Flaxseed Polysaccharide Altering Colonic Gene Expression of Lipid Metabolism and Energy Metabolism in Obese Rat

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Short report

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Abstract

Background

Obesity is one of the most serious public health challenges. Recently, we found that flaxseed polysaccharide (FP) had an anti-obesity effect through promoting lipid metabolism, inducing satiety and regulating gut microbiota, but how FP promote lipid metabolism through altering the colonic epithelial cells remains to be elucidated. In this study, a transcriptome study was performed to investigate the effect of FP altering the gene expression of colonic epithelial cells in an obese rat model.

Results

The transcriptome analysis showed that 3,785 genes were differentially expressed after FP intervention in colonic epithelial cells, including 374 down-regulated and 3,411 up-regulated genes. Through KEGG analysis, we found out three classical pathways related to lipid metabolism and energy metabolism, including PPAR signaling pathway, nitrogen metabolism and oxidative phosphorylation (OXPHOS). Moreover, qRT-PCR results showed consistent expression trends of differential genes with transcriptome analysis.

Conclusions

The anti-obesity effect of FP may be achieved by regulating the expression of lipid metabolism- and energy metabolism-related proteins acting on the PPAR (peroxisome proliferator-activated receptor) signaling pathway, nitrogen metabolism and OXPHOS pathway *in vivo*.

Introduction

Caused by the complex interactions between genes and environmental factors, including food components, diet and/or way of life, obesity is one of the most serious public health challenges facing the world [1]. Over 340 million children and adolescents aged 5–19 in 2016, as well as an estimated 38.2 million children under the age of 5 years in 2019, were overweight or obese [2]. Developing from an imbalance between energy expenditure and energy intake, obesity is a risk factor for the development of several clinical conditions, such as diabetes, cancers and cardiovascular disease [3].

Defined as the edible parts of plants or analogous carbohydrates, dietary fiber resists absorption and digestion in the small intestine, partially or completely fermented in the large intestine [4]. Dietary fiber is beneficial to weight control, mainly through influencing satiation and satiety to control appetite [5]. Besides, fiber intake also has a positive effect on the treatment of pathologies associated with obesity, namely dyslipidemia, hyperglycemia and/or hypertension [6].

Dietary fibers regulated gene expression in colonic epithelial cells and induced genes involved in the processes such as OXPHOS, TCA cycle, glycolysis and electron transport chain, all pathways responsible for generating energy by substrate oxidation [7]. Besides, PPAR γ is identified as central regulator of

transcriptional responses to fermented fibers, which regulates genes involved in energy metabolism, in particularly lipid catabolism [7]. What's more, intestinal microbiota can affect both sides of the energy balance equation, as a factor affecting the harvest of energy from dietary ingredients, and as a factor affecting host genes that regulate energy consumption and storage, for example, they can diminish mitochondrial fatty acid oxidation, suppress intestinal epithelial production of Angptl4 to facilitate lipid storage in adipose tissues, show greater inhibition of Cpt1 and so on [8].

In the previous research, we had found flaxseed polysaccharides (FP) had an anti-obesity effect via promoting lipid metabolism, inducing satiety and regulating the intestinal flora [9–10]. However, as a polysaccharide, FP can not directly enter the body to play a role, so we speculate that it can promote weight loss through the interaction with intestinal flora, change the gene expression of intestinal epithelial tissue, and induce the metabolism of fat and energy *in vivo*. However, it is not clear which intestinal epithelial tissue gene expression is changed by flaxseed polysaccharide. In the present study, we aimed to find out the significantly different genes affected by FP involved in lipid and energy metabolism in colonic epithelial cells, further investigating the mechanism of FP in weight loss.

Materials And Methods

Animals, diets, and sample preparation

18 male Sprague-Dawley (SPF grade), 4 weeks old, were bought from Guangdong Medical Laboratory Animal Center (Guangzhou, China.). Group Con (n = 6) was fed with a standard diet (D12450B), others were given high-fat diet (D12492) to establish the obesity model, which were finally divided into two groups, Group HFD and Group FPD (6 rats per group). After that, Group Con and Group HFD were fed with the control diet, AIN-93M (3.77 kcal/g), while Group FPD was fed with a 10% FP diet, replaced 10% corn starch (in AIN-93M) with equivalent FP (3.37 kcal/g). The rats were weighed weekly. The details of the diets in this research are as labeled in Table 1.

