

RESEARCH PAPER



B. vulgatus ameliorates high-fat diet-induced obesity through modulating intestinal serotonin synthesis and lipid absorption in mice

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ABSTRACT

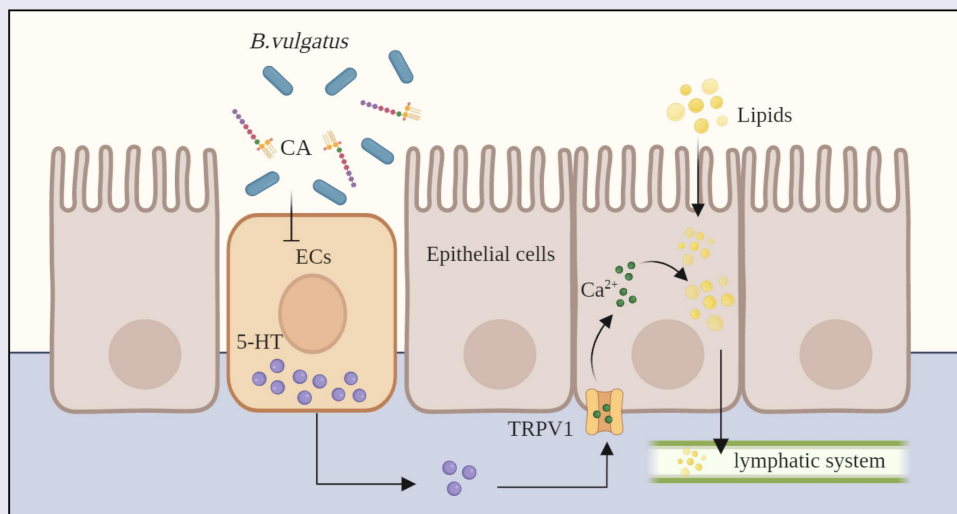
The consumption of high-fat diets (HFD) and an imbalance in gut microbiome are linked to obesity. However, the intricate connection between them and the underlying mechanisms involved in lipid digestion and absorption remain largely unclear. This study shows that after 12 weeks of HFD feeding, *C57BL/6J* mice exhibit two distinct metabolic phenotypes with significant differences in gut microbiota composition. The LOW and LOW FMT group mice with increased *Bacteroides* are protected from obesity, insulin resistance, and lipid accumulation. Supplementation with *B. vulgatus* or cholic acid (CA) alleviates HFD-induced obesity and metabolic dysfunction. This is due to the accumulation of lipid droplets and the retention of chyle particles in jejunal epithelial cells, which reduces chyle intake in the jejunal mesentery after HFD. Decreased 5-HT synthesis in the jejunal enterochromaffin cells of these mice, along with reduced chyle intake in the jejunal mesentery after HFD in *Tph1*^{ΔIEC}, suggests that intestinal 5-HT is required for host lipid absorption. TRPV1, a calcium-permeable ion channel, mediates the basolateral 5-HT-induced increase of *Isc* and ion channel open probability. This study uncovers a novel signaling axis of microbiota-metabolite-5-HT and intracellular calcium-dependent lipid absorption, which may serve as the potential therapeutic targets for treating HFD-induced obesity.

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Introduction

Obesity is a critical global public health concern of the twenty-first century, and a major risk factor for several serious diseases such as cardiovascular

disease, cancer, diabetes and osteoarthritis.^{1,2} Although genetics, behavior and hormones have an impact on body weight, obesity often results from consuming excessive calories. Studies have

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reported that consuming high-fat diets (HFD) significantly and persistently alter microbial communities in both humans and mice, leading to metabolic disorders.^{3–5} Additionally, the gut microbiota of individuals is determined by specific bacterial lineages that are linked to either thinness or obesity,^{6,7} consistent with the findings in adult female monozygotic and dizygotic twin pairs.⁸ Firmicutes and Bacteroidetes are recognized as two major bacterial phyla closely related to obesity in humans and mice.⁹ Obese individuals have a higher abundance of Firmicutes and an increased ratio of Firmicutes/Bacteroidetes, while non-obese individuals have a higher abundance of Bacteroidetes and the opposite trend of Firmicutes/Bacteroidetes ratio.¹⁰ Studies on obese animals and humans have proposed that Firmicutes are more effective than Bacteroidetes in extracting energy from food, promoting more effective caloric intake and subsequent weight gain. As the improved sequencing and analysis technologies have become available, attention has turned to strain level differences between microbes. However, an integrated view focusing on strain-level colonization of obesity has been lacking until recently.

Both imbalanced gut microbiota and excess dietary fat intake are correlated with the development of obesity. The proximal small intestine serves as the primary site for the digestion and absorption of macronutrients. Complex interactions between dietary composition, gut microbiota, intestinal endocrine, bile and pancreatic fluid release, and absorptive enterocyte function are necessary for the efficient absorption of dietary nutrients.¹¹ Dietary fats are absorbed by enterocytes in the small intestine and converted into triglyceride-rich particles, known as chylomicrons. These chylomicrons are then secreted from the basolateral side of the intestinal epithelium into the lamina propria and enter the lacteal, which is a capillary lymphatic vessel located in the core of the intestinal villi and responsible for transporting chylomicrons processed by the enterocytes into the lymphatic system. Subsequently, chylomicrons are transported through the mesenteric lymphatic vessels into the thoracic duct and then drain into the venous circulation.¹² During the process of lipid absorption, the gut basolateral membrane acts as a temporary barrier that facilitates the transport of chylomicrons, playing a crucial role in

dietary lipid transport and the onset of obesity.¹³ Nevertheless, the intricate relationship between HFD and microbiome dysbiosis as well as their underlying mechanism in lipid digestion and absorption remains largely unknown.

Microbes play an essential role in the systemic control of enteroendocrine signaling-mediated lipid digestion and absorption in the small intestine.^{14,15} Compared to specific pathogen-free (SPF) mice, HFD-fed germ-free (GF) mice have higher levels of fecal lipid, indicating the impact of gut microbiota on lipid metabolism.¹⁶ Fecal microbiota transplantation (FMT) results in significant changes in lipid metabolic pathways in peripheral tissues, highlighting the influence of gut microbiota on lipid absorption in hosts. Microbial-derived metabolites or products from specific microbial strains have also been demonstrated to affect lipid absorption in hosts. Among the microbial-derived metabolites examined, α -tocopherol, butyrate, cholate, deoxycholate, p-aminobenzoate (PABA), propionate and tyramine elevate serotonin (5-hydroxytryptamine [5-HT]) in RIN14B chromaffin cell cultures.¹⁷ 5-HT is a key signaling molecule involved in the regulation of energy balance.^{18,19} The majority of the body's 5-HT is synthesized in the gut by two types of cells, enterochromaffin (EC) cells and enteric serotonergic neurons in the myenteric plexus.^{20,21} The EC cells express tryptophan hydroxylase-1 (TPH1) to synthesize 5-HT, while enteric serotonergic neurons express TPH2 to produce 5-HT. Indigenous spore-forming bacteria (Sp) from the mouse and human microbiota promote 5-HT biosynthesis from EC cells,¹⁷ and the gut microbiota metabolite valeric acid promotes TPH2 expression in enteric serotonergic neurons. In the present study, we found that HFD-induced obesity was related to the promotion of 5-HT synthesis by metabolites of gut microbiota.

Therefore, we propose that gut microbial metabolism associated with HFD may play a crucial role in promoting 5-HT synthesis, which is a key signaling molecule and is essential for the host lipid absorption, facilitating the secretion and transportation of chyle particles and ultimately promoting the absorption of nutrients. Our study aimed to investigate the possibility of altering the gut microbiota to prevent and treat HFD-induced obesity by inhibiting the synthesis of 5-HT and thereby reducing lipid absorption.

Results

Gut microbiota participates in regulating HFD-induced obesity

After 12 weeks on the same HFD, two distinct metabolic phenotypes emerged in the *C57BL/6J* mice. The LOW group mice gained significantly less body weight (Figure 1a), along with reduced fat and increased lean body mass ratios compared to the HIGH group mice (Figure 1b). The LOW group mice also had lower liver weights (Figure 1c) characterized by less lipid accumulation (Figure 1d) and epididymal adipocytes to shrink exhibited a sharp decrease in cell size than that in HIGH group (Figure 1e,f). Consistent with less adiposity, the LOW group mice improved glucose tolerance (Figure 1g) and insulin sensitivity (Figure 1h) compared to the HIGH group. These findings suggested that LOW group mice were protected from obesity and related metabolic diseases following HFD.

Numerous studies have provided compelling evidence indicating the involvement of gut microbiota in fat storage and blood glucose regulation.^{4,22} To explore the role of gut microbiota in HFD-induced obesity, antibiotic-treated mice (Supplementary Figure S1a) were transplanted with fecal samples from HFD-fed mice belonging to either the HIGH or LOW groups. Consistent with the results from the LOW group, mice gavaged with fecal samples from the LOW group mice exhibited a reduction in body weight gain following FMT for 12 weeks (Supplementary Figure S1b). The LOW FMT group mice exhibited a lower fat mass ratio (Supplementary Figure S1c), along with decreased adiposity weight and lipid accumulation in the liver (Supplementary Figure S1d,e) and epididymal adipocytes (Supplementary Figure S1f) compared to the HIGH FMT group, with no discernible difference in lean mass ratio. These findings indicate that gut microbiota plays a role in the regulation of HFD-induced obesity.

B.vulgatus alleviates HFD-induced obesity

The diversity and composition of gut microbiota have been identified as an important contributor to metabolic associated disorders. To explore the potential effect of gut microbial dysbiosis on

HFD-induced obesity, we performed metagenomic sequencing on stool samples from mice in the HIGH and LOW groups. The microbial community diversity was analyzed using both alpha and beta diversity metrics. Shannon's index and Simpson's index were used to reflect alpha diversity, calculating the species richness and homogeneity of the microbial community. Bray-Curtis PERMANOVA beta diversity was used to evaluate the microbiota diversity among samples in each group. The results showed that the gut microbiota of the LOW group mice clustered separately from that of the HIGH group mice, with the LOW group displaying lower alpha diversity and higher beta diversity (Figure 1i,j, Supplementary Figure S2a,b).

Further analysis of the relative abundance of microbes at the phylum level showed that Firmicutes and Actinobacteria were significantly more abundant in the HIGH group mice (30.82% and 11.66%, respectively) than in the LOW group mice (22.46% and 9.47%, respectively). In contrast, Bacteroidetes and Verrucomicrobia were significantly more abundant in the LOW group mice (38.46% and 5.61%, respectively) than in the HIGH group mice (28.49% and 3.01%, respectively) (Supplementary Figure S2c ~ 2f). Previous studies have indicated that obese individuals typically show a higher ratio of Firmicutes/Bacteroidetes compared to normal-weight individuals, which is considered a possible hallmark of obesity.²³ Consistent with this observation, the HIGH group mice demonstrated a significantly increased Firmicutes/Bacteroidetes ratio compared to the LOW group mice (Supplementary Figure S2g). The LOW group mice showed an increase in abundance of Bacteroidetes and a normalized ratio of Firmicutes/Bacteroidetes, parallel to low body weight gain (Supplementary Figure S2e,g).

At the genus level, *Bacteroides*, a genus within the phylum Bacteroidetes, was significantly increased in the LOW group mice, while *Lactobacillus*, a genus within the phylum Firmicutes, was significantly more abundant in the HIGH group (Figure 1l). Linear discriminant analysis coupled with LDA Effect Size Analysis (LEfSe) identified *B. dorei* and *B. vulgatus* as key phylotypes in the LOW group, whereas multiple strains of the *Clostridium* genus and *L. reuteri* of the *Lactobacillus* genus were more abundant in the

