Food & Function

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2014, **5**, 1241

Effects of resveratrol on gut microbiota and fat storage in a mouse model with high-fat-induced obesity

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Recent studies have investigated the anti-obesity effect of resveratrol, but the pathways through which resveratrol resists obesity are not clear. In the present study, we hypothesize that resveratrol exerts anti-obesity effects that are likely mediated by mechanisms of regulating gut microbes, and in turn, improving fat storage and metabolism. Gut microbes, glucose and lipid metabolism in high-fat diet (HF) mice *in vivo* are investigated after resveratrol treatment. Several biochemical markers are measured. Fluorescence *in situ* hybridization and flow cytometry are used to monitor and quantify the changes in gut microbiota. The key genes related to fat storage and metabolism in the liver and visceral adipose tissues are measured by real-time PCR. The results show that resveratrol (200 mg per kg per day) significantly lowers both body and visceral adipose weights, and reduces blood glucose and lipid levels in HF mice. Resveratrol improves the gut microbiota dysbiosis induced by the HF diet, including increasing the Bacteroidetes-to-Firmicutes ratios, significantly inhibiting the growth of *Enterococcus faecalis*, and increasing the growth of *Lactobacillus* and *Bifidobacterium*. Furthermore, resveratrol significantly increases the fasting-induced adipose factor (*Fiaf*, a key gene negatively regulated by intestinal microbes) expression in the intestine. Resveratrol significantly decreases mRNA expression of *Lpl*, *Scd1*, *Ppar-γ*, *Acc1*, and *Fas* related to fatty acids synthesis, adipogenesis and lipogenesis, which may be driven by increased *Fiaf* expression. The Pearson's correlation coefficient shows that there is a negative correlation between the body weight and the ratios of Bacteroidetes-to-Firmicutes. Therefore, resveratrol mediates the composition of gut microbes, and in turn, through the *Fiaf* signaling pathway, accelerates the development of obesity.

Received 26th November 2013
Accepted 1st April 2014

DOI: 10.1039/c3fo60630a

www.rsc.org/foodfunction

Introduction

Obesity has emerged since the late 1990s with a stable and increasing tendency and has affected millions of individuals in the Western world.[1] Obesity, bearing the features of the metabolic syndrome (MS), has profound impacts on cardiovascular diseases, insulin resistance and type 2 diabetes.[2,3] However, the prevention and treatment of obesity remain significant challenges to medical scientists.[4] Gut microbiota, so-called second genome, play an important role in human nutrition and health, by promoting nutrient supply, preventing pathogen colonization, maintaining normal mucosal immunity, and regulating fat storage and metabolism.[5,6] Commensal microbiota contribute substantially to the overall body weight because germ-free (GF) mice have less body fat and do not become obese when fed a HFD.[5,6] Samuel *et al.* showed that cocolonization of gnotobiotic mice with *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii* increased the ability of the host to harvest and store calories from the diet.[7] In addition, analysis of GF and conventionalized, normal and *Fiaf* knockout mice established that the fasting-induced adipocyte factor (*Fiaf*) is a circulating lipoprotein lipase inhibitor and that its suppression is essential for the microbiota-induced deposition of triglycerides in adipocytes.[8] So, new targets for a better and more effective modulation of intestinal microflora are being developed.

Resveratrol, (3,5,4′-trihydroxy-*trans*-stilbene, RES), a natural polyphenolic compound, occurs in grapes and red wine and exerts antioxidant, anti-inflammatory,[9] antitumor, cardioprotective and aging-delay effects.[10] Recently, the anti-obesity effect of resveratrol has been reported; it improves thermogenesis in the brown adipose tissue[11] and attenuates the expression of key adipogenic transcription factors, including FAS, aP2, and LPL.[12] However, the proportion of resveratrol in the human diet is low.[13] In the Spanish population, the estimated dose of daily resveratrol intake is 0.9 mg per day.[14] Moreover, resveratrol has low bioavailability after oral administration but high accumulation in the intestinal tissue.[15,16] Azorín-Ortuño *et al.* showed that approximately 74.5% of the

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total resveratrol administered was recovered in the form of resveratrol, dihydroresveratrol and derived metabolites (65.1% along the gastrointestinal tract, and 0.5% in other tissues).[17] So, it may be very difficult to reach effective concentrations in metabolism regulating tissues *in vivo*, *e.g.* visceral adipose tissues. Polyphenol bioavailability and absorption may be influenced by its chemical structure, food matrix, and enterohepatic circulation; a high percentage of resveratrol is not absorbed in the small intestine, it arrives intact at the colon, where it may interact with microflora.[18] Therefore, it seems to be more reasonable to conclude that the effects of resveratrol might mediate by targeting on gut microbiota.

Few studies have investigated the effect of dietary resveratrol on the complex gut microbiota *in vivo*, and they focused mainly on a single bacterial population and polyphenols in red wine, tea, and coffee. Taken together, in the present study, we hypothesize that resveratrol exerts anti-obesity effects partly mediated by mechanisms of regulating the composition of gut microbes and this, in turn, may affect their functional relationships with host fat storage and metabolism. The effects of resveratrol on gut microbiota, obesity, and their relationships are investigated in high-fat diet-fed obesity mice.