Table 1
Detailed Recipes of Each Diet (%)

Ingredients	standard diet (D12450B)	high-fat diet (D12492)	control diet (AIN- 93M)	flaxseed polysaccharide diet
flaxseed	0.00	0.00	0.00	10.00
polysaccharide				
corn starch	33.00	0.00	46.57	36.57
dextrin	3.35	16.35	15.50	15.50
casein	19.13	26.17	14.00	14.00
sucrose	34.47	9.00	10.00	10.00
cellulose	4.78	6.54	5.00	5.00
soybean oil	2.39	3.27	4.00	4.00
lard	1.91	32.06	0.00	0.00
mineral mix ain-93	3.35	4.58	3.50	3.50
vitamin mix ain-93	0.96	1.31	1.00	1.00
L-cystine	0.29	0.39	0.18	0.18
choline bitartrate	0.24	0.33	0.25	0.25

On day 53, all rats were fasted overnight and were anesthetized with sodium pentobarbital. After execution of the rats, the colonic epithelial cells of each rat were scraped. They were frozen in liquid nitrogen shortly upon sampling and then stored at -80°C until RNA extraction. The experiments were ratified by the Research Animal Administration Center of Jinan University (Guangzhou, China), following all Institutional Animal Care and Use Committee of Jinan University guidelines for the use and care of animals.

RNA extraction

TRIzol reagent (Invitrogen, CA, USA) was used to extract the total RNA of rats (n = 6 per group) from the colonic epithelial cells according to the manufacturer's instructions. Genomic DNA was removed by Deoxyribonuclease I (TaKara, Dalian, China). We determined the RNA quality with a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and quantified it by ND-2000 (Thermo Fisher, Shanghai, China). The sequencing library was constructed by using high-quality RNA samples ($OD_{260/230} \geq 2.0$, $OD_{260/280} = 1.8 \sim 2.2$, RNA integrity number ≥ 6.5 , $> 10 \mu\text{g}$, $28\text{S}:18\text{S} \geq 1.0$) were used to construct the sequencing library.

Library preparation and Illumina HiSeq 4,000 sequencing

First, messenger RNA was isolated by using oligo (dT) beads according to the polyA selection method, fragmented by fragmentation buffer. Then, double-stranded cDNA was synthesized by using Super Script double-stranded cDNA synthesis Kit (Invitrogen, CA, USA) and random hexamer primers (Illumina, Shanghai, China). A library of 200–300 bp cDNA target fragments was selected on 2% Low Range Ultra Agarose (Bio-Rad, CA, USA), and then PCR amplification of 15 PCR cycles was carried out using Phusion DNA polymerase (NEB, Beijing, China). After the quantification with TBS380, we sequenced the paired-end RNA-Seq sequencing library with Illumina HiSeq 4000 (sequencing mode of paired-end, 2×126 bp).

Read mapping

The raw paired-end reads were trimmed and the default parameters were used for quality control with SeqPrep (<https://github.com/jstjohn/SeqPrep>) and Sickle (http://www.ensembl.org/Rattus_norvegicus/Info/Index). Then, bowtie2 (<http://bowtie-bio.sourceforge.net/index.shtml>) software was used to accurately compare clean reads with reference genome (http://www.ensembl.org/Rattus_norvegicus/Info/Index) through orientation mode. The mapping standard of bowtie was that the sequencing reading should uniquely match the genome, allowing up to two mismatches, and no insertions or deletions. Besides, the whole genome was divided into 15 kbp windows, sharing 5 kbp. The new transcription regions were defined as more than two consecutive windows without an overlapping gene region. There were at least two reads mapped per window in the same orientation. The sequencing data were annotated with the databases Ensemble, KEGG, GO, eggNOG, UniProtKB and Symbol.