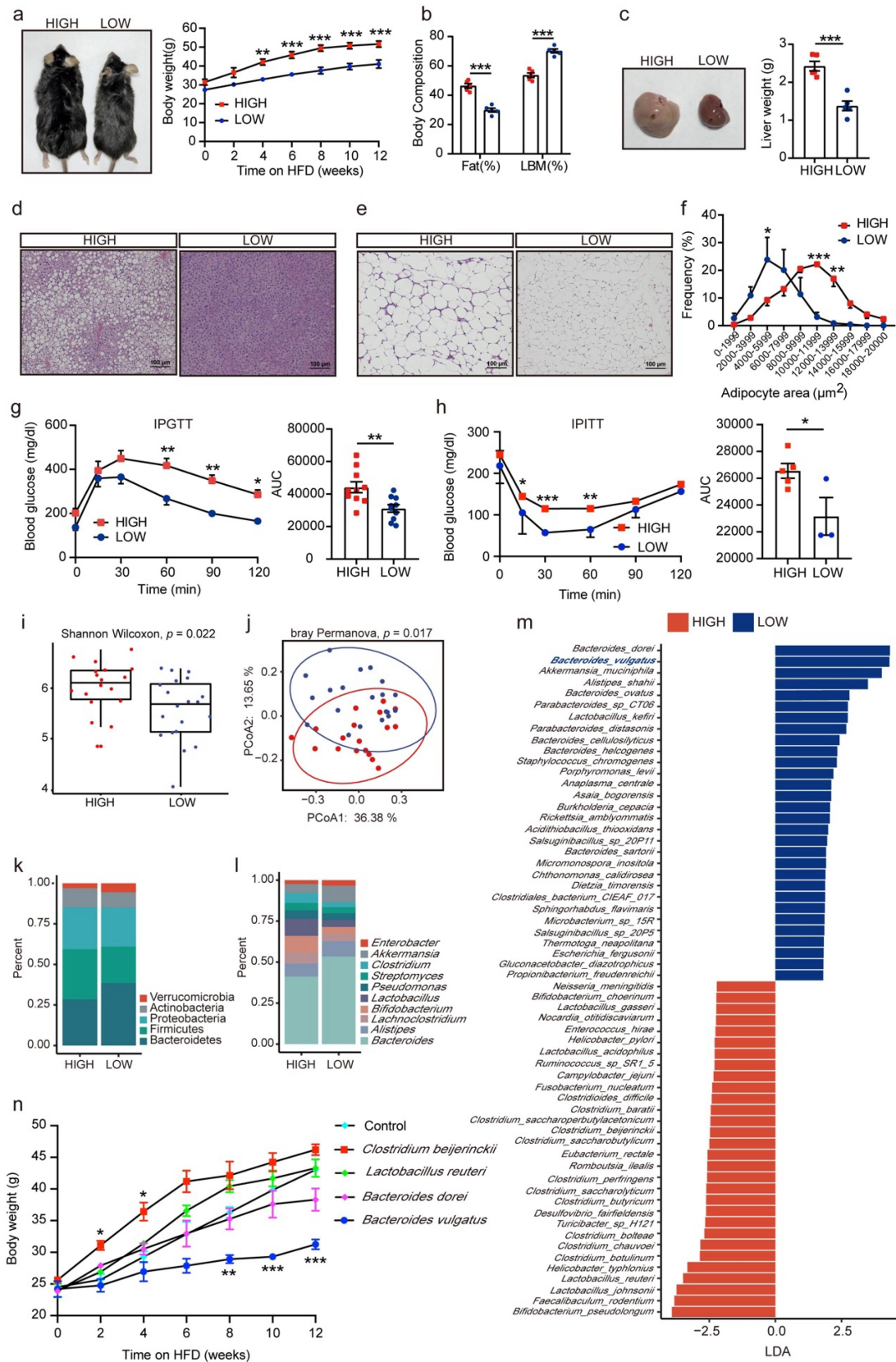


Figure 1. Gut microbiota participates in regulating HFD-induced obesity. (a~c) Body weight gain, body composition, and liver weight of HIGH and LOW group mice over 12 weeks of HFD feeding ($n = 5$); (d&e) Hematoxylin and eosin (H&E) staining of liver and epididymis fat; (f) Adipocyte area of epididymis fat ($n = 5$); (g) Oral glucose tolerance test ($n = 10 \sim 11$); (h) Insulin tolerance test ($n = 3 \sim 6$). (i&j) Alpha diversity and principal component analysis (PCoA) of fecal microbiota; (k) Phylum-level distribution of fecal microbiota; (l&m) Genus-level distribution and LDA Effect Size (LEfSe) of fecal microbiota; (n) Body weight gain of mice supplemented with *B. dorei*, *B. vulgatus*, *L. reuteri* and *C. beijerinckii* ($n = 3 \sim 4$). Data are expressed as mean $\pm$ standard deviation. Two-way ANOVA and Student's *t* test were used to analyze statistical differences; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

HIGH group (Figure 1m). To assess whether these specific strains contribute to HFD-induced obesity, *B. dorei*, *B. vulgatus*, *L. reuteri* and *C. beijerinckii* were orally administrated to mice twice a week for the first 8 weeks following a periodic antibiotic cocktail treatment. After 12 weeks of HFD feeding, only *B. vulgatus* showed a significant regulatory effect on body weight (Figure 1n).

Mice colonized with *B. vulgatus* displayed significantly less weight than Control mice after 12 weeks of HFD feeding (Figure 2a). *B. vulgatus* supplementation reduced the HFD-induced fat body mass ratio, liver weights, and epididymal adipocyte size while increasing the lean body mass ratio compared to Control mice (Figure 2b–e). Additionally, mice colonized with *B. vulgatus* displayed improved glucose tolerance (Figure 2f) and insulin sensitivity (Figure 2g). These data indicate that *B. vulgatus* supplementation alleviates HFD-induced obesity and metabolic dysfunction.

Conversely, *C. beijerinckii*-colonized mice displayed a tendency toward increased body weight compared to Control mice after 12 weeks of HFD feeding (Figure 2h). Supplementation with *C. beijerinckii* increased the HFD-induced fat body mass ratio, liver weights and epididymal adipocyte size while reducing the lean body mass ratio (Figure 2i–l). These suggest that *C. beijerinckii* supplementation may promote HFD-induced obesity.

Microbial metabolite cholic acid prevents HFD-induced obesity

Gut microbiota are known to secrete metabolites that regulate various biological and pathological processes in the host.^{23,24} Gut microbiota modify primary bile acids into secondary bile acids, which play a role in regulating lipid metabolism, glucose homeostasis, and energy balance. In this study, the alterations of fecal metabolites in the HIGH and LOW group mice were detected by using UHPLC-MS/MS. Principal component analysis revealed significant differences in fecal metabolites between the LOW and HIGH group mice (Figure 3a). Volcanic plot analysis identified cholic acid (CA), β -Muricholic acid (β -MCA), 7-ketodeoxycholic acid, and glycodeoxycholic acid as candidate markers distinguishing the HIGH from the LOW group (Figure 3b). Among the metabolites

examined, primary bile acids, particularly chenodeoxycholic acid, CA, and murine-specific primary bile acids (α -MCA and β -MCA), as well as the secondary bile acid ursodeoxycholic acid, were significantly upregulated in the LOW group mice (Figure 3c). Bile acids are generally known for their roles in facilitating the digestion and absorption of dietary fats.²⁵ However, our findings suggest that these bile acids may also play an unexpected role in reducing obesity after HFD.

To further verify the role of CA, we observed that HFD-fed mice supplemented with CA gained less weight (Figure 3d) and had a lower fat body mass ratio (Figure 3e). Additionally, CA supplementation improved glucose tolerance (Figure 3f), reduced lipid accumulation in the liver (Figure 3g), and decreased the size of epididymal adipocytes (Figure 3h) in HFD-fed mice. Our study highlights the significant role of microbial metabolites, particularly bile acids like CA, in reducing obesity in HFD-fed mice.

Gut microbiota modulates intestinal lipid absorption to reduce HFD-induced obesity

In the small intestine, lipid digestion products enter the epithelial cells, where most bind to apolipoproteins to form chyle particles. These chyle particles pass through the basolateral side of the intestinal epithelium into the lamina propria and subsequently diffuse into the lymphatic vessels.²⁶ Transmission Electron Microscope (TEM) technology was used to observe lipid transport within intestinal epithelial cells of different model mice. Compared to HIGH group mice, the LOW group mice exhibited more intracellular lipid droplets (CLDs) in their intestinal epithelial cells and a significant accumulation of chyle particles (Figure 4a). Similarly, mice supplemented with *B. vulgatus* (Figure 4c) or CA (Figure 4e) showed accumulation and retention of CLDs and chylomicrons. However, neither *B. vulgatus* colonization nor CA supplementation altered the food intake (Supplementary Figure S3a,b) or fecal lipid excretion (Supplementary Figure S3c,d) after HFD, indicating that lipid absorption on the luminal side of the jejunal epithelial cells was unaffected. Additionally, qPCR results indicated no significant differences in the expression of key enzymes and

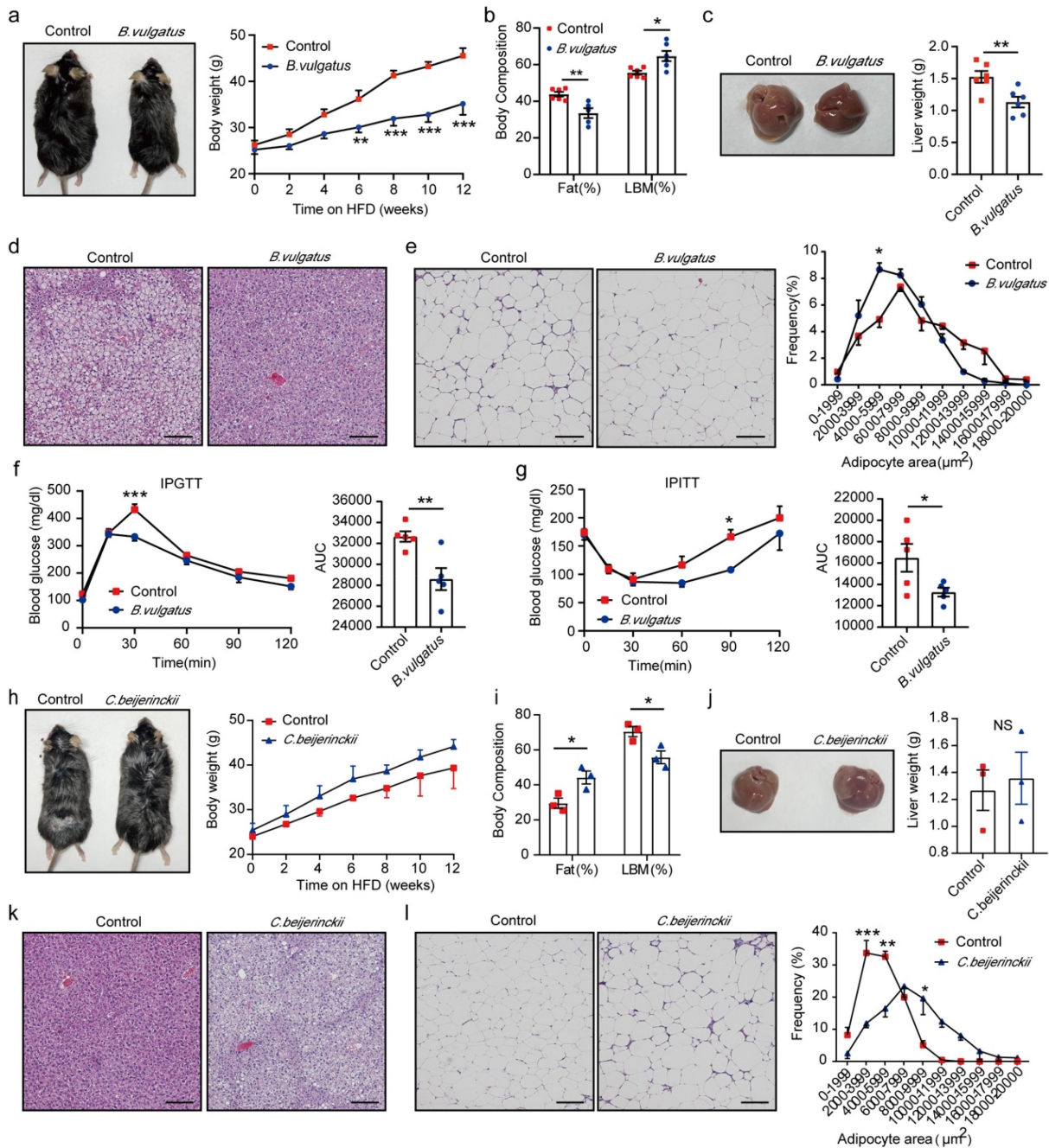


Figure 2. *B. vulgatus* alleviates HFD-induced obesity. (a~c) Body weight gain ($n = 9$), body composition ($n = 5 \sim 6$), and liver weight ($n = 6$) of Control and *B. vulgatus* supplemented mice over 12 weeks of HFD feeding; (d&e) H&E staining of liver and epididymis fat, and adipocyte area of epididymis fat ($n = 3$); (f) Oral glucose tolerance test ($n = 5$); (g) Insulin tolerance test ($n = 5$); (h~j) Body weight gain, body composition and liver weight of Control and *C. beijerinckii* supplemented mice over 12 weeks of HFD feeding ($n = 3$); (k&l) H&E staining of liver and epididymis fat, and adipocyte area of epididymis fat ($n = 3$). Data are expressed as mean $\pm$ standard deviation. Two-way ANOVA and Student's *t* test were used to analyze statistical differences; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Scale bars: 100 μ m.

components essential for chylomicron assembly, including fatty acid transporters (CD36 and Fatp4), FABP1, FABP2, ApoB, MTP, HSL, and ATGL, between Control mice and those supplemented with *B. vulgatus* (Supplementary Figure

S3e). This suggests that the gut microbiota does not influence the ingestion and assembly of chylomicrons within intestinal cells. The transport of chylomicrons from the intracellular space to the lamina propria is controlled by the gut basolateral