Materials and methods

Diets and animals

Male Kunming mice were obtained from Shanghai Slac Laboratory Animal, Co., Ltd. (Shanghai, China), which were kept in an environmentally controlled breeding room (temperature: 23 ± 2 °C, humidity: $60 \pm 5\%$, 12 hour light–dark cycle) and fed a normal diet (total calories 3.25 kcal g^{-1}, 10% calories in fat) or a high-fat diet (HF) (total calories 3.68 kcal g^{-1}, 50% calories in fat), or a high-fat diet supplemented with resveratrol (HF-RES) ($n = 8$) at a concentration of 200 mg per kg per day for 12 weeks.[19] Resveratrol (>99%) was purchased from Sigma-Aldrich (China) and mixed to homogeneity during preparation of the diets. The study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Care and Use Committee of Jiangnan University. Mice were killed by cervical dislocation after a 6 h period of fasting. The ileum, liver, and visceral adipose tissues were dissected, immersed in liquid nitrogen and stored at −80 °C. The colonic contents were collected for further analysis.

Biochemical analysis

After a 6 h fasting period, blood samples were collected and were isolated by centrifugation at 1500 × *g* at 4 °C for 10 min. Blood glucose and insulin were assayed using commercial kits (Jingmei BioTech Co. Ltd, Shenzhen, China). Blood glucose was estimated by the glucose oxidase method.[20] Concentrations of total cholesterol (TC), triglycerides, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) in serum were determined by enzymatic colorimetric methods using commercial kits (Shanghai Rongsheng Biotech Co., Ltd., Shanghai, China).

Colonic sample collection, fixation and fluorescence *in situ* hybridization (FISH)

The cell-fixation procedure was adapted from Schwiertz *et al.*[21] One volume of the colonic contents was suspended in 9 ml of phosphate-buffered saline (PBS) and vortexed with glass beads for 3 min; then 0.2 ml of the suspension was fixed in 0.6 ml fresh 4% paraformaldehyde at 4 °C overnight. After fixation, the bacteria were stored in 50% ethanol–PBS at −20 °C for further analysis.

The group- or genus-specific oligonucleotide probes targeted the bacterial 16S rRNA, including the *Bacteroides–Prevotella* group (Bac303, 5′-CCAATGTGGGGGACCTT), the *Clostridium cluster I & II* group (Chis150, 5′-TTATGCGGTATTAATC-TYCCTTT), the *Enterobacteriaceae* group (Enter1432, 5′-GTTTTGCAACCCACT), the *Lactobacillus* group (Lab158, 5′-GGTATTAGCA(C/T)CTGTTTCCA), the *Eubacterium rectale* group (Mib724, 5′-GCTTCTTAGTCARGTACCG), the *Atopobium* group (Ato291, 5′-GGTCGGTCTCTCAACCC), the *Bifidobacterium* group (Bif164, 5′-CATCCGGCATTACCACCC), the *Enterococcus faecalis* (Enf191, 5′-GAAAGCGCCTTTCACTCTTATGC), and the *Clostridium coccoides–Eubacterium rectale cluster* group (Erec482, 5′-GCTTCTTAGTCA- GGTACCG) were used for enumeration of dominant members of the gut microbiota. The EUB338 probe used to determine total counts was labeled with fluorescein isothiocyanate (FITC), while the group- or genus-specific probes were labeled at the 5′-end with indocyanine (Cy3; Sangon Biotech, Shanghai, China).

Hybridization was carried out as previously described.[22] Hybridization was performed in a 50 μl of hybridization solution containing the probe resulting in a final concentration of 4 ng μl^{-1}. After hybridization, 150 μl of hybridization solution was added, centrifuged at 4000 × *g* for 15 min at room temperature and then incubated in 200 μl of washing solution (65 mmol l^{-1} NaCl, 20 mmol l^{-1} Tris–HCl, pH 8.0, 5 mmol l^{-1} EDTA, pH 8.0, 0.01% SDS) at 37 °C for 20 min. Aliquots of 200 μl were added to 500 μl of PBS for flow cytometry with a FACS Calibur device (Becton Dickinson and Co, Franklin Lakes, NJ) as previously described.[23] The flow rate of events was generally 200–300 events per s. A total of 20 000 events were recorded for each sample. Data were analyzed on line with the use of the BD FACSDIVA software version 4.1.1 (Becton Dickinson and Co).

Histology

Randomly selected epididymal fat and liver tissues obtained from mice were washed with PBS and fixed in 4% formaldehyde for 1 h at 4 °C. Paraffin-embedded epididymal fat tissue was sectioned at 3–5 μm thickness and stained with hematoxylin–eosin (HE). Ornithine carbamoyl-transferase frozen liver tissue was sectioned at 3–5 μm thickness and stained with oil red O. Histological assessment of the tissue morphology was performed using an Olympus light microscope (Olympus, Tokyo, Japan).

