



Protective Effect of Compound Whole-Grain on High Fat and Cholesterol Diet-Induced Obesity and Lipid Accumulation in Rats

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ABSTRACT

To evaluate the effects of compound whole grains on high fat and cholesterol diet-induced obesity and lipid accumulation in rats and the possible molecular mechanisms, 40 male Sprague-Dawley rats were randomly assigned to four different diets, including reference chow diet (RCD), high fat and cholesterol diet (HFCD), city diet (CD) and compound whole-grain diet (CWD). Serum lipid profiles and glucose level were examined after 8 weeks. The molecular mechanisms underlined the effects of CWD on lipid metabolism were investigated by western blot and real-time PCR. CD exhibited fat accumulation increasing, increase in serum triacylglycerol, total cholesterol, glucose and the decrease in high density lipoprotein cholesterol. However, CWD can improve blood lipid and blood sugar levels, and at the same time improve obesity and fat accumulation in rats. CWD significantly augmented the relative level of peroxisome proliferators-activated receptor γ (PPAR γ) and suppressed the sterol regulatory element-binding protein 1c (SREBP-1c) protein expression in rats' tissues. Compound whole-grain ameliorates diet-induced obesity and hyperlipidemia by enhancing PPAR γ and reducing SREBP-1c in rats.

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Authors' Contribution

C-KZ designed the study. HZ and S-FH collected the data and drafted the manuscript. JW, S-KW analysed the results. G-JS revised the manuscript.

Key words

Compound whole-grain, Lipid profiles, Lipogenic genes, Rats

INTRODUCTIONS

The prevalence of chronic metabolic diseases such as hypertension, diabetes, atherosclerosis and obesity has increased dramatically all over the world (Karra and Batterham, 2010). Evidence suggests that nutritional imbalances and excesses, especially increased consumption of refined foods related to sedentary lifestyles, are key factors contributing to the epidemic (Boutayeb, 2006; Aune *et al.*, 2013). The intake of high refined carbohydrate and high saturated fat/cholesterol food is a common dietary pattern of urban residents in China, which leads to the increasing prevalence of hyperlipidemia and obesity in China (Yang *et al.*, 2008). Previous studies have showed that improving of food type and dietary structure can significantly improve body weight, lipid levels and reduce the risk of developing metabolic diseases (Williams *et al.*, 2008). Processed wheat flour and white rice are the most common cereals eaten by human beings. Studies have confirmed

that increasing dietary fiber and coarse grains intake by adjusting dietary carbohydrate sources can significantly improve body weight and glucose metabolism (Ye *et al.*, 2012). Therefore, the control of dietary cereals is beneficial to improve nutrition-related metabolic syndrome. Including new cereals in the diet may be a feasible and effective strategy to achieve this goal (Frølich *et al.*, 2013; Ross, 2015). Incorporating new grains or combinations of several whole grains as a staple food source in a high-fat may be an effective solution to this problem.

Compound whole grain is a kind of reconstituted whole grain product. Its original components are reconstituted according to the relative proportion of natural grains that are often eaten (Okarter and Liu, 2010). Compound whole-grain consists of intact wheat, sorghum, corn, millet and soybean including bran and germ according to preliminary study and traditional dietary habit of Chinese. The intact

Abbreviations

PPAR γ , peroxisome proliferators-activated receptor-gamma; FAS, fatty acid synthase; SREBP-1c, sterol regulatory element binding protein-1c; ACC, Acetyl-CoA carboxylase; RCD, reference chow diet; HFD, high-fat and cholesterol diet; CD, city diet; CWD, compound whole-grain diet; TC, total cholesterol; TG, triacylglycerol; HDL-C, high density lipoprotein cholesterol; HRP, horseradish peroxidase-conjugated.

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whole grain products contain a diversity of known phytochemicals, such as phytoestrogens, phenols and antioxidant, along with dietary fiber with a potential to significantly improve human health (Liu, 2007; Okarter and Liu, 2010). Previous studies have demonstrated that dietary intervention of compound whole-grain significantly ameliorated body weight, lipid and glucose metabolism in Chinese population (Jiang *et al.*, 2007; Zhang *et al.*, 2010). But up to now, the mechanism of compound whole grains improving fat and glucose metabolism is not clear. The purpose of this study was to evaluate how compound whole grains affect high fat and cholesterol diet-induced obesity and lipid accumulation in rats and related molecular mechanisms. By observing the regulation of compound whole grains on the expression of key proteins (peroxisome proliferators-activated receptor γ (PPAR γ) and sterol regulatory element binding protein-1c (SREBP-1c) (Han *et al.*, 2017; Knebel *et al.*, 2012) and genes (fatty acid synthase (FAS) and Acetyl-CoA carboxylase (ACC) (Horton, 2002; Foulle and Ferre, 2002)) revolve in fat and cholesterol metabolism to reveals the possible mechanism of compound whole grains affecting fat and cholesterol metabolism.