Differential expression analysis and functional enrichment

In order to identify the DEGs among several different samples, we calculating the expression level of each transcript by the fragments per kilobase of exon per million mapped reads (FRKM) method. RSEM (RNA-Seq by Expectation-Maximization, <http://deweylab.biostat.wisc.edu/rsem/>) was used to quantify gene abundance and utilized DESeq2 (<http://bioconductor.org/packages/stats/bioc/DESeq2/>) was used for differential expression analysis. When the adjusted P-value (Benjamin Hochberg (BH) multiple test correction method) was less than 0.05, it was considered that genes were significant differentially expressed. The difference of gene expression was observed by volcano map. KEGG (Kyoto Encyclopedia of Genes and genome database) pathway analyses and KEGG functional enrichment were performed. It was considered to be a significantly different expression level if the false discovery rate (FDR) was less than 0.05 in the multi-group comparison. By using the BLAST algorithm (blastx/blastp2.2.24+), all the genes were mapped to the KEGG gene database (GENES) to find the potential biological pathway of the genes.

Quantitative real-time PCR (qRT-PCR) analysis

TRIzol reagents (Invitrogen, CA, USA) were used to extract total RNA. Following the supplier's instructions, the cDNA was synthesized with a Bestar™ qPCR RT kit (DBI Bioscience, Shanghai, China). Then, the

gene mRNA expression was determined with a Bestar™ SybrGreen qPCR Master Mix. Briefly, a 20 µL qPCR mixture was prepared as follows: 0.5 µL of reverse primer (10 µmol/L), 0.5 µL of forward primer (10 µmol/L), 1 µL of cDNA, 8 µL of double-distilled water and 10 µL of Bestar® SybrGreen qPCR Master Mix (DBI Bioscience, Shanghai, China). Real-time PCR was performed with a Stratagene Mx3000p Real time PCR system (Agilent, Santa Clara, CA, USA). The PCR conditions were initially maintained at 95 °C for 2 min, followed by 40 cycles of 94 °C for 20 s, 58 °C for 20 s and 72 °C for 20 s. Primers were designed using Primer5 and the sequences of all the primers used are provided in Supplementary Table S1. The average cycle threshold (Ct) values were used for quantification using the $2^{-\Delta\Delta C_t}$ method, with β -actin as reference gene.

Statistical Analysis

Numerical data were presented as means $\pm$ standard deviation (SD). The difference between means was analyzed using SPSS 25.0 software (IBM Corporation, Armonk, New York). Two-tailed Student's t-tests were conducted to compare the data in different groups. Besides, $P < 0.05$ (*) or $P < 0.01$ (**) was considered as statistically significant.

Results

Gene expression profile of rat colonic epithelial cells

As showed in our previous research, the body weight of rats in Group FPD was significantly lower than that of group HFD, and finally became very similar to those in Group Con at the end of the trial, which indicated that FP induced body weight loss [9]. Approximately 52.16 million raw reads were obtained from three groups. A huge proportion (95.14%) of high-quality clean reads was generated after adapter trimming and filtering low-quality reads. The percentages of clean reads having a base quality greater or equal than Q30 were above 95.14% with 48.37–50.25% of GC content, indicating that the data produced by sequencing are of high quality. On average, 96.04% of clean reads were mapped to the reference genome, among which 90.06% were aligned to unique locations.

As shown in Fig. 1a, 80.45% of the genes were in common among all three groups. Besides, the gene composition between Group Con and Group HFD were somewhat similar, while Group FPD was obviously different compared with them (Fig. 1b). This result suggested that FP intervention would change the gene composition of colonic epithelial tissues.

Identification of Differentially Expressed Genes

The volcano plot showed the variation of mRNA expression between Group HFD and Group Con (Fig. 2a). In total, 28 DEGs, including 14 upregulated and 14 downregulated, were identified. Besides, 3,785 DEGs were identified between Group HFD and Group FPD (Fig. 2b), including 374 downregulated and 3411 upregulated DEGs ($P < 0.05$). Compared with Group HFD, there were a lot of DEGs in Group FPD, but few in Group Con. Therefore, our subsequent analysis would focus on Group HFD and Group FPD.

KEGG Pathway Analysis of Differentially Expressed Genes

The DEGs were mapped in the KEGG pathway database. Compared with Group HFD and Group FPD, the annotated genes were classified into six categories. Most of them were enriched in signal transduction. Besides, 15 DEGs were enriched in the pathway of Energy metabolism and 26 DEGs in PPAR signaling pathway (Fig. 3).