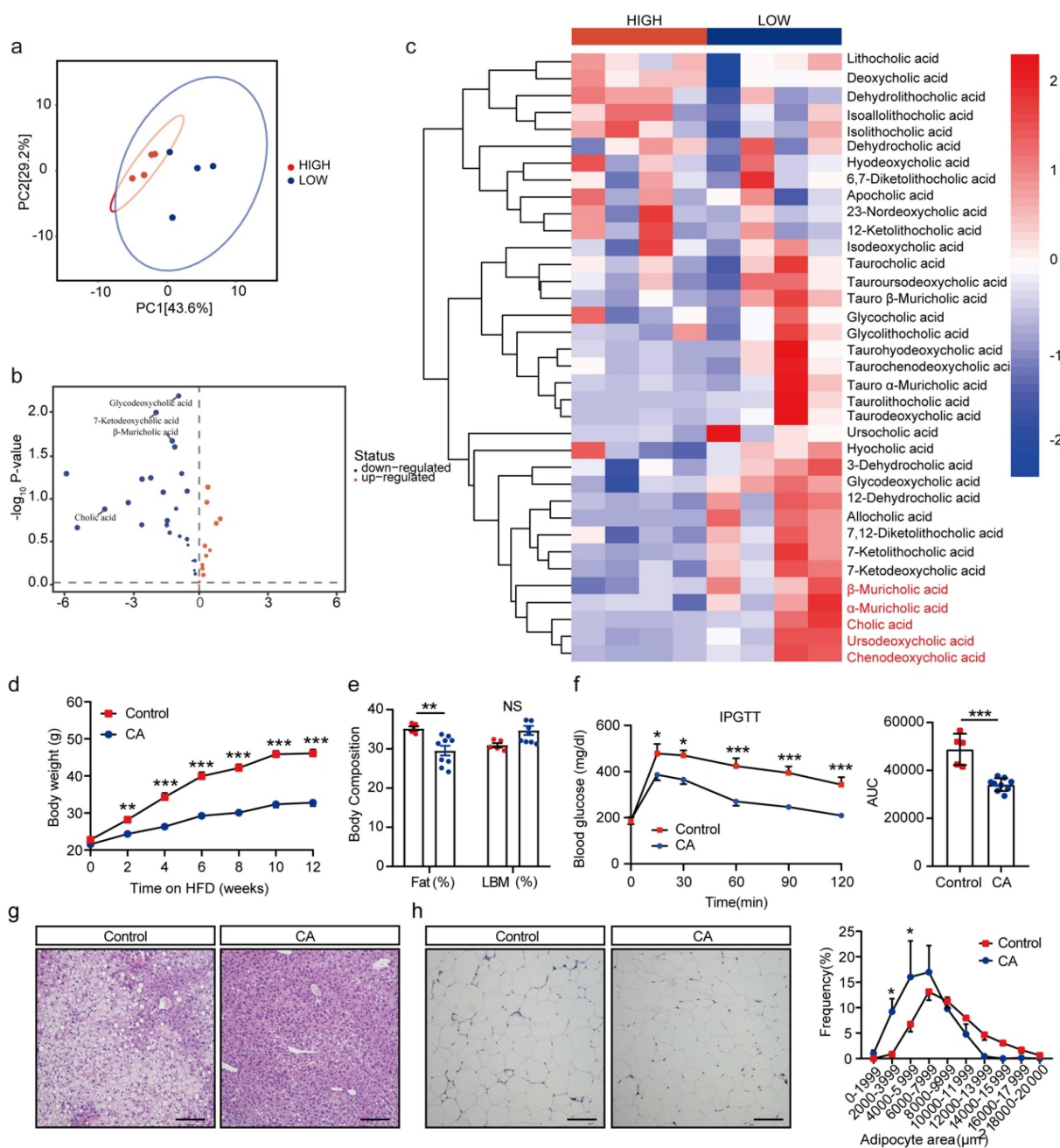


Figure 3. Microbial metabolites CA prevents HFD-induced obesity. (a~c) Principal components analysis (PCoA), volcano plot, and heat map analysis of fecal metabolites of HIGH and LOW group mice over 12 weeks of HFD feeding; (d&e) Body weight gain and body composition of Control and CA supplemented mice ($n = 5 \sim 9$); (f) Oral glucose tolerance test ($n = 5 \sim 9$); (g&h) H&E staining of liver and epididymis fat, and adipocyte area of epididymis fat ($n = 4$). Data are expressed as mean $\pm$ standard deviation. Two-way ANOVA and Student's t test were used to analyze statistical differences; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Scale bars: 100 μm .

membrane.²⁶ Mice of LOW group (Figure 4b) and supplemented with *B. vulgatus* (Figure 4d) or CA (Figure 4f) exhibited chyle particle accumulation and retention near jejunal basolateral membrane. Mice colonized with *B. vulgatus* (Figure 4g) or fed with CA (Figure 4h) showed a significant reduction in chylomicron uptake in the jejunal mesentery

following HFD, as indicated by the reduced number of chyle-filled ducts. These findings suggest that basolateral transit of lipids was slowed down, leading to delayed lipid absorption following HFD. Furthermore, these mice exhibited significantly lower levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein

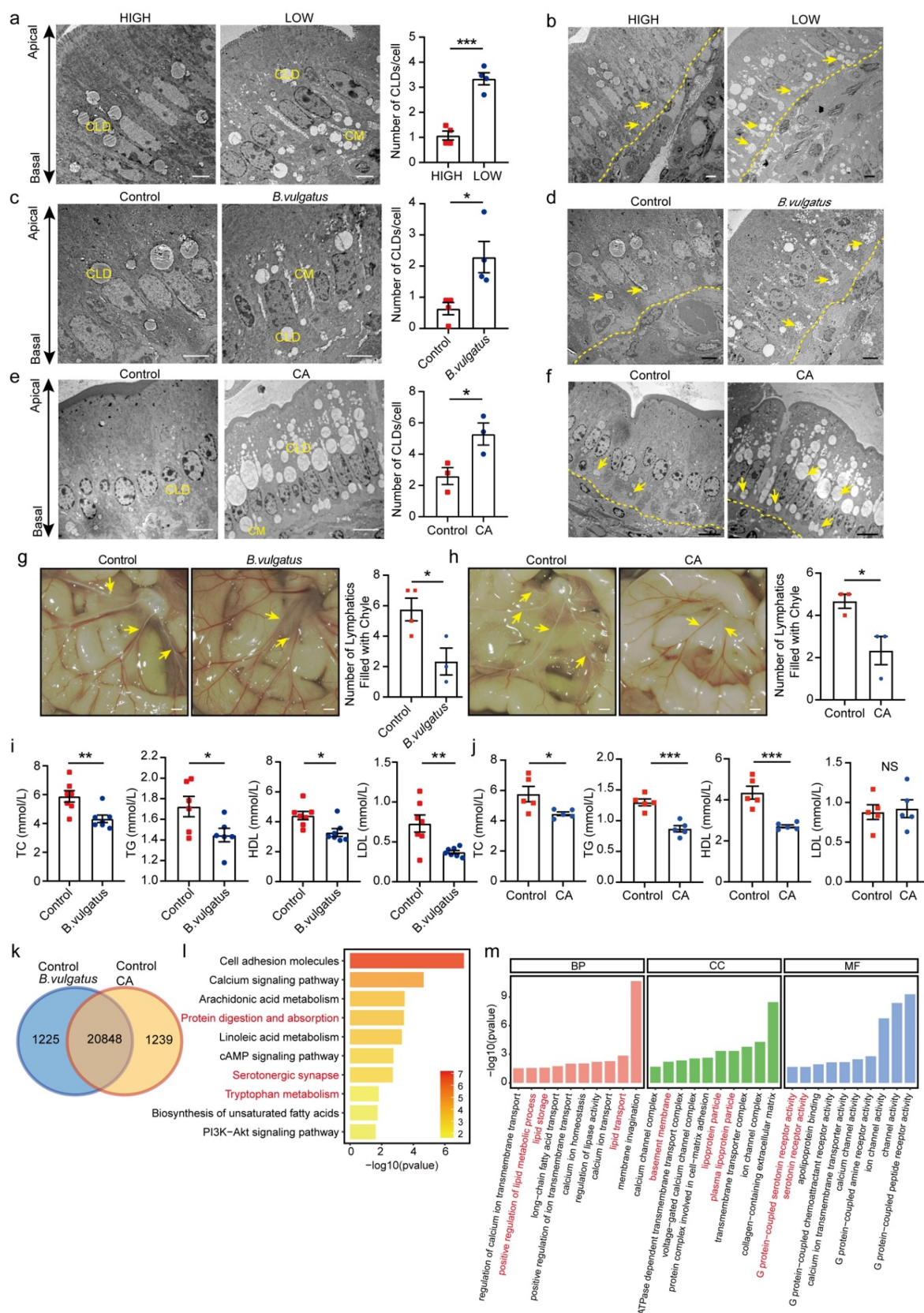


Figure 4. Gut microbiota modulates intestinal lipid absorption to reduce HFD-induced obesity. (a~f) Transmission electron microscopy of the jejunal epithelium and CLD statistical analysis in HIGH and LOW group mice, Control and *B. vulgatus* (or CA) supplemented mice ($n = 3 \sim 4$). Yellow dashed lines represent the basolateral membrane, with yellow arrows pointing to lipid aggregation on the basolateral membrane side. Scale bars: 5 μm ; (g&h) Chylous uptake in the jejunum and statistical analysis of the number of chylous

cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in their serum compared to the Control group (Figure 4i,j). These data indicate that gut microbiota hindered the transport of chyle particles in the jejunum to the chyle ducts.

The mRNA of the jejunal epithelium in Control and *B. vulgatus* supplementation mice, as well as Control and CA fed mice, was extracted and subjected to transcriptome (RNA-seq) analysis. The Venn diagram (Figure 4k) showed the overlap of differentially expressed genes identified in these two groups of model mice. KEGG enrichment analysis indicated that the top 10 most significant signaling pathways were shown in Figure 4l, including protein digestion and absorption, serotonergic synapse, and tryptophan metabolism. Additionally, we found that differentially expressed genes were enriched in lipid transport, lipid storage, and the positive regulation of lipid metabolic processes in the BP enrichment analysis; in plasma lipoprotein particle, lipoprotein particle, and the basement membrane in the CC enrichment analysis; and in G protein-coupled serotonin receptor and serotonin receptor activity pathway in MF enrichment analysis (Figure 4m). Gut microbiota regulates host peripheral 5-HT biosynthesis via metabolites,^{11,18} such as deoxycholic acid, a major secondary bile acid, which stimulates 5-HT release from EC cells in the proximal and distal colon.¹⁹ These findings suggest that the reduced transport of chyle particles in our previous experiments may be closely related to 5-HT.

Loss of intestinal 5-HT synthesis hinders host lipid absorption

Immunofluorescence double-labeling experiments confirmed the scattered distribution of cells co-stained with chromogranin A-positive (CgA⁺) and 5-HT in the jejunal epithelium. Immunofluorescence analysis revealed a significant decrease in the number of CgA⁺

enterochromaffin (EC) cells and 5-HT immunoreactive cells in the LOW group mice compared to the HIGH group (Figure 5a,b). The results of UHPLC-MS/MS and ELISA demonstrated a reduced concentration of 5-HT in both the jejunal mucosa and serum of the LOW group (Figure 5c). Similar findings were observed in mice supplemented with *B. vulgatus* (Figure 5d–f) or CA (Figure 5g–i) when compared to Control mice. The qPCR results also indicated that the gene level of CgA was significantly decreased in the mice colonized with *B. vulgatus* or fed with CA (Supplementary Figure S4a,b), which consistent with the results of immunofluorescence staining. Numerous studies have indicated that inhibiting peripheral 5-HT synthesis can reduce obesity and metabolic dysfunction.^{19,27} To investigate the role of 5-HT produced by intestinal EC cells, *Tph1* specific knockout mice in the intestinal epithelium (*Tph1*^{ΔIEC}) were utilized. These mice exhibited a complete absence of *Tph1* expression at the mRNA level (Supplementary Figure S4c) and a significant reduction at the protein level (Supplementary Figure S4d). In *Tph1*^{ΔIEC} mice, 5-HT was nearly undetectable in the intestinal epithelial tissue (Supplementary Figure S4e), and serum 5-HT levels were significantly reduced (Supplementary Figure S4f). *Tph1*^{ΔIEC} mice showed notable improvements in HFD-induced obesity, with less weight gain and improved glucose tolerance compared with *Tph1*^{fl/fl} mice (Supplementary Figure S4g,h). Additionally, neither *B. vulgatus* colonization nor CA supplementation led to further weight loss in *Tph1*^{ΔIEC} mice after HFD feeding, suggesting that the anti-obesity effects of *B. vulgatus* or CA are mediated by the inhibition of 5-HT (Supplementary Figure S4g). Furthermore, *Tph1*^{ΔIEC} mice had an increased number of CLDs in intestinal epithelial cells and significant accumulation of chyle particles. These mice also

jejunal ducts filled with chyle in Control and *B. vulgatus* (or CA) supplemented mice ($n = 3 \sim 4$). Scale bars: 1 mm. **CLD**: Cellular lipid droplets; **CM**: Chylomicron; (i&j) Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in Control and *B. vulgatus* (or CA) supplemented mice ($n = 5 \sim 7$); (k~m)Venn diagram analysis, KEGG pathway analysis, and GO pathway analysis of overlapping differentially expressed genes in the intestinal epithelium of Control and *B. vulgatus* supplemented mice and Control and CA supplemented mice ($n = 3$). Data are expressed as mean $\pm$ standard deviation. Student's *t* test was used to analyze statistical differences; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

