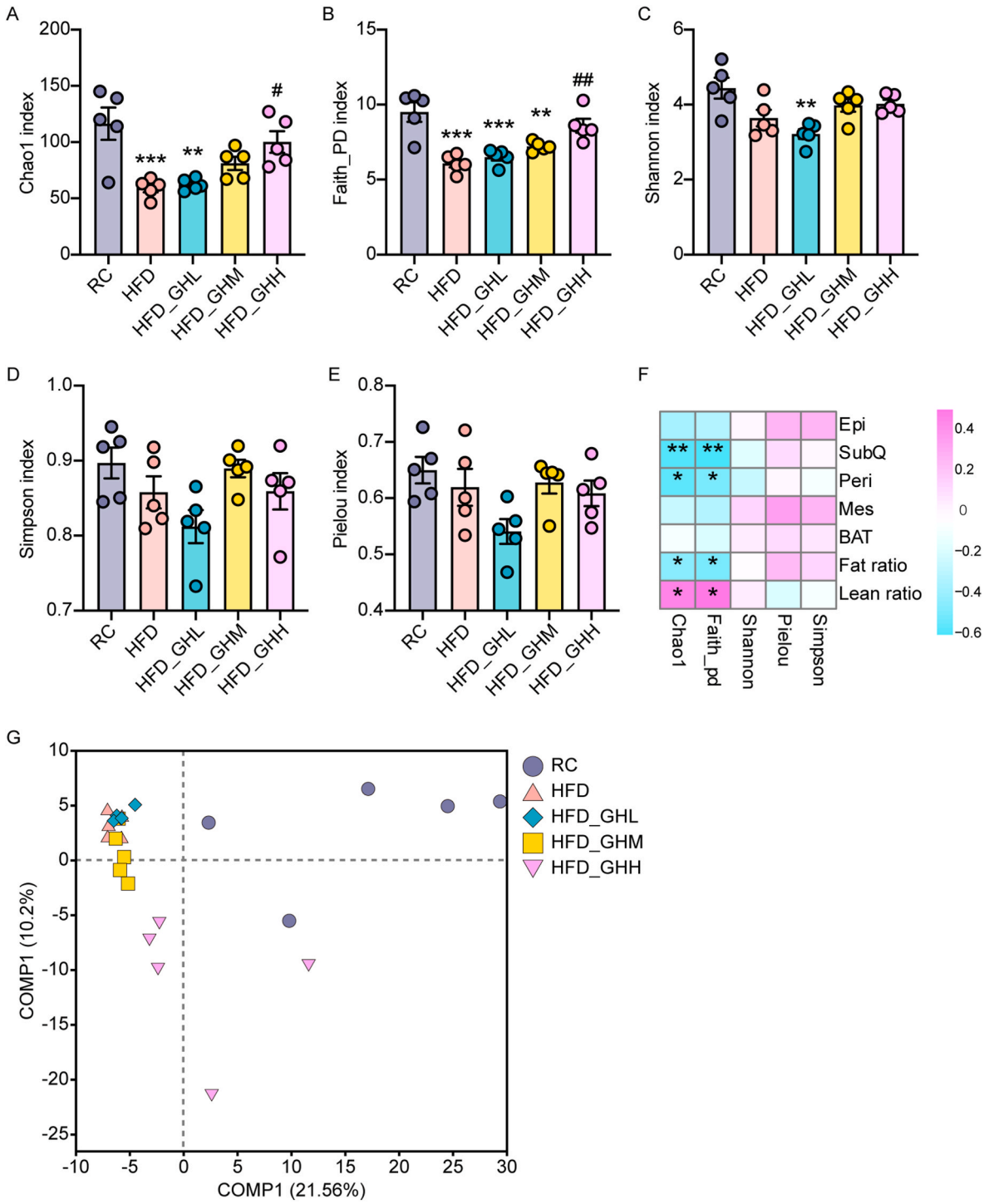


**Fig. 4.** Analysis of the α-diversity and beta-diversity of gut bacterial community. **A-E:** α-diversity indices. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group. **F:** Correlation between the α-diversity indices and obesity related phenotypes. Epi, epididymal white adipose tissue; SubQ, subcutaneous white adipose tissue; Peri, perinephric white adipose tissue; Mes, mesenteric white adipose tissue; BAT, brown adipose tissue. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. **G:** PLS-DA plot of bacterial communities in different samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### *3.4. GH reshapes taxonomic composition of gut microbiota disturbed by HFD*

The taxonomic composition variations within the bacterial community were analyzed at both the phylum and genus levels. At the phylum level, eight distinct phyla were identified across all groups, with *Firmicutes*, *Actinobacteriota, Proteobacteria*, *Verrucomicrobia* and *Bacteroidota* being the dominant phyla in the RC group (Fig. 5A). Significant differences were observed in three major phyla (Fig. 5B). *Firmicutes* abundance was notably higher in the HFD group compared to the RC group. However, in the high-dose GH-treated group (HFD_GHH), the abundance of *Firmicutes* was significantly reduced, suggesting a potential reversal of the HFD-induced shift. As for *Bacteroidota* and *Proteobacteria*, their abundances were significantly reduced upon HFD induction. GH