KEGG Pathway Enrichment Analysis of Differentially Expressed Genes

It has been reported that weight loss involves a variety of mechanisms, such as inhibiting energy intake, stimulating energy expenditure and so on. Thus, KEGG pathway enrichment analysis of 15 DEGs in 'Energy metabolism' between Group HFD and Group FPD was performed. As a result, 13 metabolic pathways with significantly differential expressions ($p < 0.05$) were identified between Group HFD and Group FPD, of which 10 pathways showed the most significant difference ($p < 0.01$). Figure 4 shows the top 13 ranked KEGG pathway of DEGs. Nitrogen metabolism occupied the strongest enrichment degree as it possessed the highest Rich factor, followed by monobactam biosynthesis. Nitrogen metabolism had the most DEGs, and the then is OXPHOS. The DEGs in the two pathways were listed in Table 2, and all of them were upregulated. Especially, there were 9 genes of carbonic anhydrase enriched in nitrogen metabolism.

Table 2
Information of three KEGG pathways and DEGs enriched

KEGG pathway	Gene name	Gene description	Regulation
Oxidative phosphorylation	Ndufa4l2	NDUFA4, mitochondrial complex associated like 2	Upregulated
	Atp6v1g2	ATPase H + transporting V1 subunit G2	Upregulated
	Cox4i2	cytochrome c oxidase subunit 4i2	Upregulated
	Cox6b2	cytochrome c oxidase subunit VI b polypeptide 2	Upregulated
Nitrogen metabolism	Cps1	carbamoyl-phosphate synthase 1	Upregulated
	Car3	carbonic anhydrase 3	Upregulated
	Car1	carbonic anhydrase I	Upregulated
	Ca5b	carbonic anhydrase 5B	Upregulated
	Car8	carbonic anhydrase 8	Upregulated
	Car13	carbonic anhydrase 13	Upregulated
	Car2	carbonic anhydrase 2	Upregulated
	Car12	carbonic anhydrase 12	Upregulated
	Car14	carbonic anhydrase 14	Upregulated
	Car4	carbonic anhydrase 4	Upregulated
PPAR signaling pathway	Plin4	perilipin 4	Upregulated
	Fabp1	fatty acid binding protein 1	Upregulated
	Fabp3	fatty acid binding protein 3	Upregulated
	Cyp8b1	cytochrome P450, family 8, subfamily b, polypeptide 1	Upregulated
	Scd	stearoyl-CoA desaturase	Upregulated
	Fabp4	fatty acid binding protein 4	Upregulated
	Lpl	lipoprotein lipase	Upregulated
	Acsl6	acyl-CoA synthetase long-chain family member 6	Upregulated
	Plin1	perilipin 1	Upregulated
	Pltp	phospholipid transfer protein	Upregulated

KEGG pathway	Gene name	Gene description	Regulation
	Angptl4	angiopoietin-like 4	Upregulated
	Rxrg	retinoid X receptor gamma	Upregulated
	Adipoq	adiponectin, C1Q and collagen domain containing	Upregulated
	Acsbg2	acyl-CoA synthetase bubblegum family member 2	Upregulated
	Fabp5	fatty acid binding protein 5, epidermal	Upregulated
	Fabp2	fatty acid binding protein 2	Upregulated
	Aqp7	aquaporin 7	Upregulated
	Cpt1c	carnitine palmitoyl transferase 1c	Upregulated
	Acsl4	acyl-CoA synthetase long-chain family member 4	Upregulated
	Pparg	peroxisome proliferator-activated receptor gamma	Upregulated
	Acsl1	acyl-CoA synthetase long-chain family member 1	Upregulated
	Acsbg1	acyl-CoA synthetase bubblegum family member 1	Downregulated
	Gk	glycerol kinase	Downregulated

Expression correlation analysis and Protein–Protein Interaction (PPI) Network Analysis of DEGs

The DEGs enriched in PPAR signaling pathway were further analyzed. Based on the correlation of gene expression, the correlation coefficient between gene and gene is obtained by Spearman algorithm. To systemically analyze the functions of these DEGs, we mapped them to PPI data and obtained an PPI network (Fig. 5). The network showed the association between 23 DEGs (Table 2). The result suggested that the upregulation of Pparg, Lpl, Fabp1 and Acsl1 might play an important role in colonic epithelial tissues.