RNA preparation and real-time qPCR analysis

Total RNA was prepared from tissues using TRIzol® (Biomiga) and treated with DNAse, according to a previously described

Table 1 Gene-specific primers used in qRT-PCR assays[a]

Gene name	Abbreviation	Primer sequences (5′–3′)	Amplicon size (bp)
Fasting-induced adipose factor	*Fiaf*	F: CAATGCCAAATTGCTCCAATT, R: TGGCCGTGGGCTCAGT	82
Lipoprotein lipase	*Lpl*	F: ACTGCCACTTCAACCACAG, R:CCCAATACTTCGACCAGG	361
Cholesterol 7α-hydroxylase	*Cyp7a1*	F: CTAAGGAGGATTTCACTTGC, R: ACTGGTCCAAAGGTGGACAT	350
Stearoyl-CoA desaturase 1	*Scd1*	F: AGGCTTCTGGGCCTTATGTG, R: TGCTTCTCTCGCCAGGAATAC	128
Peroxisome proliferator activated receptor γ	*Ppar-γ*	F: ATGTCTCACAATGCCATCAGGTT, R: GCTCGCAGATCAGCAGACTCT	116
Acetyl-CoA carboxylase 1	*Acc1*	F: AAGTCCTTGGTCGGGAAGTATACA, R: ACTCCCTCAAAGTCATCACAAACA	126
Fatty acid synthase	*Fas*	F: TGGTGAATTGTCTCCGAAAAGA, R: CACGTTCATCACGAGGTCATG	149

[a] F, forward; R, reverse.

method.[24] The total RNA was reverse transcribed (RT) and PCR amplified using the AccuPower® Greenstar qPCR kit (Bioneer). QRT-PCR assays were performed for each 10 μl reaction containing cDNA corresponding to 1 ng of total RNA and 900 nM gene-specific primers (Table 1) with the following parameters: 1 cycle of 95 °C for 5 min; 45 cycles of 95 °C for 20 s, 60 °C for 30 s and 72 °C for 20 s; and 1 cycle of 72 °C for 5 min. β-Actin was chosen as the housekeeping gene. Each assay was performed in duplicate in the same run. All assays were performed in triplicate with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Gene expression was calculated as ΔCT using β-actin as a reference, and was expressed relative to the control group normalised to a value of 1.

Statistics

Differences between two groups were analyzed by the one-way analysis of variance (ANOVA) test followed by the Tukey's test. When variances were not homogeneous, data were analyzed by the non-parametric Kruskal–Wallis test followed by the Student–Newman–Keuls (SNK) test. The data were expressed as means with their standard errors. Pearson's correlation coefficient was used to determine the relationship of the body weight and the colonic bacterial content. A P value of less than 0.05 was considered significant. Analysis was performed using SPSS 11.5 (SPSS, Inc., Chicago, IL, USA).

Results

Effect of a high-fat diet and RES treatment on the body weight and adiposity weight

The phenotype data indicated that the animals on the control diet had a normal body, liver, and abdominal fat weight. Compared with normal chow diet-fed mice, HF mice showed significant increases in the body, liver, and adipose weights (Fig. 1). Especially, there was a 1.6-fold increase in the adiposite index of HF mice compared with the CT group. However, RES treatment significantly inhibited increases in body and fat weights in HF mice, which suggests that it exerts anti-obesity effects.

Effect of RES on the level of blood glucose, insulin, and lipid in high-fat diet-fed mice

There was a significant increase in blood glucose and insulin levels of HF mice compared with the CT group (Table 2). RES significantly decreased blood glucose to that of control levels and significantly decreased blood insulin compared with HF mice. In addition, HF mice showed significant increases in serum total cholesterol but had no significant change in serum triglycerides compared with normal controls (Table 2). RES significantly inhibited increases in serum total cholesterol in HF mice; HDL-C levels decreased ($P < 0.05$), and LDL-C levels increased ($P < 0.05$) in HF-fed mice. After RES treatment, both the levels of HDL-C and LDL-C in plasma were significantly improved.

Effect of high-fat diet and RES on the composition of gut microbiota

FISH analysis, as a quantitative molecular-based technique, was applied to assess the microbiota composition. The relative abundance of the major bacterial groups indicated that, even when considering a degree of interindividual variation, the HF diet induced the gut microbiota dysbiosis (Fig. 2A). The results of principal coordinate analysis showed that HF microbiomes were clearly different from those in CT and HF-RES mice (Fig. 2B). Moreover, the principal coordinate analysis scores plot in Fig. 2B supports the presence of overlapping clusters between HF-RES and CT groups, which illustrated the relative similarity of CT and RES treated mice in microbiotal profiles. *Lactobacillus* was reduced by 1.8-fold in HF mice and *Bifidobacterium* was decreased by 1.9-fold, whereas *Enterococcus faecalis* was increased by 1.8-fold in the HF group compared with CT mice (Fig. 2C). After RES treatment, *Lactobacillus* and *Bifidobacterium* were significantly increased, whereas *Enterococcus faecalis* was significantly decreased to that of control levels (Fig. 2C). In