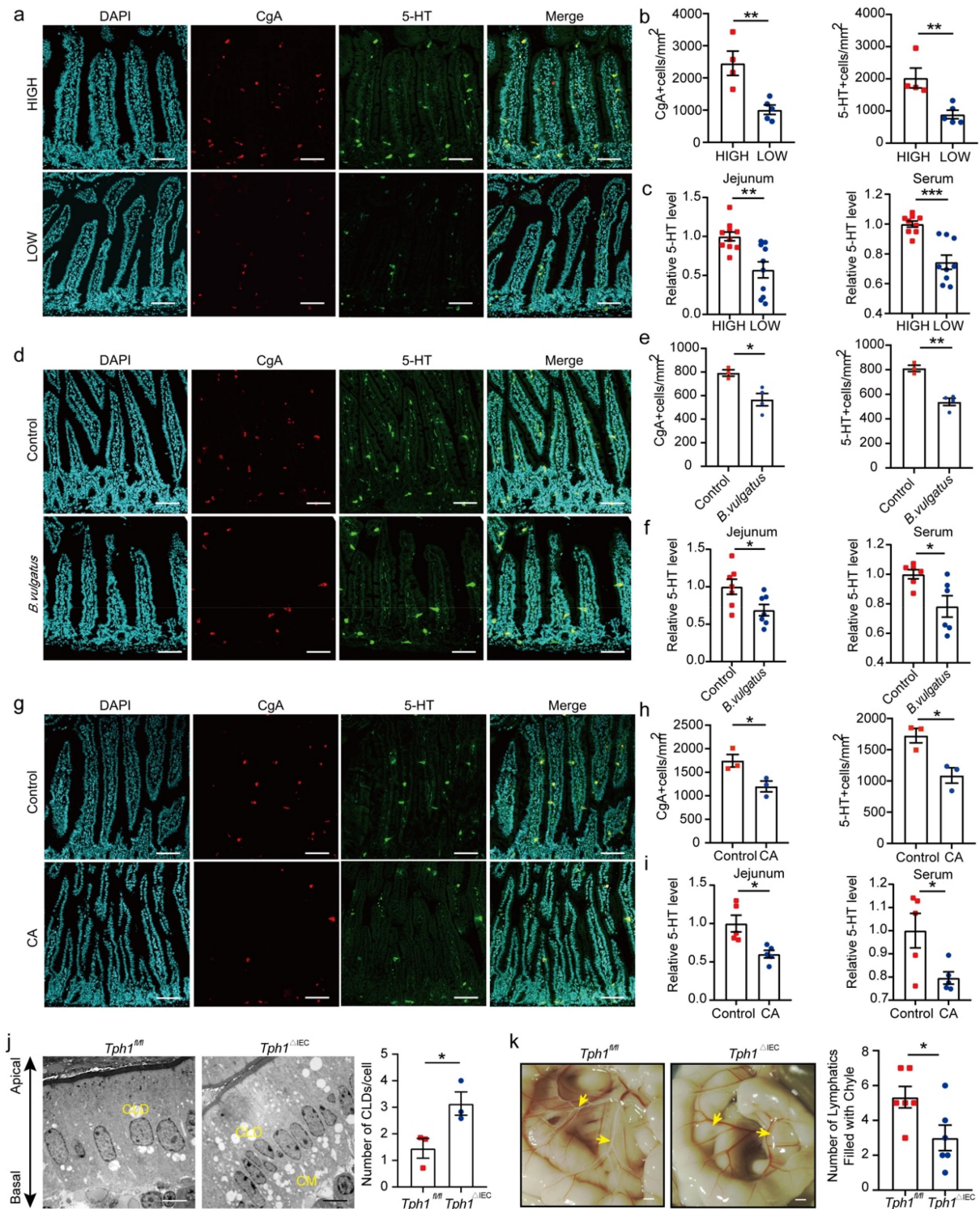


Figure 5. Loss of intestinal 5-HT synthesis hinders host lipid absorption. (a~i) Immunofluorescence labeling of CgA (chromogranin A, red) and 5-HT (green), along with the number of CgA-positive (CgA⁺) EC cells and 5-HT-positive (5-HT⁺) cells in the jejunum and relative 5-HT levels in the jejunal mucosa and serum of HIGH and LOW group mice, Control and *B. vulgatus* (or CA) supplemented mice ($n = 3 \sim 10$). Scale bars: 50 μ m; (j) Transmission electron microscopy of the jejunal epithelium and CLD statistical analysis in *Tph1*^{fl/fl} and *Tph1* ^{Δ IEC} mice ($n = 3$). Scale bars: 5 μ m; (k) Representative images of chylous uptake in the jejunum of *Tph1*^{fl/fl} and *Tph1* ^{Δ IEC} mice with statistical analysis of the number of chylous jejunal ducts filled with chyle ($n = 6$). Scale bars: 1 mm. Data are expressed as mean $\pm$ standard deviation. Student's *t* test was used to analyze statistical differences; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

exhibited a marked reduction in chyle particle uptake in the jejunal mesentery after HFD, as determined by the number of chyle filled ducts (Figure 5j,k). These suggested that the loss of intestinal 5-HT synthesis hindered the transport of chyle particles from the jejunum to the chyle ducts.

Increased intracellular calcium promotes 5-HT induced lipid absorption

The intestinal epithelium plays a crucial role in nutrient absorption, transportation and secretion of various active molecules.²⁸ To identify the key molecules involved in the transport of chyle micro-particles in the jejunal epithelium, we collected jejunal epithelial tissues from all the four groups of mice for RNA-seq analysis. A Venn diagram illustrated the overlap of differentially expressed genes among these groups (Figure 6a). GO enrichment analysis showed significant enrichment of intersecting differential genes in ion transport-related pathways (Figure 6b). Short-circuit current (*I*_{sc}) is a classic method for detecting ion transport. The basolateral, not apical, 5-HT-induced increase of *I*_{sc} in the jejunum of HIGH group mice was much higher than in the LOW group (Supplementary Figure S5 and Figure 6c), indicating enhanced substance transport mediated by 5-HT. Supplementing with *B. vulgatus* and metabolite CA significantly reduced 5-HT-induced changes of *I*_{sc} in the jejunum (Figure 6d,e). KEGG enrichment analysis further highlighted significant enrichment of intersecting genes in the calcium signaling pathway, serotonergic synapse, and tryptophan metabolism (Figure 6f). Transient receptor potential vanilloid subfamily member 1 (TRPV1), a Ca²⁺-permeable ion channel, is implicated in modulating intestinal permeability²⁹ and lipid metabolism.³⁰ Capsazepine, the most commonly used specific antagonist of TRPV1,^{31–35} was employed to block TRPV1 activity. Blocking TRPV1 with capsazepine significantly reduced the 5-HT-induced increase of *I*_{sc} in the jejunal epithelium of Control mice, while a decreasing trend was observed in the jejunal mucosal epithelium of mice colonized with *B. vulgatus* (Figure 6g), suggesting that TRPV1 is involved in 5-HT-induced jejunal substance transport. To further investigate the role

of calcium signaling in the process, we transfected TRPV1 plasmids into HEK293 cells and found that 5-HT increased the open probability (*NP*_o) of ion channels, which was significantly reduced by the TRPV1 inhibitor capsazepine (Figure 6h). Additionally, 5-HT increased both the average and Δfold change in fluorescence intensity of fluo-4 in HEK 293 cells transfected with TRPV1, while pretreatment with capsazepine completely blocked this effect (Figure 6i). These data suggest that the 5-HT-induced increase in intracellular calcium in intestinal epithelial cells is mediated via TRPV1. The secretion of chylomicrons in intestinal epithelial cells requires the participation of intracellular calcium ions.²⁶ Our findings suggest that the increase in intracellular calcium mediated by intestinal 5-HT has a positive regulatory effect on the transport of lipid droplets and chyle particles in intestinal epithelial cells.

Discussion

Various studies suggest that the intestinal microbiota structure is one of the most critical factors affecting individual energy homeostasis.³⁶ Individuals prone to obesity possess a unique intestinal microbiota that promotes energy intake and storage from specific diets which differs from that of non-obese individuals.¹¹ Although lower diversity is generally considered a characteristic of obesity in humans and mice, some studies report that the bacterial diversity in obese individuals is higher than in those with a normal BMI.^{37,38} Our study found that after administering an HFD for 12 weeks, the Shannon index and Simpson index, which reflect the alpha diversity of the gut microbiota, were significantly higher in the HIGH group mice compared to the LOW group mice. Principal components analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS) plots, which reflect beta diversity of the gut microbiota, revealed distinct differences in microbiota composition between these two groups. Over the last decade, the Firmicutes/Bacteroidetes ratio has been frequently considered as a possible hallmark for obesity.³⁹ Similarly, our results indicated that the HIGH group mice had the highest proportion of Firmicutes, whereas the LOW group mice had the highest proportion of Bacteroides. The HIGH

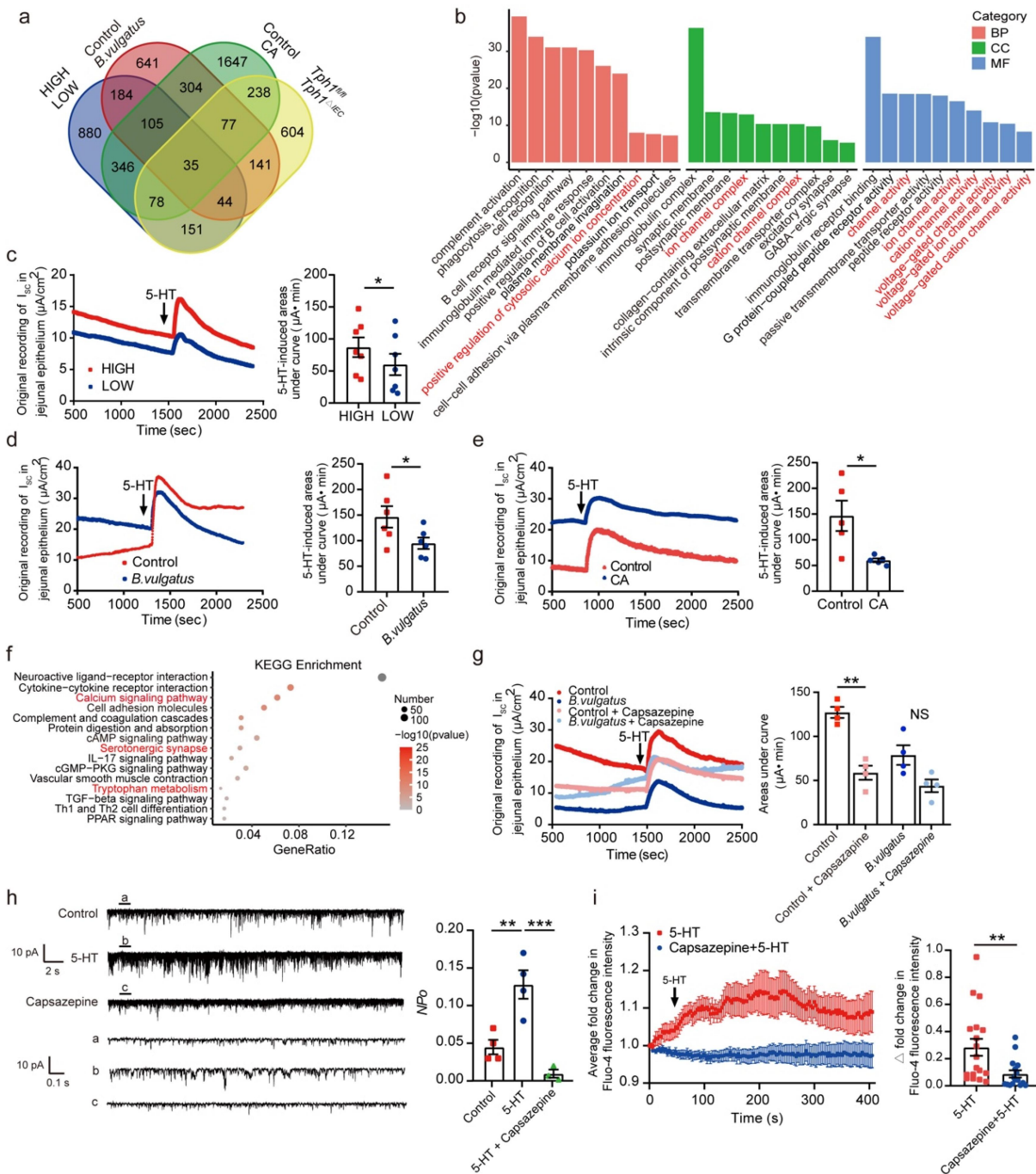


Figure 6. Increased intracellular calcium promotes 5-HT induced lipid absorption. (a) Venn diagram analysis and (b) GO pathway analysis of overlapping differentially expressed genes in the intestinal epithelium across HIGH and LOW group mice, Control and *B. vulgatus* supplemented mice, Control and CA supplemented mice, as well as *Tph1^{fl/fl}* and *Tph1^{ΔIEC}* mice ($n = 3$); (c~e) Short circuit current (I_{sc}) recordings (Left) and 5-HT-induced changes (Right) in jejunal epithelium of HIGH and LOW group mice, Control and *B. vulgatus* (or CA) supplemented mice, with 5-HT addition marked by an arrow; (f) KEGG pathway analysis of overlapping differentially expressed genes in the four group mice ($n = 3$); (g) I_{sc} recordings (Left) and the effect of TRPV1 inhibitor capsazepine on 5-HT-induced changes (Right) in Control and *B. vulgatus* supplemented mice; (h) Cell-attached patch clamp showing open probability (NPo) of ion channels in Control and capsazepine-pretreated cells with 5-HT (Left), magnification (Middle), and 5-HT-induced NPo changes (Right). The membrane potential was held at -100 mV; (i) The average and Δ fold change in fluo-4 fluorescence intensity in HEK 293 cells transfected with TRPV1 treated with 5-HT or capsazepine + 5-HT. 5-HT: $10 \mu M$. Data are expressed as mean $\pm$ standard deviation. One-way ANOVA and Student's t test were used to analyze statistical differences; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

group mice exhibited a significant increase of Firmicutes/Bacteroidetes ratio compared to the LOW group mice. To accurately identify specific differential strains, we utilized third-generation sequencing and metagenomic analysis. *Bacteroides vulgatus* (*B. vulgatus*), a member of the Bacteroides genus, was the highly abundant in the feces of the LOW group mice (Figure 1) and demonstrated a significant reduction in obesity, as shown in mice with *B. vulgatus* supplementation (Figure 2).