Verification of differentially expressed genes

In order to verify the results of transcriptome sequencing, 17 important DEGs were chosen for quantitative RT-PCR. The results of qRT-PCR are shown in Fig. 6. The expression trends were consistent with those obtained by RNA-seq, suggesting that the RNA-seq datas reliably reflected the gene expression alterations.

Discussion

This experiment investigated the gene expression of colonic epithelial cells affected by FP intervention in an obese rat model. After FP intervention, 3,785 DEGs were found in the obese rats, 15 of which were enriched in energy metabolism. Among them, 10 DEGs were enriched in nitrogen metabolism, including 9 genes of carbonic anhydrase and Cps1. Four DEGs were enriched in OXPHOS, including Cox4i2, Cox6b2, Atp6v1g2 and Ndufa4l2. Besides, 23 DEGs in PPAR signaling pathway showed strong connection.

As showed in our previous research, obese rats in Group FPD showed the reduction of body weight loss as well as body fat [9]. It is well-known that obesity is always related to an imbalance between the accumulation and consumption of lipids and energy. Thus, since FP intervention had been proved to lose weight, we speculated that it could improve lipid metabolism and energy metabolism. Our present results were consistent with our previous report, and more importantly, we found that FP intervention did improve the lipid metabolism, mainly by regulation of the PPAR signaling pathway in colonic epithelial tissues.

FP intervention promoted lipid metabolism of the colonic epithelial tissue

Most of the dietary lipids are long-chain TGs, which are hydrolyzed in the intestine to produce FAs and monoglycerides [11]. Then, they will enter enterocytes. As the members of the lipid-binding protein superfamily, FABPs bind to saturated and unsaturated LCFA and other hydrophobic ligands such as monoacylglycerols, mediating FA transportation to different cell organelles [12]. Thus, FP intervention upregulated the expression of Fabp1- Fabp5, heightening the transshipment of FA. After entering enterocytes, FFAs and glycerol arrive at the crossroads of several pathways; they can be metabolized within mitochondria, or be re-esterified to TGs, packaged into chylomicrons (CMs), and secreted from enterocytes to the lymph [13], which results in elevated TG levels in the circulation. CMs are the main contributors to the serum lipid level, and the decreased clearance or increased production of them can lead to hypertriglyceridemia [14]. High intake of dietary fat increases postprandial plasma TG levels significantly, and the regulation of lipid metabolism in intestinal epithelial cells could affect postprandial hyperlipidemia [15]. Most dietary fatty acids are processed as CM-TG, which can be later metabolized at the tissue level by LPL, releasing FA for tissue uptake [16]. Thus, FP intervention upregulated Lpl, inhibiting the release of CMs and the rise of plasma TG levels.

As a key enzyme in glycerol metabolism, GK catalyzes the conversion of glycerol to glycerol 3-phosphate, which is the main substrate in TG synthesis [17]. As a pore-forming transmembrane protein, the aquaglyceroporin AQP7 facilitates the transport of glycerol across cell membranes [18]. AQP7 involves in rapid fluid movement through the villus epithelium in the small and large intestine [19]. Therefore, FP intervention downregulated Gk to inhibit the synthesis of TG, and upregulated Aqp7 to facilitate the transport of glycerol that would be metabolized in other tissues.

It is also very important to accelerate the oxidation of FAs, which in turn helps inhibit their re-esterification. In the present study, the upregulation of Pparg and Rxrg was found. Several prior studies have demonstrated that administering mice PPAR agonists can induce the expression of lipid oxidation genes and β -oxidation in intestinal cells, and inhibit enterocytic lipid accumulation and post-prandial lipidemia [20]. RXR is a heterodimeric partner of PPARs, and LCFA may increase transcriptional activity of PPARs by acting as a physiological ligand of RXR [21]. Researchers found that the activation of PPAR γ could control pathways connected with FA metabolism and mitochondrial function [13]. Thus, FP intervention enhanced the activation of PPAR γ and RXR, altering DEGs of colonic epithelial tissue to perform biological responses and transcription of genes related to lipids.