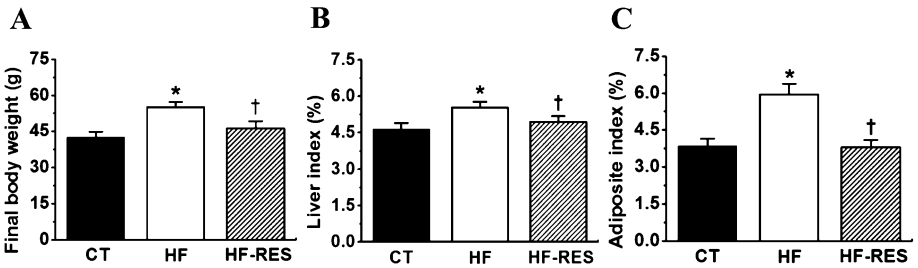

Fig. 1 Phenotypes of mice groups fed on different diets. (A) Average body weight, (B) liver weight (percentage of body weight) and (C) visceral and subcutaneous adipose tissue weight (percentage of body weight). Values are means with their standard errors ($n = 8$). Mean values were significantly different compared with those of the CT group: * $P < 0.05$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$. CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HF supplemented with RES.

Table 2 Serum biochemical analyses from CT and HF mice with or without RES treatment (mean values with their standard errors, $n = 8$)[a]

	CT		HF		HF-RES	
	Mean	SEM	Mean	SEM	Mean	SEM
Blood glucose (mmol l^{-1})	5.54	0.31	9.05**	0.42	5.75††	0.22
Blood insulin (pmol l^{-1})	110.50	9.18	468.18**	20.91	245.13**††	15.11
Serum total cholesterol (mmol l^{-1})	3.30	0.11	4.18*	0.11	3.32†	0.23
Serum triglycerides (mmol l^{-1})	1.13	0.12	1.30	0.13	1.18	0.16
HDL-C (mmol l^{-1})	1.68	0.09	1.12*	0.09	1.61†	0.14
LDL-C (mmol l^{-1})	1.58	0.40	2.22*	0.18	1.61†	0.13

[a] CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HFD supplemented with RES. HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Mean values were significantly different compared with those of the CT group: * $P < 0.05$, ** $P < 0.01$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$, †† $P < 0.01$.

addition, in our study, a high-fat diet resulted in 2.2-fold lower Bacteroidetes-to-Firmicutes ratios than those of CT mice, whereas RES supplemented diets resulted in a higher abundance of Bacteroidetes and a lower abundance of Firmicutes (Fig. 2D).

Relationship between body weight and gut microbiota

The correlations between body weight and the colonic bacterial content were calculated (Fig. 3). Body weight was positively and significantly correlated with *Enterococcus faecalis* ($P < 0.05$) (Fig. 3C), and body weight was negatively correlated with *Lactobacillus* and *Bifidobacterium* (Fig. 3A and B). In addition, a negative correlation was found between the body weight and the ratios of Bacteroidetes-to-Firmicutes ($r = -0.805$, $P = 0.002$) (Fig. 3D).

The suppressive effect of RES on *Fiaf* expression in the intestine

Fiaf expression was significantly decreased by 2.1-fold in the intestines of HF mice compared with normal controls, and *Lpl* (lipoprotein lipase) expression was significantly increased by 1.9-fold (Fig. 4A and B). However, after RES treatment, *Fiaf* expression in the intestines of HF mice was significantly increased and even higher than the CT group, but had no significant change. Moreover, *Lpl* expression showed decreasing tendency in HF-RES mice compared with normal controls, and had a significant change (Fig. 4A and B).

Effect of RES on gene expressions in the liver

Glucose and insulin are known to induce the expression of lipogenic enzymes in the liver.[25] Oil Red O staining of liver sections confirmed that there was severe steatosis seen in HF mice compared with the CT group, which was well prevented in RES treatment mice (Fig. 5A). QRT-PCR assays confirmed that the HF diet was accompanied by 4.5-fold statistically significant elevations in the liver mRNAs encoding one key regulator of fatty acid release from triglyceride rich lipoproteins, *Lpl* (Fig. 5B). Intriguingly, *Lpl* expression was significantly decreased in the intestine (Fig. 4B). In addition, HF mice showed a decrease in *Cyp7a1* expression, which encodes cholesterol 7α-hydroxylase and can catalyze the initial step in cholesterol catabolism and bile acid synthesis. Although HF diet feeding reduced hepatic *Cyp7a1* expression, RES treatment up regulates this expression (Fig. 5C). The biomarker of fatty acid biosynthesis, *Scd1*, was significantly increased under HF-fed conditions, as compared to normal diet-fed mice (Fig. 5D). In contrast, RES ingestion normalized the expression of *Scd1* (Fig. 5D).