**Table 1**
PERMANOVA analysis for cecal bacterial communities at ASV level.

| | RC | HFD | HFD_GHL | HFD_GHM | HFD_GHH |
|---|---|---|---|---|---|
| RC | | F = 4.004<br>P = 0.0078 | F = 3.688<br>P = 0.0066 | F = 3.394<br>P = 0.0093 | F = 1.649<br>P = 0.1753 |
| HFD | | | F = 1.316<br>P = 0.1241 | F = 1.473<br>P = 0.0577 | F = 1.773<br>P = 0.0076 |
| HFD_GHL | | | | F = 1.322<br>P = 0.1156 | F = 1.661<br>P = 0.0074 |
| HFD_GHM | | | | | F = 1.135<br>P = 0.2147 |

treatment at high dose restored their levels to those comparable to the RC group. The low and middle doses of GH did not significantly affect the abundances of these three phyla.

At the genus level, a heatmap displaying the top 30 most abundant genera highlighted variations across the groups (Fig. 5C). Notably, the HFD group showed a significant increase in the abundances of *Lactobacillus*, *Romboutsia*, *Clostridium sensu stricto 1* and *Faecalibaculum,* while the abundances of an uncultured genus in *Ruminococcaceae*, *Ruminococcaceae UCG−004* and two genera in *Muribaculaceae* were decreased. Moreover, the low and middle doses of GH did not significantly affect the abundance of these genera impaired by HFD. High-dose GH treatment reversed the changes in all these genera except for *Lactobacillus*,