Most cellular FA metabolic pathways need to be preferentially activated by thioesterification, and acyl-CoA synthetase catalyzes the formation of active FA [22]. ACSLs catalyze the conversion of free long-chain fatty acids into their acyl-coenzyme A (CoA) forms [23]. The transport of long-chain acyl-CoA esters into the mitochondria matrix is mediated by the CPT system, among which CPT1 is responsible for converting acyl-CoAs into acylcarnitine to shuttle across the mitochondria membranes [24]. Thus, FP intervention upregulated Acsl1, Acsl4, Acsl6 and Cpt1c, which promoted the conversion of FA to acyl-CoA and the start of mitochondrial fatty acid β -oxidation. Besides, SCFAs and MCFAs permeate the inner mitochondrial membrane in the non-esterified form, which are activated into their CoA-derivatives in the mitochondrial matrix [25]. Afterwards, these FAs participate in β -oxidation and the TCA cycle. The final products include CO₂, NADH and FADH₂.

In addition to accelerate FA oxidation, FP intervention also heightened cholesterol catabolism. As the key enzyme in the bile acid formation pathway, Cyp8b1 was found to be inhibited expressed in high fat diet mice, which inhibited the catabolism of cholesterol into bile acids, causing the elevated cholesterol [26]. Researchers found the elevation of PLTP activity results in rapid disposal of cholesterol from body through increased conversion into bile acids and subsequent excretion [27]. Thus, FP intervention upregulated Cyp8b1 and Pltp to accelerate cholesterol catabolism. Besides, FP intervention also played an important role in the prevention of inflammation. Two or more members of the perilipin family of lipid droplet surface proteins decorate lipid droplets in chordates [28]. As metabolically dynamic cellular organelles, lipid droplets are specialized in storing free fatty acids, the excess of which have toxic effects and can lead to inflammation [29]. Researchers found that Plin1 deficiency secreted pro-inflammatory lipid metabolites, exacerbating adipose tissue inflammation [29]. The colonic epithelial cells responded to elevated FAs by increasing expression of Perilipin under FP intervention, inhibiting inflammation. Adiponectin is encoded by the Adipoq gene, controls the metabolism of glucose and FA and has a general anti-inflammatory effect [30]. Adiponectin protects against murine colitis and maintains intestinal homeostasis through by modulating adaptive immunity and interactions with its receptor AdipoR1 [31]. Angptl4 protected against acute colonic inflammation and that its absence worsened the severity of inflammation [32]. As the critical enzyme in FA metabolism, SCD is responsible for the conversion of saturated FAs to unsaturated FAs, and oleic acid is one of the major products [33]. Researchers found that gut-specific SCD-1 activity is necessary to control intestinal epithelial inflammation and synthesize

oleate, and that dietary oleic acid provides protection against intestinal inflammation *in vivo* [34]. Thus, FP intervention upregulated Plin1, Plin4, Adipoq, Angptl4 and Scd to regulate and attenuate inflammation.

In conclusion, FP intervention modulated PPAR signaling pathways to suppress triglyceride accumulation mainly by stimulating fatty acid oxidation, as well as reduce inflammation.

FP intervention promoted energy metabolism of the colonic epithelial tissue

We found plenty of CAs were upregulated. As isozymes, CAs catalyze the carboxylation of water to form carbonic acid, which spontaneously decomposes into protons and bicarbonate ($\text{H}_2\text{O} + \text{CO}_2 \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+$) [35]. The CAs we found includes several isoforms: cytosolic (CA I, CA II, CA III, CA XII), membrane-bound (CA IV, CA XII and CA XIV), mitochondrial (CA V) and catalytically inactive (CA VIII) [36–38]. The luminal mucus from the colon and stomach of guinea pigs, mice and humans showed a large amount of CA activity, by which the hydration rate of CO_2 was increased by 1000–2000 times, about 1 / 10 of the rate found in red cells [39]. Thus, as showed in Fig. 7, FP intervention up-regulated CAs and accelerated the consumption of CO_2 , reducing the accumulation of it in cells and enhancing the TCA cycle. This would further increased energy consumption.

We also found the upregulation of Ca5b and Cps1. Localized in mitochondria, Cps1 catalyzes the condensation of metabolic ammonia and HCO_3^- to carbamyl phosphate [40]. Researchers suggested CA V in enterocytes provided bicarbonate ions to CPS1 and facilitated the conversion of ammonia to citrulline, the first two steps of the urea cycle, which played an important role in detoxification and disposal of ammonia produced in the gut [41]. Approximately 12–18 g of protein reaches the human colon daily, and the main pathway of amino acid fermentation is deamination, leading to the production of ammonia, most of which is absorbed and metabolized into urea by the liver and excreted in urine [42]. Thus, FP intervention reduced the accumulation of bicarbonate ions in mitochondria, enhancing the initiation of the urea cycle and ammonium removal.