Furthermore, the abundance of *B. vulgatus* and genes encoding bile salt hydrolase (BSH) was elevated in patients with polycystic ovary syndrome (PCOS), thereby influencing the metabolism of glycine deoxycholic acid (GDCA) and taurine ursodeoxycholic acid (TUDCA) in the host.⁴⁰ The classic route for the synthesis of primary bile acid involves the production of cholic acid (CA) and chenodeoxycholic acid (CDCA) from cholesterol. The synthesis, metabolism, and biological transformation of bile acids require the involvement of intestinal microbiota, including *Bacteroides fragilis* (*B. fragilis*), which plays a vital role in the enterohepatic circulation of bile acids by facilitating the binding and hydrolysis of bile salts during the biological transformation process.⁴¹ The hydrolysis of amino acid residues in bile acids by BSH is a common phenomenon among archaea and bacteria.⁴² Bacteria with BSH activity include Bacteroides, Clostridium, Lactobacillus, and Bifidobacterium.⁴³ The abundance of BSH gene in intestinal microbiota is significantly correlated with several diseases, including type 2 diabetes, breast cancer, ulcerative colitis, Parkinson's disease and others.⁴⁴ Additionally, HFD feeding alters the structure of intestinal microbiota by affecting the secretion of bile acids.⁴⁵ Administration of gut commensal *Christensenella minuta* alleviated features of metabolic disease in HFD-induced obese mice associated with a significant increase in acylated bile acids with 3-O-acylation.⁴⁶ The targeted screening of bile acid metabolites in our study revealed that the LOW group mice had a higher content of primary cholates than that in the HIGH group, which corresponded to the metagenome sequencing results in the previous study. The high abundance of Bacteroides in the LOW group was closely related to the high content of primary cholates. To confirm the findings of the targeted

screening of bile acid metabolites, we conducted a supplementation experiment on the highly expressed primary cholate metabolite CA in the LOW group. Supplementation with metabolite CA significantly inhibited the obesity phenotype compared to the Control group (Figure 3), supporting the possibility of Bacteroides and metabolite CA participating in obesity regulation.

The small intestine is a vital organ for nutrient absorption, primarily through the numerous intestinal epithelial cells. The subepithelial lamina propria of the small intestine contains a central chylous tube and a network of capillaries responsible for transporting various absorbed nutrients.⁴⁷ After entering the intestinal lumen, the lipids are digested and re-esterified into complex lipid molecules in intestinal epithelial cells. Most lipids in these cells are reorganized into lipoproteins, forming chyle particles that enter the chyle ducts of the lamina propria through the intercellular space, and eventually into the lymphatic system for transport throughout the body.²⁶ Additionally, some lipids within the cell may form cytoplasmic lipid droplets for energy storage or undergo fatty acid oxidation for energy supply.^{47,48} It has been reported that treatment with *B. vulgatus* Bv46 restores the intestinal flora composition, enhances the bioconversion of cholesterol to bile acids, which accelerates energy expenditure, promotes cholesterol excretion, and improves lipid homeostasis of HFD-feeding rats.⁴⁹ However, our study focused on the process of intestinal lipid absorption. The results of qPCR and transcriptome pathway enrichment analyses in intestinal epithelial tissues showed no significant changes in genes related to the assembly and secretion of chylomicrons within intestinal cells. We found retention of lipid droplets and chyle particles in intestinal epithelial cells of mice supplemented with *B. vulgatus* or metabolite CA (Figure 4). This retention hindered the secretion of chyle particles from the jejunum to the intestinal lymph and reduced serum lipids.

5-HT is a monoamine neurotransmitter that plays a crucial role in the body. Recent studies have shown that the levels of 5-HT in both the serum and intestine of germ-free mice were significantly decreased compared to the control group.⁵⁰ This indicates that intestinal microbiota is a main regulator of 5-HT production in the intestine.¹⁷

Intestinal microbiota produces fecal metabolites, such as α -tocopherol, p-aminobenzoate, and tyramine that stimulate the synthesis of 5-HT in intestinal chromaffin cells, which then supply 5-HT to the mucosa, lumen, and circulating platelets. The use of different antibiotics can cause a structural disorder in the intestinal microbiota, which affects the level of serum 5-HT,⁵¹ however, the exact strains involved in the regulation of 5-HT are not yet clear. Bacteria regulate the expression of 5-HT by modulating the intestinal chromaffin cell network in mice, mainly reflected in the expression of intestinal chromaffin marker chromaffin protein A and numbers of intestinal chromaffin cells that produce 5-HT.⁵²

5-HT has been observed to impact the levels of glycolipid metabolism-related metabolites in peripheral blood and liver, leading to an acceleration of bile acid metabolism.⁵³ The synthesis of 5-HT is positively correlated with the production of deoxycholic acid (DCA), and this synthesis is primarily regulated by *Clostridium* sp.,¹⁷ which suggests that the synthesis of 5-HT can participate in the regulation of glycolipid metabolism through bile acid metabolism, with the intestinal microbiota acting as a mediator. Our results also showed that *B. vulgatus* and CA supplementation led to a decrease in the expression of 5-HT in both intestinal epithelium and serum (Figure 5), indicating that *B. vulgatus* and the metabolite CA may be involved in the regulation of obesity by inhibiting the production of intestinal 5-HT. Previous studies have indicated that bile acids, including CA, are critical regulators of epithelial function in both health and disease.²³ Under physiological conditions, CA represent an intrinsic stimulus that dictates intestinal stem cell proliferation to sustain daily epithelial regeneration.⁵⁴ In colitis, CA potentiates intestinal epithelial injury by suppressing intestinal stem cell renewal. However, the mechanisms underlying these findings remain unexplored, which is a limitation of the present study.

We conducted a transcriptomic analysis on the jejunal epithelium of four different groups of model mice: obesity model (HIGH and LOW groups), *B. vulgatus* supplementation mice, metabolite CA supplementation mice, and *Tph1*^{ΔIEC} mice. Through this analysis, we identified intersection differential genes enriched in the ion

transport-related pathways using GO pathway enrichment analysis and the calcium signaling pathway using KEGG pathway enrichment analysis (Figure 6). Calcium is known to play a critical role in various biological processes,⁵⁵ and TRPV1 is functionally involved in regulating Ca²⁺ homeostasis of organelles and cells.³⁰ The experimental evidence indicates that TRPV1 knockout mice are resistant to obesity induced by a HFD,¹⁰ suggesting the importance of TRPV1 in lipid homeostasis maintenance.⁵⁶ TRPV1 activity is potentiated by the PLC/PKC and cAMP/PKA pathways,^{49,57} which are known to be involved in the modulation of TRPV1 by 5-HT through activation of 5-HT_{2A} and 5-HT₇ receptors.⁵⁸ Additionally, 5-HT-induced calcium signals are primarily mediated by Ca²⁺ influx and are regulated by the 5-HT_{2B/2C}-PKC ϵ pathway.⁵⁹ Therefore, the proposed mechanism suggests that 5-HT enhances Ca²⁺ influx through TRPV1 by activating specific serotonin receptors (such as 5-HT_{2A}, 5-HT_{2B/2C}, and 5-HT₇) and downstream signaling pathways (such as PKA and PKC pathway), which potentiate TRPV1 function and increase calcium influx into the cell. In our study, blocking TRPV1 with capsazepine significantly reduced 5-HT-induced the increase of *Isc* and the *NPo* of TRPV1 (Figure 6), suggested that TRPV1 is involved in the jejunal substance transport induced by 5-HT. There is a complex interaction between 5-HT and TRPV1, and 5-HT-triggered GPCR cascades are able to decrease the threshold for TRPV1 activation and increase the number of receptors at the cell surface.⁶⁰ In the entire small intestine of mammals, chyle particles enter the lymphatic system through cell exocytosis, and Ca²⁺ is known to play a crucial role in the secretion of chyle particles.²⁶ Our study found that 5-HT induced increments of average and Δ fold change fluorescence intensity of fluo-4 were increased in HEK 293 cells transfected with TRPV1, which enhanced the secretion and transport of chyle particles. Pretreatment with the TRPV1 inhibitor capsazepine completely blocked 5-HT induced changes of fluorescence intensity (Figure 6), suggest that the positive regulatory effect of intestinal 5-HT on the transport.

In summary, our findings revealed that HFD-associated gut microbial metabolism regulates intestinal 5-HT synthesis, which is essential for the

host lipid absorption. We confirmed that *B. vulgatus* and metabolite cholic acid reduce HFD-induced obesity via inhibiting 5-HT synthesis from EC cells and innovatively found that 5-HT increased intracellular calcium in intestinal epithelial cells by activating TRPV1, thereby promoting lipid absorption. This study uncovers a novel signaling axis of microbiota-metabolite-5-HT and lipid absorption, which provides potential therapeutic targets for the treatment of HFD-induced obesity.

Methods

Ethics statement

All animal care and experimental procedures were performed in accordance with the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences Adhoc Committee on Animal Research. All the procedures employed in our animal experiments were approved by the Animal Ethics Committee of the Capital Medical University (AEEI-2023-061). All efforts were made to minimize animal suffering, and the minimum number of animals necessary to produce reliable scientific data was used.

Animal care

Eight-week-old C57BL/6J SPF male mice were purchased from Vital River Laboratory Animal Technology Co Ltd. (Beijing, China) and fed in the Experimental Animal Center of Capital Medical University. All the mice were housed in a specific-pathogen-free facility (SPF) under a 12-h dark–light cycle with ad libitum access to food and water.

Obesity mouse model

The production of obese model was repeated three times, using 40 wild-type eight-week-old male SPF mice each time. Mice were given a 60 kcal% high-fat diet (HFD, MP Biomedicals, USA) for 12 weeks, and a significant difference was observed in the body weight of mice. The weight of HFD-induced mice with low body weight gain was similar to that of mice on a normal diet fed simultaneously (33.4 ± 1.7 , $n = 4$). Then, we used the body weight of male

wild-type mice at twenty-week-old on a normal diet provided by Jackson's laboratory (34.2 ± 2.9 , $n = 40$) as the standard for screening the LOW group mice, and the proportion of LOW group mice is 19.4%. To compare the differences, we selected the mice with the highest body weight as the HIGH group mice in the same proportion. Collect fresh feces from mice during 9–12 weeks of the feeding period, freeze the feces of HIGH and LOW group mice in liquid nitrogen, and store them at -80°C .

Fecal microbiota transplantation (FMT)

The production of FMT models was repeated twice, using 10 wild-type eight-week-old male SPF mice each time. Mice were treated with water containing a combination of antibiotics^{61–64} (neomycin sulfate 0.5 mg/mL, metronidazole 0.5 mg/mL, ampicillin sodium 0.5 mg/mL, vancomycin 0.25 mg/mL) for 2 weeks. The antibiotic treated mice were randomly divided into two groups feeding with HFD 12 weeks and transplanted with the feces of HIGH group and LOW group mice. We dissolved 100 mg of mixed feces in 5 mL of sterile PBS, vortex mixed thoroughly, and allowed to settle at room temperature for 10 min before collecting the supernatant for gavage. To ensure the effectiveness of microbiota colonization, each mouse was given gavages twice per week for 12 weeks.

Strain replenishment

The production of *B. vulgatus* or *C. beijerinckii* replenishment models was repeated three times, using 10 wild-type eight-week-old male SPF mice each time. Mice were treated with water containing a variety of antibiotics (neomycin sulfate 0.5 mg/mL, metronidazole 0.5 mg/mL, ampicillin sodium 0.5 mg/mL, vancomycin 0.25 mg/mL) for 2 weeks. The antibiotic treated mice were randomly divided into two groups: (a) Control: the medium of strain was taken for gavage and fed with HFD; (b) Strain: the concentration of $6.0\text{--}7.0 \times 10^8$ CFU/mL strain suspension was administered by gavage and fed with HFD. Mice were given a 60 kcal% HFD for 12 weeks. To ensure the effect of strain colonization, each mouse was administered twice a week for 12 weeks. Weights of the mice were recorded every 2 weeks.