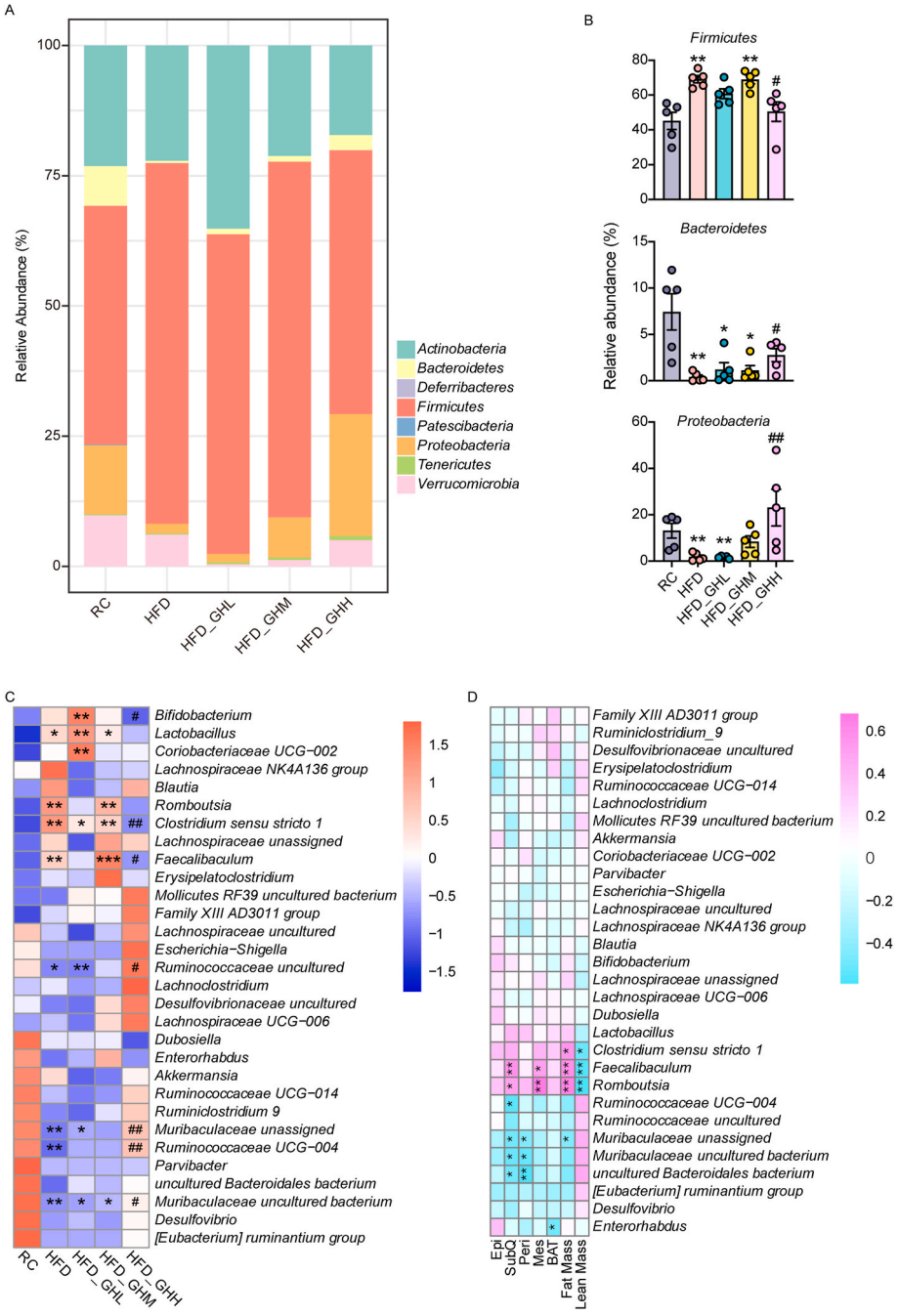


**Fig. 5.** Taxonomic composition of gut bacterial community. **A:** Composition of the bacterial community in the mice under different treatment at the phylum level. **B:** Comparison of the phyla relative abundance among different groups. **C:** Heatmap illustrating the variations in top 30 genera among different groups. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group. **D:** Correlation between the genera and obesity related phenotypes. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

indicating its potential to modulate microbiota composition disrupted by the HFD. However, the low and middle doses of GH did not significantly impact these genera. Additionally, correlation between genera and obesity-related phenotypes was analyzed (Fig. 5D). *Clostridium sensu stricto 1*, *Faecalibaculum* and *Romboutsia* were positively correlated with fat mass and adipose tissue mass (epididymal and mesenteric fat), and negatively correlated with lean mass. This indicates their association with obesity in the HFD group. Conversely, *Ruminococcaceae UCG−004*, two genera in *Muribaculaceae* and an uncultured *Bacteroidales* bacterium were negatively correlated with epididymal or perinephric fat mass, suggesting a potential protective role against fat accumulation.