Generated from the TCA cycle and β -oxidation, NADH and FADH_2 provide electrons to the ETC [43]. The electrons are transferred to O_2 at complex IV to produce H_2O , and the pumped protons produce an electrochemical gradient on the inner membrane of mitochondria, which is the driving force for complex V to produce ATP [44]. As a component of the ETC complex I subunit, NDUFA4L2 fine-tunes complex I activity, and mediates mitochondrial activation of OXPHOS [45]. COX6B2 facilitates the assembly of complex IV to support mitochondrial respiration and OXPHOS-induced generation of ATP [46]. We also found the upregulation of ATP6V1G2, which encoded subunit G2 of vacuolar ATPase (V-ATPase), transporting protons from the cytoplasm into the lysosome and maintaining lysosomal acidification [47]. V-ATPases are highly conserved proton pumps, and V1 is the peripheral membrane subcomplex of them, which contains the sites of ATP hydrolysis [48]. Thus, FP intervention upregulated Ndufa4l2 and Cox6b2 to heighten OXPHOS, accelerating the generation of ATP and stimulating energy consumption. Besides, FP intervention also upregulated Atp6v1g2, hydrolyzing ATP to provide energy for H^+ transport.

As the TCA progress and oxygen consumption in the ETC increase, however, the oxygen concentration in the colonic epithelial cells decreases. As the major consumers of oxygen in the cells, mitochondria are severely affected by decreased oxygen availability, and thus OXPHOS is adapted to hypoxia by remodeling ETC as well as the activity of the TCA cycle [49]. The COX4 subunit optimizes respiratory chain function according to oxygen-controlled expression of its isoforms COX4i1 and COX4i2, and COX4i2 facilitates a decrease in cytochrome c oxidase affinity to oxygen [50]. Both NDUFA4L2 and COX4i2 can reduce the capacity for mitochondrial oxygen consumption and act to limit mitochondrial ROS production during hypoxia, by reducing the activity of complex I and cytochrome c oxidase respectively [51]. Researchers found the deterioration of respiratory capacity due to differences at the level of respiratory chain complexes in obese individuals, among which, particularly, electron transport at the level of complexes I and IV was most affected [52]. Therefore, FP intervention upregulated Ndufa4l2 and Cox4i2, preventing mitochondria from hypoxia, which kept the energy expenditure steady and healthy.

As we have mentioned above, the Adipoq gene of colonic epithelial tissues that encode adiponectin was upregulated. In the previous researches, we had found FP intervention significantly upregulated adiponectin probably via the gut-brain axis. There is growing evidence that the metabolic benefits of bariatric surgery are the result of complex gastrointestinal regulation, which produces signals that "educate" the brain to adapt to the new gut environment, and these signals somehow lower the defense levels of body fat [53]. Besides, the gut-brain axis also plays a key role in the control of energy balance [54]. Therefore, FP intervention might improve lipid metabolism and energy metabolism via the gut-brain axis to achieve some of the impressive effects of bariatric surgery with medications.

Conclusion

In summary, this study further sheds light on the mechanisms involved in the reported effects of FP intake. FP intervention had directly or indirectly altered the gene expression in epithelial cells, which further changed the host's lipid metabolism and energy metabolism, finally resulting in weight loss. By altering gene expression of the PPAR signaling pathway in the colonic epithelial cells, FP intervention accelerated FA catabolism to reduce accumulation. By promoting energy metabolism, FP intervention facilitated the transformation of FA oxidation products to ATP. As shown here, this study offers convincing evidence that FP may lose weight by regulating some potential key genes, which are involved in lipid metabolism and energy metabolism. Proving the role of FP in the prevention of obesity and obesity-related chronic diseases, these novel findings may provide a powerful and novel mean to clarify FP as an efficient functional food candidate in therapy of obesity.