Cholic acid replenishment

The production of cholic acid replenishment models was repeated twice, using 10 wild-type eight-week-old male SPF mice each time. Mice were randomly divided into two groups: (a) Control: fed with HFD for 12 weeks; (b) CA: HFD supplemented with 0.5% cholic acid (sigma V900488, USA) for 12 weeks. Weights of the mice were recorded every 2 weeks.

Tph1^{ΔIEC} mice

Tph1^{flox/flox} mice were hybridized with *villin*-Cre to produce *Tph1*^{ΔIEC} mice. PCR was used for genotyping. The verified primers used in the present study were as follows: Flox Common Reaction A, 5'- TCT GCT CTG TTT CTC CTT GTT CTA -3'; Reverse Reaction A, 5'- AAA GTG ACA GCA ACA CTG TTA GTC -3'; Cre Common Reaction A, 5'- CCA TAG GAA GCC AGT TTC CCT TC -3'; Reverse Reaction A, 5'- TTC CAG GTA TGC TCA GAA AAC GC -3'. The expected PCR products were as follows: mutant (*Tph1*^{ΔIEC}) = 265 bp; heterozygote = 265 bp and 216 bp; wild type (WT) = 216 bp. Eight-week-old male mice were given a 60 kcal% HFD for 12 weeks. Weights of the mice were recorded every 2 weeks.

Bacterial DNA extraction

Total DNA in stool was isolated using the SPINeasy DNA Kit for Feces (MP Biomedicals). DNA concentration was determined using the NanoDrop™ One (Thermo Scientific). The results were normalized by multiplying the DNA volume (50 μL) by the DNA concentration and dividing by the mass of mouse feces. After antibiotic water treatment, there is a significant reduction in the concentration of bacterial DNA in the feces.

Observation of mesenteric lymphatic vessels

Following euthanasia and laparotomy, the mesenteric lymphatics around the jejunum were exposed, and images were acquired using a stereo-microscope. Numbers of chyle-filled lymphatic vessels were measured using ImageJ software.

Body composition

After 12 weeks HFD feeding, the mice were put into the body composition analysis and imaging system (PET/CT/SPECT three-in-one system for small animals), and the total body fat and lean body mass (LBM) were measured *in vivo*.

Metabolic tests *in vivo*

Metabolic tests *in vivo* were performed with adult male mice 1 week before euthanasia. Intraperitoneal glucose tolerance tests (IPGTT) were carried out using mice that had been fasted overnight for 12 h. Mice received 1.2 g/kg of glucose i.p., and blood was collected from the tail vein at specific time points (0, 15, 30, 60, 90, and 120 min post-injection). Blood glucose concentrations were determined using a portable glucometer (Contour Glucometer; Bayer). For insulin tolerance tests (IPITT), mice were fasted for 6 h and then injected with human insulin (i.p. 1 U/kg; Beyotime). Subsequently, blood glucose levels were determined at time points similar to those used for the IPGTT assay.

Histological analyses

All animals were euthanized by decapitation. The abdominal wall was opened, and the jejunum was quickly removed and immersed in Krebs-Henseleit solution (K-HS) for other experiments. Liver and epididymal adipose tissue (eWAT) were fixed in 10% neutral formalin solution for 24 h at room temperature. Samples were then dehydrated by immersion in ethanol 100% for 24 h and processed for paraffin embedding. Paraffin sections of 5 μm were stained with hematoxylin and eosin. Images were obtained using a SCN400 slide scanner and Digital Image Hub software (Leica Biosystems, Wetzlar, Germany). Adipocyte size and distribution in epididymal adipose tissues were calculated from three random fields per sample for eWAT using ImageJ software.

Immunofluorescence staining

Mice were fed with HFD for 12 weeks, the intestinal tissue was taken from the small intestine and

cleaned in cold PBS, then fixed in 10% neutral formalin solution for 24 h. After dehydration in gradient ethanol for 24 h, the intestinal samples were embedded in paraffin and cut into 5 μ m longitudinal sections. Section staining steps: after dewaxing the sample, antigens were repaired in 95°C 10 mM EDTA (pH = 9.0) for 15 min, and when the temperature cooled to room temperature, slides were washed with PBS, sealed with 5% BSA for 1 h, and stained using the primary antibodies, rabbit anti-CgA polyclonal antibody (1:500; Abcam #ab254322)⁶⁵, rat anti-5-HT monoclonal antibody (1:50; Abcam #ab6336)⁶⁶ overnight at 4°C. The next day, Alexa fluor 594 or 488 conjugated secondary antibodies, goat anti-rabbit IgG (1:1000 Invitrogen #A-11012) and goat anti-rat IgG (1:50 proteintech #SA00003-11), were incubated for 1 h at room temperature. For 5-HT (488) and CgA (594) staining, the number of positive staining spots on each intestinal villus in each field of view for the same sample was counted, and the average value for each sample was calculated. ImageJ software was used to artificially color, perform background correction, and merge monochrome images. The area of intestinal villi in each field of view for the same sample was circled using ImageJ software, and the average area was determined. The data were standardized by dividing the average number of positive cells by the average area of intestinal villi in the same sample.

High-performance liquid chromatography analysis (HPLC)

Each sample (50 ~ 60 mg) was homogenized in 150 μ L of cold solution A (0.4 M perchloric acid, protected from light), incubated on ice for 1 h, and then centrifuged for 20 min at 12,000 rpm at 4°C. The clear supernatant was transferred to a new clear tube, and then solution B (20 mM potassium citrate, 300 mM dipotassium hydrogen phosphate, 2 mM EDTA·2Na, half the volume of the transferred supernatant) was added and vortexed. The samples were further incubated on ice for another 1 h and then centrifuged for 20 min at 12,000 rpm at 4°C. The clear supernatant was transferred to a new clear tube and stored at -80°C. All samples were injected into the HPLC system (Liquid

chromatography-2695/2165, Waters, Central Laboratory of Capital Medical University).

Transmission electron microscope (TEM)

The preparation of transmission electron microscope samples was completed by the cell platform electron microscope room of the Biomedical Testing Center of Tsinghua University. Briefly, the intestinal tissue was fixed in 2.5% glutaraldehyde 2.0% paraformaldehyde solution at 4°C overnight to prepare 1 μ m semi thin section. An optical microscope is used to locate, observe, and select the appropriate section. After the location was determined, ultrathin sections were made and installed on the copper mesh, stained with 5% uranium acetate and lead citrate.

RNA extraction and quantitative real-time PCR (rt-qPCR)

Use FastRNA™ SPIN Kit for Yeast (MP Biomedicals, USA) extracted total RNA from isolated intestinal epithelial tissue (isolated the epithelial fraction from intestinal tissue). The purified RNA was reverse transcribed using FastKing RT Kit (With gDNase) kit (Tiangen, China), and the cDNA template was mixed with SYBR PowerUp Master Mix (Invitrogen, USA). Real-time PCR was performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Relative quantities (Δ cycle threshold values) were obtained by normalizing against those of β -actin (*Actb*). The primer sequences are shown in Supplementary Table S1.

Western blotting

Isolated intestinal epithelial tissue was lysed in RIPA buffer (C1053, Applygen Technologies) containing protease inhibitor cocktail (#04693116001, Roche) and phenylmethane-sulfonyl fluoride (PMSF) (#ST506, Beyotime). Protein concentration was measured using the BCA protein assay kit (#23225, Thermo Scientific). Protein samples were separated by using 4–20% SDS/PAGE (#B1005, Applygen Technologies). After blocking for 1 h in Tris-buffered saline-Tween (TBS-T)

containing 10% nonfat milk at room temperature and then incubated with primary antibodies overnight at 4°C, followed by incubation for 1 h at room temperature with the appropriate horseradish peroxidase-conjugated secondary antibodies, protein signals were detected by chemiluminescence (AI600 image system, GE Healthcare). The primary antibodies were rabbit anti-TPH1 polyclonal antibody (1:1,000 diluted; abcam; ab52954)⁶⁷ and mouse anti- β -actin monoclonal antibody (1:1,000 diluted; CST; #3700).

Short-circuit current (I_{SC}) measurement

I_{SC} is an electrophysiological experimental method used to detect the transport of electrogenic ions. The epithelium can be simplified to an equivalent circuit model, where the cellular pathway acts as a resistor in series with a battery, in parallel with a resistor representing the paracellular pathway. The jejunal mucosa was mounted between two halves of the Ussing chambers (exposed area of 0.30 cm²), equipped with water-jacketed gas lifts and were bathed on both sides with 5 ml K-HS, gassed with 95% O₂ and 5% CO₂, and maintained at 37°C by circulating the solution through a reservoir. The tissue was continuously voltage-clamped to zero potential difference by the application of an external current, and the current generated is I_{SC} . This current is generated by the ion flow on the biofilm, so the generated I_{SC} is the total active ion flow passing through the epithelial tissue. The tissues were allowed to rest for approximately 20 min to stabilize the I_{SC} prior to the addition of drugs. The transepithelial potential difference for each mucosa was measured with Ag/AgCl reference electrodes (Physiologic Instruments, San Diego, CA, USA; P2020S) connected to a preamplifier that was connected to a voltage clamp amplifier (Physiologic Instruments, San Diego, CA, USA; VCC MC6). 5-HT (10 μ M)-induced change in the short circuit current was calculated by the area under the curve for 15 min after the stimulation.

HEK293 cell culture and transfection

HEK293 cells were purchased from Procell Life Science and Technology (China) and cultured in DMEM (C11995500BT, Gibco) with 10% fetal bovine serum (10100147, Gibco). HEK293 cells

were seeded in 35 mm dishes 12 h before transfection. Lipofectamine 3000 (L3000015, Invitrogen) was used to transfect plasmid (2.5 μ g) in HEK293 cells.

Calcium imaging

The HEK293 cells were seeded in glass bottom cell culture dishes (NEST, China) and transfected as described above. A 1 mM Fluo-4 AM (F14021, Thermo) stock solution was diluted to 5 μ M Fluo-4 AM working solution using Hank's balanced salt solution (HBSS). Then, cells were incubated with 5 μ M Fluo-4 AM working solution for 30 min at 37°C. The fluorescence signal acquisition instrument is OLYMPUS FLUOVIEW FV3000 (OLYMPUS, Japan), and the excitation wavelength is 488 nm. Image J software was used to analyze the fluorescence intensity. Image analysis was performed as reported earlier.⁶⁸

Patch-clamp experiment

Patch-clamp recordings were performed at room temperature with a HEKA EPC-10 patch-clamp amplifier and recorded by associated PatchMaster software (HEKA Electronic, Germany). Cell-attached patch configuration of patch-clamp technique was used. For HEK293 cells, the bath solution contained 140 mM NaCl, 4 mM CsCl, 1 mM MgCl₂, 1 mM EGTA, 10 mM HEPES, 10 mM Glucose, with pH adjusted to 7.4; and the pipette solution contained 140 mM CsCl, 10 mM NaCl, 1 mM MgCl₂, 5 mM EGTA, 10 mM HEPES, 2 mM Mg-ATP, with pH adjusted to 7.3. Membrane potential was holding at -100 mV. Patch pipettes were resisted between 4 and 6 M Ω . Data analyze was performed as reported earlier.⁶⁸

Metagenomic data analysis

The total DNA in fecal samples was extracted utilizing MagBeads FastDNA Kit (MP Biomedicals). The purity of extracted DNA was evaluated by the Nanodrop, the concentration was detected by the Qubit instrument, and the integrity of DNA chain was detected by agarose gel electrophoresis.

The native barcoding genomic DNA (with EXP-NBD104, EXP-NBD114, and SQK-LSK109) of

Oxford Nanopore Technologies Co., Ltd. was used to construct the metagenomic sequencing library. A brief operation process was as follows: Since the end of the DNA was repaired, it could be connected with Barcode was purified by method using magnetic bead, and then different DNA was connected with their own specific Barcodes under the action of ligase. DNA strand was then connected with a sequencing connector to prepare a DNA library. The DNA library was loaded into the sequencing chip, and the chip was performed with the MinION Mk1C instrument.

Raw fast5 sequencing signals were generated using the MinKNOW instrument. The fast5 files were base called using Guppy Basecalling Software (v6.5.7) to obtain fastq files. The passed reads were then trimmed for adapters and split with barcode using guppy_barcode (v6.5.7). The parameter “-enable_trim_barcodes” was utilized to remove reads with internal barcode adapters. Statistical analysis of the MinION sequencing data was performed in R and visualized.

Metagenomic sequencing reads were mapped to the nucleotide database of NCBI (nt-NCBI) using the k-mer-based metagenomic classification tool Centrifuge (v1.0.4). The gut microbial composition was represented as the relative abundance of taxa. The relative abundance of each taxon at phylum, class, order, family, genus, and species levels was computed as the proportion of reads assigned to the respective taxonomical clade among the total classified reads.