MATERIALS AND METHODS

Animals and experimental diets

40 ten-week-old male Sprague-Dawley rats (SLAC Laboratory Animal Company, Shanghai, China) weighing between 200 and 220 g were housed in a single stainless-steel cage for 12 hours in a light-dark cycle at constant humidity (60%) and temperature (21 \pm 2°C). Distilled water was provided sufficiently. The Animal Welfare Committee of Southeast University (Nanjing, China) and Chinese Zoological Society approved the experimental design. All investigation procedures conformed to the principles outlined in the Chinese Laboratory Animal Nursing and Use Regulations (Han *et al.*, 2012).

Standard diet was fed with all rats were for one week, and then rats was randomly assigned to the following four groups ($n = 10$ for each group): reference chow diet (RCD) as control group, high-fat and cholesterol diet (HFCD) as model group, city diet (CD) group and compound whole-grain diet (CWD) group. Table 1 lists the specific compositions of the four experimental diets. All dietary formulations meet the minimum requirements of the AIN-76 dietary standard. The CD was based on the dietary pattern of Chinese urban residents, with high fat, cholesterol accompanied by refined carbohydrates (Han *et al.*, 2012). For CWD, the main dietary carbohydrate source was compound whole wheat which replaced the starch content of processed rice and wheat of CD, and the

other ingredients were the same as CD. Compound whole-grain was mixed with wheat, corn, sorghum, millet and soybean including bran and germ according to preliminary study and Chinese traditional dietary customs. The grains and soybeans were ground into a powder and passed through a 1 mm sieve, mixed with other ingredients, and then subjected to irradiation sterilization to prepare an experimental feed. All rats were given an equal amount of diet daily throughout the experiment. Ensure that all rats consume equal feed rations per day during the experiment by using a feeding strategy (Brandsch *et al.*, 2006). The weight of rats was recorded periodically every week.

Table 1.- Composition of the experimental diets.

Diet ingredients (g/kg diet)	HFD	RCD	CD ^a	CWD
Casein	215	230	215	215
Maize starch	258	295	-	-
Sucrose	265	310	-	-
Wheat starch	-	-	261.5	-
White rice	-	-	261.5	-
Compound whole-grain ^b	-	-	-	523
Lard	100	-	100	100
Cellulose	50	50	50	50
Bean oil	-	70	-	-
Egg yolk powder ^c	50	-	50	50
cholesterol	15	-	15	15
Bile salt	2	-	2	2
AIN-76 mineral mix	30	30	30	30
AIN-76 vitamin mix	10	10	10	10
DL-methionine	3	3	3	3
Choline chloride	2	2	2	2
Total protein	233.9	233.5	280.2	301.4
Total carbohydrate	537	611	448	421.4
Total fat	127.8	70.3	133.6	146.9
Total cholesterol	17.5	0	17.5	17.5
Total dietary fibre	50.3	50.3	56	120.6
Total energy (kJ/g)	16.8	15.9	16.2	17.2

^a Patterned after the composition of the diet of city residents of modern China

^b Compound whole-grain is a reconstituted whole grain product, which is mixed with wheat, corn, sorghum, millet and soybean including bran and germ according to Chinese traditional dietary customs.

^c Contains 5% (w/w) cholesterol

RCD, reference chow diet; HFD, high-fat and cholesterol diet; CD, city diet; CWD, compound whole-grain diet.

Tissue sample management

After 12 hours of fasting at the 8th week, all animals

were sacrificed by anesthesia. Blood samples from rats were collected in test tubes and then centrifuged to separate the serum. The rat tissues of interest were removed and weighted. A portion of the liver was immediately excised and frozen to -20°C and then stored in RNAlater solution (Quigen, USA) until used for total RNA extraction. Before being stored at -80°C for western blot analysis, another part of liver and epididymal adipose tissues were placed in cryogenic storage containers and flash-frozen in liquid nitrogen.

Serum analysis

At the beginning of the experiment, the fourth weekend and the eighth weekend, after the rats were fasted for 12 hours, blood samples were taken from the tail vein. Using the commercial enzyme kits (Nanjing Jianchen Institute of Bioengineering, China) to determine the concentrations of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and glucose in rat serum.