### 3.5. GH improves the putative metabolic function of gut microbiota

Based on the COG and KEGG databases, we predicted the functional pathways of the gut microbiota. In terms of COG function, the gut microbiota in the RC group exhibited the highest proportion in the category "Function unknown (S)", followed by functions related to "Amino acid transport and metabolism (E)", "Translation, ribosomal structure and biogenesis (J)" and "Carbohydrate transport and metabolism (G)". No "Extracellular structures (W)" were found in any of the groups (Fig. 6A). HFD significantly reduced the abundances of functions related to "Energy production and conversion (C)", "Amino acid transport and metabolism (E)", "Coenzyme transport and metabolism (H)", and "Secondary metabolites biosynthesis, transport and catabolism (Q)". High-dose GH treatment (but not low or middle doses) restored these functions to levels comparable to the RC group.

At the level 3 KEGG category, 66 pathways exhibited significant differences among the groups (Fig. 7). High-dose GH treatment significantly reduced the abundance of seven pathways induced by HFD, including "Cyanoamino acid metabolism", "Secondary bile acid biosynthesis", "Glycerolipid metabolism", "Galactose metabolism", "Starch and sucrose metabolism", "Mismatch repair", and "Glucagon signaling pathway". These pathways are related to metabolism (e.g., lipid, carbohydrate) and organismal system (e.g., glucagon signaling). In contract, six pathways that were decreased by HFD were significantly increased by high-dose GH treatment, including "alpha-Linolenic acid metabolism", "Ethylbenzene degradation", "Geraniol degradation", "Microbial metabolism in diverse environments", "Nitrotoluene degradation", and "Adipocytokine signaling pathway". These restored pathways involve metabolic functions and organismal signaling processes, indicating an improvement in the overall metabolic health of the gut microbiota due to high-dose GH.

## 4. Discussion

The present study investigated the effects of different doses of growth hormone (GH) on adipose tissue mass and gut microbiota in mice with HFD-induced obesity. Our findings provide valuable insights into how GH influences fat mass reduction, potentially by modulating gut microbiota.

Our study demonstrated that GH administration, particularly at the high dose (1.5 IU/kg), effectively reduced adipose tissue mass in HFD-fed mice. This reduction was most notable in subcutaneous and mesenteric fat depots, aligning with previous clinical studies that demonstrated the lipolytic effects of GH on white adipose tissue [9,10].

Previous research has suggested the existence of a mutual interplay between GH signaling and gut microbiota, through which they collectively sustain essential physiological processes, including body growth and metabolism [20]. Furthermore, it has been implicated that alterations in GH levels can lead to impaired gut microbiota, potentially contributing to the pathogenesis of diseases such as GH-secreting