Alpha-diversity and beta-diversity were calculated at the genus level using functions from the R package vegan (v.2.5). To identify the different abundant taxa between different sample groups, linear discriminant analysis effect size (LEfSe) was conducted using the LEfSe software.

Metabolomics data analysis

Bile acid separation was carried out using an UHPLC System (Vanquish, Thermo Fisher Scientific), equipped with a Waters ACQUITY UPLC BEH C18 column (150 × 2.1 mm, 1.7 μm, Waters). The mobile phase A was 1 mmol/L ammonium acetate and 0.1% acetic acid in water, and the mobile phase B was acetonitrile. The column temperature was set at 50°C. The auto-

sampler temperature was set at 4°C, and the injection volume was 1 μL. A Q Exactive HFX mass spectrometer (Thermo Fisher Scientific) was applied for assay development. Typical ion source parameters were as follows: spray voltage = +3500/-3100 V, sheath gas (N₂) flow rate = 40, aux gas (N₂) flow rate = 15, sweep gas (N₂) flow rate = 0, aux gas (N₂) temperature = 350°C, capillary temperature = 320°C.

Data were scaled and logarithmic transformed to minimize the impact of both noise and high variance of the variables. After these transformations, PCoA (Principal components analysis, PCoA), an unsupervised analysis that reduces the dimension of the data, was carried out to visualize the distribution and the grouping of the samples. Ninety-five percent confidence interval in the PCoA score plot was used as the threshold to identify potential outliers in the dataset. Fold changes were calculated as mass response ratio between two arbitrary groups (HIGH vs. LOW). The variable contribution of the OPLS-DA model was ranked by the variable importance in the projection (VIP). Metabolites passing the threshold of VIP > 1 were considered as significantly different between the HIGH and LOW groups, which were validated at a univariate level with $p < 0.05$.

RNA sequencing

Total RNA was extracted with Trizol (15596018, Thermo Scientific) from the intestinal epithelial tissue. RNA integrity testing and library construction were performed as reported earlier.⁶⁹ The quality evaluation of RNA-Seq raw reads was performed with the FastQC (v.0.11.9). Reads were pre-processed with trim_galore (v0.6.6) software to trim adaptor sequences and reads with low quality. Clean reads from each library were independently aligned to *Mus musculus* (mm10), using STAR (v2.7.2a) software with default parameters, followed by gene expression quantification which was performed with featureCounts (v1.6.3). The differential expression analysis was carried out with the Bioconductor R package DESeq2. $p \leq 0.05$, log₂ (Fold Change) > 2 or log₂ (Fold Change) < -2 were set as the thresholds for significantly differential expression. Then, hierarchical clustering analysis of DEGs was performed to

explore gene expression patterns. We used clusterProfiler software to perform Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the DEG sets.

Statistical analysis

All statistics and graphs were generated using GraphPad Prism, version 8.0 (GraphPad Software, San Diego, Calif., USA). The data were given as arithmetic means $\pm$ SEM; “*n*” refers to the number of mice or the number of cells from different batches. Statistical analyses were performed by Student’s unpaired *t*-test, One-way ANOVA, and Two-way ANOVA after confirming the normal distribution of data and homogeneity of the variance. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 were assumed to denote a significant difference.

Abbreviations

HFD	High-fat diet
<i>Bacteroides vulgatus</i>	<i>B.vulgatus</i>
LBM	lean body mass
FMT	Fecal microbiota transplantation
SPF	specific pathogen free
GF	germ-free
PCoA	Principal Component Analysis
LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis effect size
eWAT	epididymal white adipose tissue
AUC	Area under curve
CA	Cholic acid
β -MCA	β -Muricholic acid
TC	Total Cholesterol
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
TG	Triglyceride
5-HT	5-hydroxytryptamine
CgA	Chromogranin A

NMDS

BSH

CLD

CM

TPH1

TPH2

ECs

TEM

*I*_{SC}

Non-Metric Multi-Dimensional Scaling
bile salt hydrolases
cellular lipid droplets
Chyle microparticle
tryptophan hydroxylase-1
tryptophan hydroxylase-2
enterochromaffin cells
transmission electron microscope
Short-circuit current

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Disclosure statement

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Author contributions

W.W., X.L. and H.H. designed and led the study. X.W. and R. A. performed the majority of animal experiments and analyzed the mouse phenotypes. F.X. cultured the bacteria used in vitro and in vivo experiments. X.W., X.F. and F.X. performed Patch-clamp experiments and Short-circuit current (*I*_{SC}) measurement. HW.H. analyzed metagenomic data analysis, metabolomics data analysis and RNA sequencing analysis. L.M., J.Z. and P.L. performed the cell biology and molecular experiments. X.W. and X.F. wrote and edited large parts of the manuscript and provided additional data. X.W. and X.F. made the figures. W.W. provided funding

acquisition. All authors contributed to the data analysis. The authors read and approved the final manuscript.

Data availability statement

All the metagenomics sequencing data has been deposited in the Genome Sequence Archive in BIG Data Center (<https://bigd.big.ac.cn/>), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under the accession number: CRA012033 (BioProject: PRJCA018657). All transcriptome sequencing reads data was deposited in the Genome Sequence Archive in BIG Data Center, under the accession number: CRA012026 (BioProject: PRJCA018608). Metabolomics mass spectrometry data has been deposited in the Genome Sequence Archive in BIG Data Center, under the accession number: OMIX004646 (BioProject: PRJCA018694), and statistical analysis results are shown in Supplementary Table S2.

References

- Wang Y, Zhao L, Gao L, Pan A, Xue H. Health policy and public health implications of obesity in China. *Lancet Diabetes Endocrinol.* 2021;9(7):446–461. doi:10.1016/S2213-8587(21)00118-2.
- Piche ME, Tchernof A, Despres JP. Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res.* 2020;126(11):1477–1500. doi:10.1161/CIRCRESAHA.120.316101.
- Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, et al. The gut microbiota and host health: a new clinical frontier. *Gut.* 2016;65(2):330–339. doi:10.1136/gutjnl-2015-309990.
- Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JL. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA.* 2004;101(44):15718–15723. doi:10.1073/pnas.0407076101.
- Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? *Gut microbes.* *Gut Microbes.* 2017;8(2):130–142. doi:10.1080/19490976.2016.1270811.
- Aron-Wisnewsky J, Warmbrunn MV, Nieuwdorp M, Clement K. Metabolism and metabolic disorders and the microbiome: the intestinal microbiota associated with obesity, lipid metabolism, and metabolic health-pathophysiology and therapeutic strategies. *Gastroenterology.* 2021;160(2):573–599. doi:10.1053/j.gastro.2020.10.057.
- Zeybel M, Arif M, Li X, Altay O, Yang H, Shi M, Akyildiz M, Saglam B, Gonenli MG, Yigit B, et al. Multiomics analysis reveals the impact of microbiota on host metabolism in hepatic steatosis. *Adv Sci (Weinh).* 2022;9(11):e2104373. doi:10.1002/adv.202104373.
- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. *Nature.* 2009;457(7228):480–484. doi:10.1038/nature07540.
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JL. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444(7122):1027–1031. doi:10.1038/nature05414.
- Cocci P, Moruzzi M, Martinelli I, Maggi F, Micioni Di Bonaventura MV, Cifani C, Mosconi G, Tayebati SK, Damiano S, Lupidi G, et al. Tart cherry (*Prunus cerasus* L.) dietary supplement modulates visceral adipose tissue CB1 mRNA levels along with other adipogenesis-related genes in rat models of diet-induced obesity. *Eur J Nutr.* 2021;60(5):2695–2707. doi:10.1007/s00394-020-02459-y.
- Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: metabolism and perspective in obesity. *Gut Microbes.* 2018;9(4):308–325. doi:10.1080/19490976.2018.1465157.
- Wang H, Chen S, Tang Y, Nie K, Gao Y, Wang Z, Su H, Wu F, Gong J, Fang K, et al. Berberine promotes lacteal junction zippering and ameliorates diet-induced obesity through the RhoA/ROCK signaling pathway. *Phytomedicine.* 2024;124:124155268. doi:10.1016/j.phymed.2023.155268.
- Tytgat GN, Rubin CE, Saunders DR. Synthesis and transport of lipoprotein particles by intestinal absorptive cells in man. *J Clin Invest.* 1971;50(10):2065–2078. doi:10.1172/JCI106700.
- Martinez-Guryn K, Hubert N, Frazier K, Urllass S, Musch MW, Ojeda P, Pierre JF, Miyoshi J, Sontag TJ, Cham CM, et al. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell Host Microbe.* 2018;23(4):458–469. e5. doi:10.1016/j.chom.2018.03.011.
- Zong X, Zhang H, Zhu L, Deehan EC, Fu J, Wang Y, Jin M. Auricularia auricula polysaccharides attenuate obesity in mice through gut commensal *Papillibacter cinnamivorans*. *J Adv Res.* 2023;52:52203–52218. doi:10.1016/j.jare.2023.08.003.
- Rabot S, Membrez M, Bruneau A, Gerard P, Harach T, Moser M, Raymond F, Mansourian R, Chou CJ. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J.* 2010;24(12):4948–4959. doi:10.1096/fj.10-164921.
- Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell.* 2015;161(2):264–276. doi:10.1016/j.cell.2015.02.047.

18. Tecott LH. Serotonin and the orchestration of energy balance. *Cell Metab.* **2007**;6(5):352–361. doi:[10.1016/j.cmet.2007.09.012](https://doi.org/10.1016/j.cmet.2007.09.012).
19. Crane JD, Palanivel R, Mottillo EP, Bujak AL, Wang H, Ford RJ, Collins A, Blumer RM, Fullerton MD, Yabut JM, et al. Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. *Nat Med.* **2015**;21(2):166–172. doi:[10.1038/nm.3766](https://doi.org/10.1038/nm.3766).
20. Jiang L, Han D, Hao Y, Song Z, Sun Z, Dai Z. Linking serotonin homeostasis to gut function: nutrition, gut microbiota and beyond. *Crit Rev Food Sci Nutr.* **2023**;64(21):1–20. doi:[10.1080/10408398.2023.2183935](https://doi.org/10.1080/10408398.2023.2183935).
21. Zhu P, Lu T, Wu J, Fan D, Liu B, Zhu X, Guo H, Du Y, Liu F, Tian Y, et al. Gut microbiota drives macrophage-dependent self-renewal of intestinal stem cells via niche enteric serotonergic neurons. *Cell Res.* **2022**;32(6):555–569. doi:[10.1038/s41422-022-00645-7](https://doi.org/10.1038/s41422-022-00645-7).
22. Muller PA, Matheis F, Schneeberger M, Kerner Z, Jove V, Mucida D. Microbiota-modulated CART(+) enteric neurons autonomously regulate blood glucose. *Science.* **2020**;370(6514):314–321. doi:[10.1126/science.abd6176](https://doi.org/10.1126/science.abd6176).
23. Hegyi P, Maleth J, Walters JR, Hofmann AF, Keely SJ. Guts and Gall: bile acids in regulation of intestinal epithelial function in health and disease. *Physiol Rev.* **2018**;98(4):1983–2023. doi:[10.1152/physrev.00054.2017](https://doi.org/10.1152/physrev.00054.2017).
24. Araujo JR, Tazi A, Burlen-Defranoux O, Vichier-Guerre S, Nigro G, Licandro H, Demignot S, Sansonetti PJ. Fermentation products of commensal bacteria alter enterocyte lipid metabolism. *Cell Host Microbe.* **2020**;27(3):358–375.e7. doi:[10.1016/j.chom.2020.01.028](https://doi.org/10.1016/j.chom.2020.01.028).
25. Poland JC, Flynn CR. Bile acids, their receptors, and the gut microbiota. *Physiology (Bethesda).* **2021**;36(4):235–245. doi:[10.1152/physiol.00028.2020](https://doi.org/10.1152/physiol.00028.2020).
26. Tso P, Balint JA. Formation and transport of chylomicrons by enterocytes to the lymphatics. *Am J Physiol.* **1986**;250(6 Pt 1):G715–26. doi:[10.1152/ajpgi.1986.250.6.G715](https://doi.org/10.1152/ajpgi.1986.250.6.G715).
27. Yabut JM, Crane JD, Green AE, Keating DJ, Khan WI, Steinberg GR. Emerging roles for serotonin in regulating metabolism: new implications for an ancient molecule. *Endocr Rev.* **2019**;40(4):1092–1107. doi:[10.1210/er.2018-00283](https://doi.org/10.1210/er.2018-00283).
28. Gustafsson JK, Johansson MEV. The role of goblet cells and mucus in intestinal homeostasis. *Nat Rev Gastroenterol Hepatol.* **2022**;19(12):785–803. doi:[10.1038/s41575-022-00675-x](https://doi.org/10.1038/s41575-022-00675-x).
29. Karwad MA, Macpherson T, Wang B, Theophilidou E, Sarmad S, Barrett DA, Larvin M, Wright KL, Lund JN, O'Sullivan SE. Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARalpha. *FASEB J.* **2017**;31(2):469–481. doi:[10.1096/fj.201500132](https://doi.org/10.1096/fj.201500132).
30. Abdalla SS, Harb AA, Almasri IM, Bustanji YK. The interaction of TRPV1 and lipids: insights into lipid metabolism. *Front Physiol.* **2022**;13:131066023. doi:[10.3389/fphys.2022.1066023](https://doi.org/10.3389/fphys.2022.1066023).
31. Yan X, Zhang S, Zhao H, Liu P, Huang H, Niu W, Wang W, Zhang C. ASIC2 synergizes with TRPV1 in the mechano-electrical transduction of arterial baroreceptors. *Neurosci Bull.* **2021**;37(10):1381–1396. doi:[10.1007/s12264-021-00737-1](https://doi.org/10.1007/s12264-021-00737-1).
32. Wang L, Wang DH. TRPV1 gene knockout impairs postischemic recovery in isolated perfused heart in mice. *Circulation.* **2005**;112(23):3617–3623. doi:[10.1161/CIRCULATIONAHA.105.556274](https://doi.org/10.1161/CIRCULATIONAHA.105.556274).
33. Hong S, Fan J, Kemmerer ES, Evans S, Li Y, Wiley JW. Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. *Gut.* **2009**;58(2):202–210. doi:[10.1136/gut.2008.157594](https://doi.org/10.1136/gut.2008.157594).
34. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron.* **1998**;21(3):531–543. doi:[10.1016/s0896-6273\(00\)80564-4](https://doi.org/10.1016/s0896-6273(00)80564-4).
35. Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CS, Yeats JC. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br J Pharmacol.* **1992**;107(2):544–552. doi:[10.1111/j.1476-5381.1992.tb12781.x](https://doi.org/10.1111/j.1476-5381.1992.tb12781.x).
36. Lee JY, Tsolis RM, Baumler AJ. The microbiome and gut homeostasis. *Science.* **2022**;377(6601):eabp9960. doi:[10.1126/science.abp9960](https://doi.org/10.1126/science.abp9960).
37. Davis SC, Yadav JS, Barrow SD, Robertson BK. Gut microbiome diversity influenced more by the westernized dietary regime than the body mass index as assessed using effect size statistic. *Microbiologyopen.* **2017**;6(4). doi:[10.1002/mbo3.476](https://doi.org/10.1002/mbo3.476).
38. Chavez-Carbajal A, Nirmalkar K, Perez-Lizaur A, Hernandez-Quiroz F, Ramirez-Del-Alto S, Garcia-Mena J, Hernandez-Guerrero C. Gut microbiota and predicted metabolic pathways in a sample of Mexican women affected by obesity and obesity plus metabolic syndrome. *Int J Mol Sci.* **2019**;20(2):438. doi:[10.3390/ijms20020438](https://doi.org/10.3390/ijms20020438).
39. Osborne G, Wu F, Yang L, Kelly D, Hu J, Li H, Jasmine F, Kibriya MG, Parvez F, Shaheen I, et al. The association between gut microbiome and anthropometric measurements in Bangladesh. *Gut Microbes.* **2020**;11(1):63–76. doi:[10.1080/19490976.2019.1614394](https://doi.org/10.1080/19490976.2019.1614394).
40. Qi X, Yun C, Sun L, Xia J, Wu Q, Wang Y, Wang L, Zhang Y, Liang X, Wang L, et al. Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome. *Nat Med.* **2019**;25(8):1225–1233. doi:[10.1038/s41591-019-0509-0](https://doi.org/10.1038/s41591-019-0509-0).
41. Stellwag EJ, Hylemon PB. Purification and characterization of bile salt hydrolase from *Bacteroides fragilis* subsp. *fragilis*. *Biochim Biophys Acta.* **1976**;452(1):165–176. doi:[10.1016/0005-2744\(76\)90068-1](https://doi.org/10.1016/0005-2744(76)90068-1).