RES inhibits storage of triglycerides in adipocytes

Histochemical studies allowed us to conclude that the high-fat diet-induced increase in the epididymal fat pad weight reflecting adipocyte hypertrophy (Fig. 6A). Moreover, qRT-PCR analyses of epididymal fat pad RNA revealed that the expressions of key genes responsible for adipogenesis (*Ppar*-γ) and lipogenesis

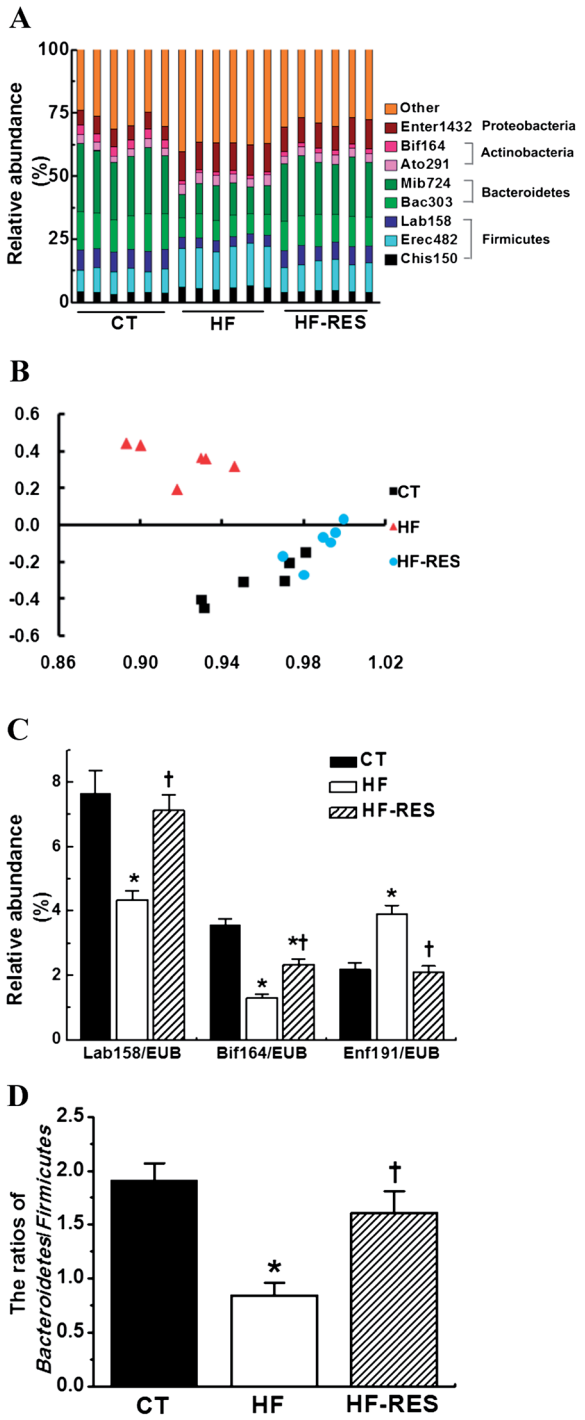

Fig. 2 Microbial profiling analysis of the intestinal contents. (A) The division-level composition of gut microbiome comparing CT, HF, and HF-RES animals ($n = 6$). (B) The principal component analysis (PCA) of the fluorescence *in situ* hybridization data from intestinal bacterial communities ($n = 6$). (C) Colonic bacterial content of the *Lactobacillus* group, the *Bifidobacterium* group, and *Enterococcus faecalis*. Bacterial quantities are expressed as a percentage of EUB338 ($n = 8$). (D) The Bacteroidetes-to-Firmicutes ratios contributed by the indicated target bacterial populations ($n = 8$). Mean values were significantly different compared with those of the CT group: * $P < 0.05$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$. CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HF supplemented with RES.

(*Acc1* and *Fas*) were significantly increased by 3.2-fold, 2.6-fold, and 2.1-fold, respectively, after a high-fat diet (Fig. 6B). However, RES treatment significantly suppressed the expression of *Ppar-γ*, *Acc1* and *Fas*, suggesting that RES inhibits storage of triglycerides in the adipocytes (Fig. 6B).

Discussion

Although obesity arises from interactions of genetic and environmental factors,[26] a high-fat diet plays an important role in the induction of visceral obesity in animal models.[27] Most sets of existing evidence on resveratrol suggest that this compound has anti-obesity effects.[11,12,19] But the exact mechanisms of how resveratrol shows anti-obesity effects are not clear. Because of very low systemic bioavailability but very high accumulation in colon tissue,[15,16] we hypothesize that resveratrol might have anti-obesity effects by targeting gut microbiota, and in turn, improving fat storage and metabolism.