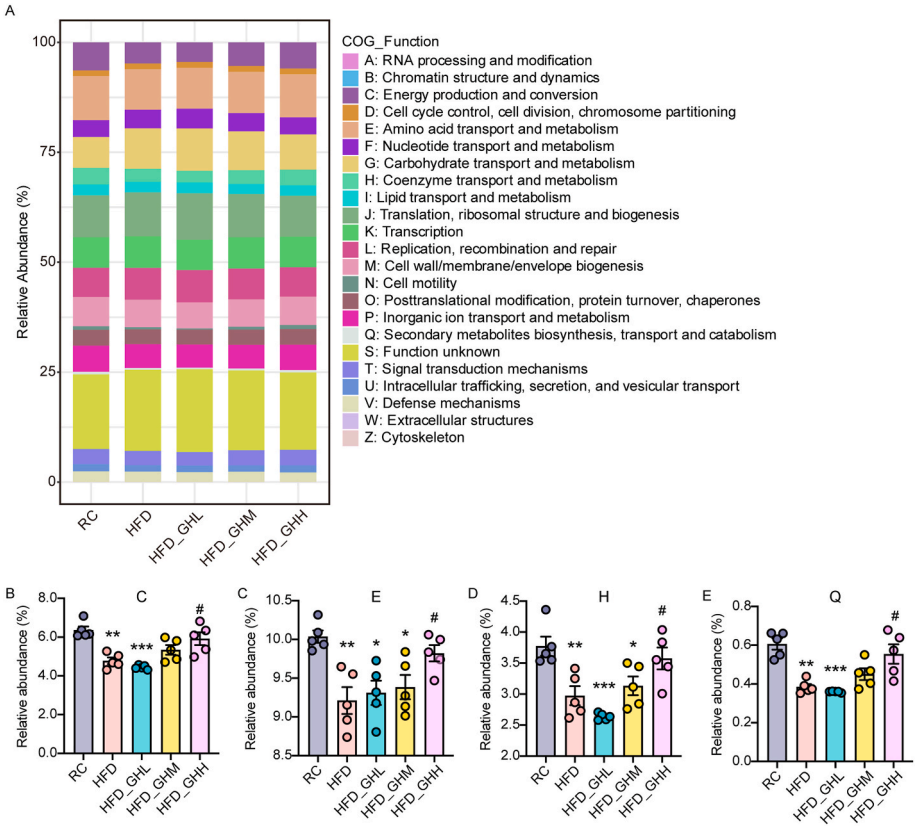


**Fig. 6.** Prediction of gut microbiota function based on COG database. **A:** Classification of COG function in all the groups. **B-E:** Bar plot illustrating the COG functions that were significantly varied among the groups. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group.


**Fig. 7.** Heatmap illustrating the predicted KEGG pathways that were significantly varied among the groups. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, compared to the RC group; #: $P < 0.05$, ##: $P < 0.01$, ###: $P < 0.001$, compared to the HFD group.

pituitary adenoma [21]. Despite the direct lipolytic activity of GH in white adipose tissue and anti-lipogenic effect in hepatocyte [22], the effects of GH treatment on gut microbiota in obese individuals and its role in obesity were still unclear. Microbial richness, an important measure of community biodiversity, reflects the functional stability of bacterial communities [23]. In the present study, we observed that high-dose GH treatment significantly increased the α-diversity indices, especially the richness of gut microbiota, which has been diminished by HFD. This suggested that GH can enhance the stability of gut microbiota, potentially contributing to improved metabolic function in GH-treated mice. Furthermore, the restoration of microbial diversity observed in our study contrasts with earlier findings in GH gene-disrupted mice, where α-diversity was unaffected under regular chow condition [15]. These findings indicate that GH may exhibit distinct gut

microbiota-modulating effects depending on the nutritional environment.

It is well established that the gut microbiota composition, particularly the abundance of *Bacteroidetes* and *Firmicutes* and their ratio, is closely associated with obesity. Specifically, the obese microbiome exhibits an enhanced capacity to harvest energy, as evidenced by the enrichment of pathways involved in starch/sucrose metabolism, galactose metabolism, and ABC transporters [24]. This observation is recapitulated in our HFD-induced mouse model, which is characterized by an increased abundance of *Firmicutes*, a decreased abundance of *Bacteroidetes*, and enriched metabolic pathways involved in starch and sucrose metabolism, galactose metabolism, and ABC transporters. High-dose GH reversed these alterations, restoring the balance between these two phyla, suggesting its potential to correct gut dysbiosis linked to obesity.