42. Jones BV, Begley M, Hill C, Gahan CG, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci USA*. 2008;105(36):13580–13585. doi:[10.1073/pnas.0804437105](https://doi.org/10.1073/pnas.0804437105).
43. Song Z, Cai Y, Lao X, Wang X, Lin X, Cui Y, Kalavagunta PK, Liao J, Jin L, Shang J, et al. Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome*. 2019;7(1):9. doi:[10.1186/s40168-019-0628-3](https://doi.org/10.1186/s40168-019-0628-3).
44. Jia B, Park D, Hahn Y, Jeon CO. Metagenomic analysis of the human microbiome reveals the association between the abundance of gut bile salt hydrolases and host health. *Gut Microbes*. 2020;11(5):1300–1313. doi:[10.1080/19490976.2020.1748261](https://doi.org/10.1080/19490976.2020.1748261).
45. Zheng X, Huang F, Zhao A, Lei S, Zhang Y, Xie G, Chen T, Qu C, Rajani C, Dong B, et al. Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice. *BMC Biol*. 2017;15(1):120. doi:[10.1186/s12915-017-0462-7](https://doi.org/10.1186/s12915-017-0462-7).
46. Liu C, Du MX, Xie LS, Wang WZ, Chen BS, Yun CY, Sun XW, Luo X, Jiang Y, Wang K, et al. Gut commensal *Christensenella minuta* modulates host metabolism via acylated secondary bile acids. *Nat Microbiol*. 2024;9(2):434–450. doi:[10.1038/s41564-023-01570-0](https://doi.org/10.1038/s41564-023-01570-0).
47. Ko CW, Qu J, Black DD, Tso P. Regulation of intestinal lipid metabolism: current concepts and relevance to disease. *Nat Rev Gastroenterol Hepatol*. 2020;17(3):169–183. doi:[10.1038/s41575-019-0250-7](https://doi.org/10.1038/s41575-019-0250-7).
48. D'Aquila T, Hung YH, Carreiro A, Buhman KK. Recent discoveries on absorption of dietary fat: presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes. *Biochim Biophys Acta*. 2016;1861(8 Pt A):730–747. doi:[10.1016/j.bbalip.2016.04.012](https://doi.org/10.1016/j.bbalip.2016.04.012).
49. Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M. Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain*. 2005;1:13. doi:[10.1186/1744-8069-1-3](https://doi.org/10.1186/1744-8069-1-3).
50. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA*. 2009;106(10):3698–3703. doi:[10.1073/pnas.0812874106](https://doi.org/10.1073/pnas.0812874106).
51. Huttner A, Bielicki J, Clements MN, Frimodt-Moller N, Muller AE, Paccaud JP, Mouton JW. Oral amoxicillin and amoxicillin-clavulanic acid: properties, indications and usage. *Clin Microbiol Infect*. 2020;26(7):871–879. doi:[10.1016/j.cmi.2019.11.028](https://doi.org/10.1016/j.cmi.2019.11.028).
52. Modasia A, Parker A, Jones E, Stentz R, Brion A, Goldson A, Defernez M, Wileman T, Ashley Blackshaw L, Carding SR. Regulation of enteroendocrine cell networks by the major human gut symbiont bacteroides thetaiotaomicron. *Front Microbiol*. 2020;11:11575595. doi:[10.3389/fmicb.2020.575595](https://doi.org/10.3389/fmicb.2020.575595).
53. Watanabe H, Akasaka D, Ogasawara H, Sato K, Miyake M, Saito K, Takahashi Y, Kanaya T, Takakura I, Hondo T, et al. Peripheral serotonin enhances lipid metabolism by accelerating bile acid turnover. *Endocrinology*. 2010;151(10):4776–4786. doi:[10.1210/en.2009-1349](https://doi.org/10.1210/en.2009-1349).
54. Sorrentino G, Perino A, Yildiz E, El Alam G, Bou Sleiman M, Gioiello A, Pellicciari R, Schoonjans K. Bile acids signal via TGR5 to activate intestinal stem cells and epithelial regeneration. *Gastroenterology*. 2020;159(3):956–968 e8. doi:[10.1053/j.gastro.2020.05.067](https://doi.org/10.1053/j.gastro.2020.05.067).
55. Lertsuwan K, Wongdee K, Teerapornpuntakit J, Charoenphandhu N. Intestinal calcium transport and its regulation in thalassemia: interaction between calcium and iron metabolism. *J Physiol Sci*. 2018;68(3):221–232. doi:[10.1007/s12576-018-0600-1](https://doi.org/10.1007/s12576-018-0600-1).
56. Zhao JF, Ching LC, Kou YR, Lin SJ, Wei J, Shyue SK, Lee TS. Activation of TRPV1 prevents OxLDL-induced lipid accumulation and TNF- α -induced inflammation in macrophages: role of liver X receptor α . *Mediators Inflamm*. 2013;2013:1–14. doi:[10.1155/2013/925171](https://doi.org/10.1155/2013/925171).
57. Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature*. 2000;408(6815):985–990. doi:[10.1038/35050121](https://doi.org/10.1038/35050121).
58. Su YS, Chiu YY, Lin SY, Chen CC, Sun WH. Serotonin receptor 2B mediates mechanical hyperalgesia by regulating transient receptor potential vanilloid 1. *J Mol Neurosci*. 2016;59(1):113–125. doi:[10.1007/s12031-015-0693-4](https://doi.org/10.1007/s12031-015-0693-4).
59. Evans C, Howells K, Suzuki R, Brown AJH, Cox HM. Regional characterisation of TRPV1 and TRPA1 signalling in the mouse colon mucosa. *Eur J Pharmacol*. 2023;954:954175897. doi:[10.1016/j.ejphar.2023.175897](https://doi.org/10.1016/j.ejphar.2023.175897).
60. Frank JA, Moroni M, Moshourab R, Sumser M, Lewin GR, Trauner D. Photoswitchable fatty acids enable optical control of TRPV1. *Nat Commun*. 2015;6(1):67118. doi:[10.1038/ncomms8118](https://doi.org/10.1038/ncomms8118).
61. Zhao R, Coker OO, Wu J, Zhou Y, Zhao L, Nakatsu G, Bian X, Wei H, Chan AWH, Sung JYJ, et al. Aspirin reduces colorectal tumor development in mice and gut microbes reduce its bioavailability and chemopreventive effects. *Gastroenterology*. 2020;159(3):969–983 e4. doi:[10.1053/j.gastro.2020.05.004](https://doi.org/10.1053/j.gastro.2020.05.004).
62. Rey K, Manku S, Enns W, Van Rossum T, Bushell K, Morin RD, Brinkman FSL, Choy JC. Disruption of the gut microbiota with antibiotics exacerbates acute vascular rejection. *Transplantation*. 2018;102(7):1085–1095. doi:[10.1097/TP.0000000000002169](https://doi.org/10.1097/TP.0000000000002169).
63. Li Q, Ma L, Shen S, Guo Y, Cao Q, Cai X, Feng J, Yan Y, Hu T, Luo S, et al. Intestinal dysbacteriosis-induced IL-25 promotes development of HCC via alternative activation of macrophages in tumor microenvironment. *J Exp Clin Cancer Res*. 2019;38(1):303. doi:[10.1186/s13046-019-1271-3](https://doi.org/10.1186/s13046-019-1271-3).
64. Zhou W, Yang T, Xu W, Huang Y, Ran L, Yan Y, Mi J, Lu L, Sun Y, Zeng X, et al. The polysaccharides from the fruits of *Lycium barbarum* L. confer anti-diabetic effect

- by regulating gut microbiota and intestinal barrier. *Carbohydr Polym.* **2022**;291:291119626. doi:[10.1016/j.carbpol.2022.119626](https://doi.org/10.1016/j.carbpol.2022.119626).
65. Shan TD, Yue H, Sun XG, Jiang YP, Chen L. Rspo3 regulates the abnormal differentiation of small intestinal epithelial cells in diabetic state. *STEM Cell Res Ther.* **2021**;12(1):330. doi:[10.1186/s13287-021-02385-8](https://doi.org/10.1186/s13287-021-02385-8).
66. Norkin M, Ordonez-Moran P, Huelsken J. High-content, targeted RNA-seq screening in organoids for drug discovery in colorectal cancer. *Cell Rep.* **2021**;35(3):109026. doi:[10.1016/j.celrep.2021.109026](https://doi.org/10.1016/j.celrep.2021.109026).
67. Yi G, Safdar B, Zhang Y, Li Y, Liu X. A study of the mechanism of small-molecule soybean-protein-derived peptide supplement to promote sleep in a mouse model. *RSC Adv.* **2020**;10(19):11264–11273. doi:[10.1039/d0ra00389a](https://doi.org/10.1039/d0ra00389a).
68. Ren J, Cheng Y, Wen X, Liu P, Zhao F, Xin F, Wang M, Huang H, Wang W. Bk(ca) channel participates in insulin-induced lipid deposition in adipocytes by increasing intracellular calcium. *J Cell Physiol.* **2021**;236(8):5818–5831. doi:[10.1002/jcp.30266](https://doi.org/10.1002/jcp.30266).
69. Xin F, Cheng Y, Wen X, Zhang J, Shi X, Liu P, Ren J, Lu W, Liu F, Li Z, et al. BK channel depletion promotes adipocyte differentiation by activating the MAPK/ERK pathway. *STEM Cells.* **2024**;42(2):146–157. doi:[10.1093/stmcls/sxad082](https://doi.org/10.1093/stmcls/sxad082).