Gut microbes, as environmental factors, may regulate fat storage and affect energy metabolism homeostasis, by acceleration of the processing of dietary polysaccharides and induction of *de novo* lipogenesis.[6,28] In the present study, both the relative abundance of the major bacterial divisions and principal coordinate analysis showed that, the HF diet induced the gut microbiota dysbiosis. These findings are in line with Cani *et al.*,[29] who found that the high-fat diet disrupted gut microbiota by promoting the growth of endotoxin producers. Resveratrol treatment significantly improved the gut microbiota dysbiosis induced by the HF diet (Fig. 2A and B). Few studies have investigated the effect of dietary resveratrol on the complex gut microbiota, and they focused mainly on selected bacterial populations. In addition, there was a decrease in the *Lactobacillus* and *Bifidobacterium* frequencies and an increase of *Enterococcus faecalis* in HF mice compared with CT mice, which agree with our previous studies that a high-fat diet significantly decreases the number of *Lactobacillus* and increases the number of *Enterococcus faecalis*.[30] After resveratrol intake, the numbers of *Lactobacillus* and *Bifidobacterium* were significantly increased. In contrast, *Enterococcus faecalis*, an important, multidrug-resistant nosocomial pathogen, was significantly decreased in HF-RES mice. Similar results were reported by Larrosa *et al.*,[31] who observed that the increase of *Lactobacillus* and *Bifidobacterium* counts in feces of the DSS-Res group became significantly increased after 20 days of resveratrol intake.

Bacteroidetes and Firmicutes are two main communities that affect energy metabolism homeostasis.[32] Previous studies show that obese mice have more Firmicutes and less Bacteroidetes than lean controls.[32–34] Similar results were found in the present study that HF diet induced obesity mice have lower Bacteroidetes-to-Firmicutes ratios than CT mice. We also found that resveratrol altered colonic microbiota, and consequently the *Bacteroides*/Firmicutes balance. Taken together, these findings could suggest a possible prebiotic effect of resveratrol on the microbiota, which may influence the host metabolism. The mechanism of how resveratrol affects the gut microbiota remains an important field of future research. Recent studies showed that polyphenols are associated with antimicrobial

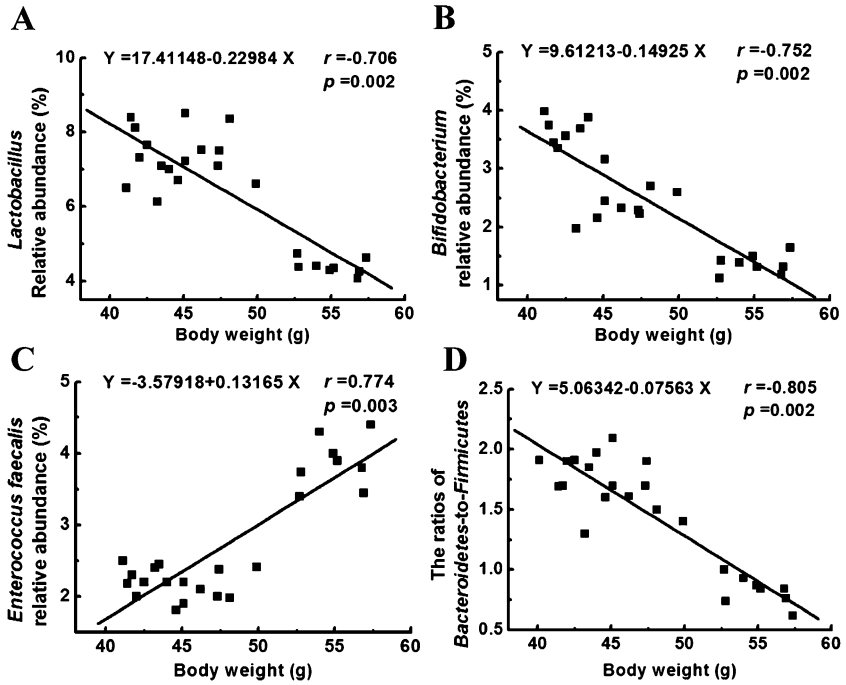

Fig. 3 Correlation between the body weight and the colonic bacterial content of (A) the *Lactobacillus* group, (B) the *Bifidobacterium* group, (C) *Enterococcus faecalis*, and (D) the ratios of Bacteroidetes-to-Firmicutes. Mice were fed either a control diet (CT) or a high-fat diet (HF), or a high-fat diet supplemented with resveratrol (HF-RES). Bacterial quantities are expressed as relative abundance (%). Body weight values (g) are presented as means ($n = 8$ animals per group), with standard errors represented by vertical bars.