At the genus level, high-dose GH treatment increased the abundance of beneficial bacteria such as *Ruminococcaceae* and *Muribaculaceae*. *Ruminococcaceae* is negatively correlated with obesity and acts as a protective factor for subcutaneous adipose tissue generation [25,26], largely due to its improvement of energy metabolism [27]. *Ruminococcaceae* produces short-chain fatty acids (SCFAs), particularly butyrate, which is known to reduce adiposity and improve insulin sensitivity. Butyrate stimulates the expression of PYY and GLP-1 by activating GPR41, thereby contributing to improved glucose homeostasis [28,29]. The increase in *Muribaculaceae* in the high-dose GH group further supported the beneficial metabolic effects observed. *Muribaculaceae* is involved in carbohydrate metabolism and inflammatory processes, and its increase is associated with reduced fat accumulation and improved glucose homeostasis [30]. Furthermore, *Muribaculaceae* produces acetate and succinate by which it increases mitochondrial respiration [31], and succinate has been shown to effectively reduce obesity in HFD-induced mice [32]. In contrast, GH suppressed genera like *Clostridium sensu stricto 1* which are linked to obesity [33,34]. In addition, high-dose GH also enhanced the metabolic capabilities of the gut microbial pathways related to starch and sucrose metabolism, galactose metabolism, and secondary bile acid biosynthesis, which are crucial in the pathophysiology of obesity [24,35]. Overall, our results indicate that high-dose of GH can alleviate obesity through modulating the gut microbiota and its metabolic functions.

While the precise mechanisms by which GH modulates gut microbiota remain to be fully elucidated, our findings suggest two potential models of action. First, it is probable that GH enhances gut microbiota diversity and composition by improving intestinal barrier integrity. Our study showed that GH therapy, particularly at higher doses, increases the expression of tight junction proteins which are essential for maintaining intestinal permeability and regulating microbial populations [36]. A robust intestinal barrier limits the translocation of harmful bacterial components (e.g., lipopolysaccharides), which can drive systemic inflammation and metabolic dysfunction associated with obesity. By enhancing intestinal barrier integrity, GH may contribute to the creation of a more stable gut environment, which in turn fosters the growth of beneficial bacteria, including *Ruminococcaceae* and *Muribaculaceae*. Second, bile acid metabolism appears to be a crucial mediator in GH's effects on gut microbiota. We previously demonstrated that hepatic GHR modulates the gut microbiota by regulating the bile acid metabolism in the liver, thereby altering the bile acid profile in the gut [17]. Bile acids not only directly suppress the growth of specific bacteria [37], but also exert the gut microbiota modulating effects via its downstream signaling, such as FXR pathway [38]. Here, we observed that GH modulated the abundances of *Bacteroidetes* and *Firmicutes* in a manner opposite to that observed in hepatic GHR knockout mice. Additionally, high-dose GH therapy reversed the HFD-induced change in bile acid biosynthesis pathway, providing additional evidence that the gut microbiota responds to changes in bile acid composition caused by activated hepatic GH signaling. These findings imply that GH exerts its effects on gut microbiota, at least in part, through enhanced intestinal permeability and bile acid metabolism.

In conclusion, our study highlights the therapeutic potential of GH in obesity management, primarily through its effects on gut microbiota. High-dose GH treatment significantly reduces adipose tissue mass and reverses HFD-induced alterations in gut microbiota diversity and composition. These findings suggest that GH's beneficial effects on obesity may, at least in part, be mediated by its modulation of the gut microbiota. Further research is warranted to fully unravel the underlying mechanisms and evaluate the long-term safety and efficacy of GH therapy for treating obesity and related metabolic disorders. Overall, our study contributes to the growing understanding of the complex interplay between GH, adipose tissue, and gut microbiota, opening new avenues for therapeutic strategies targeting obesity and related metabolic disorders.

## CRediT authorship contribution statement

**Yu Wang:** Writing – original draft, Visualization, Investigation, Formal analysis. **Liyuan Ran:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Fang Zhang:** Investigation, Formal analysis. **Haolin Li:** Investigation. **Qianqian Cha:** Funding acquisition, Formal analysis. **Kun Yang:** Investigation. **Haoan Wang:** Investigation. **Yingjie Wu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Zichao Yu:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

## Availability of data and materials

The datasets of the current study are available from the corresponding author on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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