Abbreviations

FP, flaxseed polysaccharide; PPAR, peroxisome proliferator-activated receptor; OXPHOS, oxidative phosphorylation; LPL, Lipoprotein lipase; TG, triglycerides; FA, fatty acid; FABP, fatty acid-binding proteins; DEGs, differentially expressed genes; VLDL, very low density lipoprotein; ACSL, acyl-CoA synthetase

enzymes specific for long chain; SCD, Stearoyl-CoA desaturase; AQP7, aquaglyceroporin 7; Gyl, Glycerol kinase; Angptl4, Plasma angiopoietin-like 4; CAs, Carbonic anhydrases; CPS1, Carbamyl phosphate synthetase I; COX6B2, Cytochrome c oxidase subunit 6B2; PLTP, Phospholipid transfer protein; UC, ulcerative colitis; FFA

free fatty acids; LCFA, long chain fatty acids; MCFAs:medium-chain fatty acids; TCA cycle:tricarboxylic acid cycle; ETC:electron transport chain; ROS:reactive oxygen species; CMs:chylomicrons

Declarations

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Authors' contributions

XL, JQ and GZ designed the study, performed research, analyzed data and prepared the manuscript. XP contributed to the scientific idea, supervised the study, and revised the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Analysis of the relationship between three groups. a, Venn diagram Venn; b, PCA analysis. Group Con indicates rats fed D12450B during the obesity model building and AIN-93M thereafter. Group HFD indicates rats fed D12492 during model building and AIN-93M thereafter. Group FPD indicates rats fed D12492 during model building and an FP containing diet thereafter.

Figure 2

DEGs between different treatment groups. The horizontal axis indicates expression changes (log) of the genes in different treatment groups while the vertical axis shows the differences of gene expression. The discrepancy was more significant with smaller p-values and bigger $-\log_{10}$ (adjusted p-value). Splashes were for different genes, among which grey dots were genes with no significant discrepancy, red dots were genes significantly up-regulated and blue dots were significantly down-regulated genes.

Abbreviations: DEGs, differentially expressed genes; Group Con indicates rats fed D12450B during the obesity model building and AIN-93M thereafter. Group HFD indicates rats fed D12492 during model building and AIN-93M thereafter. Group FPD indicates rats fed D12492 during model building and an FP containing diet thereafter.

Figure 3

KEGG function classification of DEGs in Group HFD and Group FPD Abbreviations: DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes. Group HFD indicates rats fed D12492 during model building and AIN-93M thereafter. Group FPD indicates rats fed D12492 during model building and an FP containing diet thereafter.

Figure 4

Bubble diagram of 15 DEGs between Group HFD and Group FPD. The vertical axis indicates KEGG pathway and the horizontal axis represents the Rich factor. The enrichment degree was stronger with a bigger Rich factor. The size of dots indicates the number of genes. Abbreviations: DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes. Group HFD indicates rats fed D12492 during model building and AIN-93M thereafter. Group FPD indicates rats fed D12492 during model building and an FP containing diet thereafter.

Figure 5

The PPI network of 23 DEGs in PPAR signaling pathway. Nodes stand for the proteins (genes), and edges stand for the interactions of proteins. Abbreviations: DEGs, differentially expressed genes; PPI, Protein–Protein Interaction.

Figure 6

The qRT-PCR verification for RNA-Seq analysis. * * means P < 0.01. * means P < 0.05.

Figure 7

Illustration of potential regulatory mechanism involved in mitochondrial energy metabolism of FP intervention in the colonic epithelial cell. FP intervention upregulated ACS and CPT-1, promoting the transformation of FA to acyl-CoAs and the transfer into mitochondria for β -oxidation and TCA cycle. Then, CO_2 , NADH and FADH_2 were generated. FA intervention upregulated the relative proteins of complexes I and IV, promoting oxidative phosphorylation and generated H_2O . CA V catalyzed H_2O and CO_2 into HCO_3^- , and then co-catalyzed with CPS1 for the transformation of NH_3 and HCO_3^- to Citrulline. Therefore, the anti-obesity effect of FP intervention functioned through promoting the catabolism of FA to facilitate lipid metabolism and energy metabolism. Note that rectangles in green indicate upregulation of proteins in group FPD compared with group HFD, while that in grey indicates no significantly alteration. Group HFD indicates rats fed D12492 during model building and AIN-93M thereafter. Group FPD indicates rats fed D12492 during model building and an FP containing diet thereafter.

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