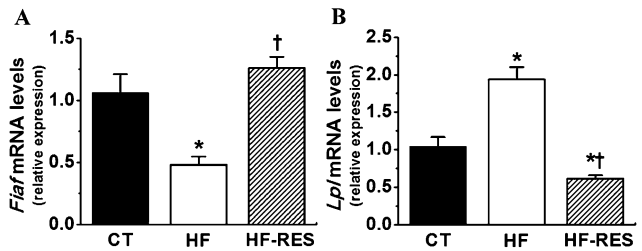

Fig. 4 Effects of high-fat diet and RES supplementation on *Fiaf* (A) and *Lpl* (B) mRNA expressions in ileum tissue ($n = 6$ per group). Each sample was assayed in triplicate; values are means, with their standard errors represented by vertical bars; mean values were significantly different compared with those of the CT group: * $P < 0.05$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$. CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HF supplemented with RES.

activity, exerting their inhibitory effect on certain bacterial groups by binding to bacterial membranes.[35,36] Differences in cell surface structures could explain why Gram-positive bacteria are more sensitive to the bactericidal effects of these compounds than are Gram-negative species.[37] On one hand, in our study, resveratrol has an inhibitory effect on the growth of these bacteria, which includes *Enterococcus faecalis* and the *Enterobacteriaceae* group—an important pathogen closely related to obesity and insulin resistance on a high-fat diet but not on a normal chow diet.[38] However, on the other hand, this study showed that resveratrol intake positively affected the growth of the *Lactobacillus* group, the *Bifidobacterium* group, and the *Bacteroides* phylum. These findings are consistent with previous studies. For instance, Vendrame *et al.*[39] described that wild blueberries, a rich source of polyphenols, significantly increased the number of *Bifidobacterium* spp. and *Lactobacillus acidophilus*, and Neyrinck *et al.*[40] demonstrated that a polyphenol-rich extract of pomegranate peel increased the caecal content weight and caecal pool of *Bifidobacteria* spp. and *Lactobacillus* spp. The antimicrobial activity of resveratrol could not explain these results. Therefore, there are additional mechanisms, by which resveratrol mediates the composition of gut microbiota, which should be further elucidated.

Conventionalization of adult GF mice with a normal microbiota (CONV-D mice) produces a 60% increase in the body fat content accompanied by increased *Fiaf* mRNA expression within 14 days. After knockout of the *Fiaf* gene, the increased total body fat content of CONV-D mice markedly attenuates.[8] By analysis of GF and conventionalized, normal and *Fiaf* knockout mice, Backhed *et al.*[8] showed that *Fiaf* is a circulating lipoprotein lipase inhibitor and that its suppression is essential for the microbiota-induced deposition of triglycerides in adipocytes. In our study, HF diet inhibited the mRNA expression of *Fiaf* in the intestine. Conversely, resveratrol significantly promoted the expression of *Fiaf* in the intestines of HF mice, which may be associated with the prebiotic effect of resveratrol on the microbiota. The increased *Fiaf* expression enhances energy metabolism by inhibiting LPL in the intestine and liver, which is a key regulator of fatty acid release from triglyceride rich lipoproteins in the muscles and heart.[41] Decreased adipocyte LPL activity leads to decreased cellular uptake of fatty acids and

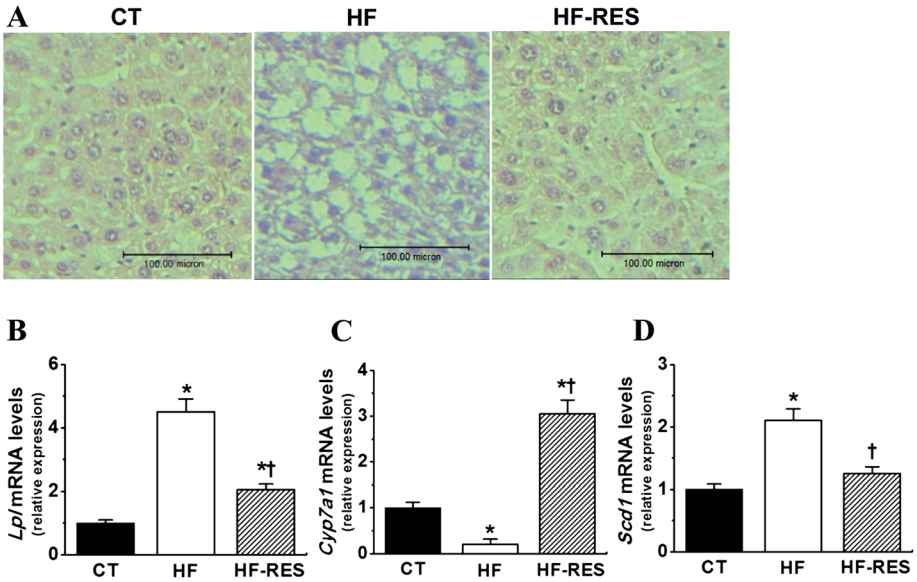

Fig. 5 Effects of high-fat diet and RES supplementation on lipogenesis in visceral livers. (A) Oil red O stains of paraformaldehyde-fixed liver sections prepared from CT, HF, and HF-RES mice (bars: 100 μm). qRT-PCR assays of liver RNAs reveal significant changes in the expression of mediators or biomarkers of lipogenesis, such as *LPL* (B), *Cyp7a1* (C), and *Scd1* (D). Each relative mRNA expression was determined by the ratio of intensity to β-actin production. Values are means, with their standard errors represented by vertical bars ($n = 6$). Mean values were significantly different compared with those of the CT group: * $P < 0.05$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$. CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HF supplemented with RES.

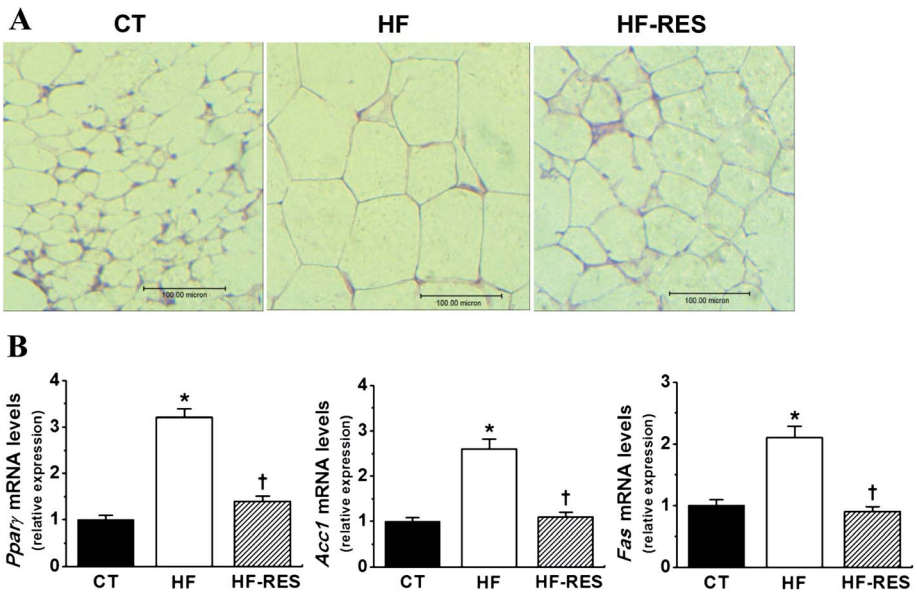

Fig. 6 Effects of high-fat diet and RES supplementation on adipocyte hypertrophy through the PPAR signaling pathway in OP mice. (A) The corresponding hematoxylin- and eosin-stained epididymal fat sections are shown. (B) qRT-PCR assays for gene expression of the PPAR signaling pathway in epididymal fat pads. Values are means, with their standard errors represented by vertical bars ($n = 6$). Mean values were significantly different compared with those of the CT group: * $P < 0.05$. Mean values were significantly different compared with those of the HF group: † $P < 0.05$. CT, normal chow diet-fed mice; HF, high-fat diet-fed mice; HF-RES, mice fed with HF supplemented with RES.

adipocyte triglyceride accumulation. The stearoyl-CoA desaturase 1 (*Scd1*) gene is involved in the synthesis and regulation of unsaturated fatty acids from saturated fatty acids.[42] The *Cyp7a1* gene encodes the enzyme cholesterol 7α-hydroxylase, which catalyzes the initial step in cholesterol catabolism subsequent to the induction of SREBP-1 and lipogenesis.[43] In the liver tissue, resveratrol promoted the expression of *Cyp7a1* and normalized *Scd1* expression to that of the control level, suggesting that resveratrol mainly enhances energy metabolism through the fatty acid and cholesterol catabolism pathways.

Ppar-γ, *Acc1* (acetyl-CoA carboxylase 1), and *Fas* (fatty acid synthase) are marker genes responsible for adipogenesis or lipogenesis in visceral adipose tissues. Resveratrol suppressed the expression of *Ppar-γ*, *Acc1* and *Fas* in HF mice. *Ppar-γ*, *Acc1*, and *Fas* play important roles in adipocyte differentiation,[44] *de novo* fatty acid biosynthesis[45] and fatty acid synthesis,[46] respectively. Activation of *Ppar-γ* can increase insulin sensitivity.[47] Taken together, these may explain why resveratrol has anti-hyperlipidemic and anti-obesity effects.

Conclusion

The composition of gut microbiota regulated by resveratrol may result in *de novo* lipogenesis, and systemically increasing expression of the *Fiaf* gene in the intestine, in turn, suppressing expressions of the lipoprotein lipase gene (*Lpl*) and the fatty acid biosynthesis gene (*Scd1*) in the liver, and inhibiting expressions of adipogenesis or lipogenesis genes (*Ppar-γ*, *Acc1*, and *Fas*) in visceral adipose tissues. Taken together, these activities may contribute to significant anti-obesity effects. In this study, we show evidence that pharmacological regulation of gut microbes by resveratrol can lead to development of an anti-obesity strategy.

Non-conflict of interest statement

The authors confirm that there is no conflict of interest with any financial organization regarding the results presented in this research manuscript.

Abbreviations

CT	Control diet
FISH	Fluorescence *in situ* hybridization
HF	High-fat
HF-RES	High-fat diet supplemented with resveratrol
qPCR	Quantitative PCR
RES	Resveratrol

Acknowledgements

This work was supported by a grant from the National Natural Science Foundation of China (no. 31201805), the Fundamental Research Funds for the Central Universities (no. JUDCF10058), the 12th 5 Year Plan for Science and Technology Development (no. 2012BAD33B05).

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