Western blot analysis

Analyzing protein expression of PPAR γ and SREBP-1c of liver and epididymal adipose tissues by using the western blot technique. The Bradford kit (Keygen Biotechnology Company, China) was used to determine protein concentration. The same amount of protein was loaded into 10% SDS polyacrylamide gel and then transferred to the PVDF membrane (Milipore, USA) at a constant current of 200 mA for 60 minutes. The non-specific binding site was blocked by incubation containing 5% skimmed dry milk in TBST (Tris-HCl 10 mmol/L, NaCl 50 mmol/L and Tween-20 0.05 %, pH 7.6) for 1 hour at room temperature. The blots were incubated overnight with anti-SREBP-1C and anti-PPAR- γ (Santa Cruz, USA) antibodies under 4°C conditions. Antigen-antibody complexes were washed with horseradish peroxidase coupled anti-goat IgG antibody at room temperature and observed for 2 hours (cell signal, USA). Chemiluminescence ECL detection system was used to detect antibody reactivity (Keygen Biotechnology Company, China). Monoclonal mouse antibodies against β -actin (cell signal, USA) were used to as control protein loading. At least three impressions were performed to confirm the repeatability of these results. Band intensity was measured using Image Tool 3.0 software and normalized by β -actin measurement.

Quantitative real-time PCR

Trizol solution (Invitrogen, USA) was used to isolate total RNA. Reverse transcription of 1 μg total RNA into cDNA using M-MuLV reverse transcriptase (Fermentas,

USA). Using SYBR Green chemical real time PCR to quantify the relative gene abundance in 20 μL PCR reaction system containing 1 μL cDNA, 8 μL SYBR Green Master Mix (Toyobo, Japan), 2 μL Plus solution with 1.2 μL sense and antisense primers (10 μM). The PCR parameters as follows: initial at 50°C for 2 min, 5 min denaturation, then 40 cycles, denaturation for 15 seconds at 95°C and denaturation for 1 min at 60°C were performed. Real-time PCR analysis was performed by an iCycler Real-Time Detection System (Applied Biosystems 7300). The gene expression was calculated by the $2^{-\Delta\Delta\text{Ct}}$ values and standard curve method (Livak and Schmittgen, 2001). The reference control was GAPDH. To evaluated the specificity of the amplified PCR products, melting curve analysis was performed (Han *et al.*, 2012).

The primers were designed by Premier 5.0 software, and then primers were synthesized by Sangon Biotech (Shanghai, China). Primers sequences were as follows: GAPDH, 5'-AGTGCCAGCCTCGTCTCATAG-3'(forward), 5'-CCTTGACTGTGCCGTTGA-3'(reverse) FAS, 5'-AGCCCCCTCAAGTGCACAGTG-3'(forward), 5'-TGCCAATGTGTTTCCCTGA-3'(reverse). ACC, 5'-GGACCACTGCATGGAATGTTAA-3'(forward) 5'-TGAGTGACTGCC GAAACATCTC-3'(reverse).

Statistical methods

The data presented are in the form of mean along with standard deviation. One-way ANOVA was used to evaluate the significance of the differences among groups, and then Tukey *post-test* was performed. All statistical tests were 5% significant. SPSS software was used for statistical analysis (Version 15.0, SPSS, Inc., Chicago, IL, USA).

RESULTS

Body weight and organ/body weight ratio

The changes in mean body weight and organ/body weight ratios in each group is listed in Table II. There was no significant difference in mean body weight among the four groups before the experiment ($P > 0.05$).

All groups showed a slow increase in weight during the 8-wk experimental period. At the fourth week of the experiment, rats fed HFCD weighted heavier than control group fed RCD (424.32 ± 15.13 vs. 409.12 ± 11.82 g, $P < 0.05$).

At the end of the experiment, the weight gain of CWD-fed rats was significantly lower than that of CD-fed rats ($P < 0.05$) (Table II). The final average body weight of CD group was the same as that of HFCD group, while that of CWD group was comparable with that of RCD group. In addition, compared with CD and HFCD, CWD reduced the proportion of liver weight to body weight and visceral

fat index. There was no significant difference in the ratio of kidney weight/body weight, heart weight/body weight, spleen weight/body weight among the four groups.

Serum lipid profile and glucose

Before the experiment, serum concentrations of TG (Fig. 1A), TC (Fig. 1B), HDL-C (Fig. 1C) and glucose (Fig. 1D) were similar in the four groups of rats. At the 4th weekend of the experiment, HFCD-fed rats alone showed significant elevations in serum TC level, and reduction in serum HDL-C concentration compared to rats fed RCD ($P < 0.05$). But there was no difference in the level of serum TG and glucose in the two groups. At the end of the experiment, the levels of blood lipid and glucose in CD-fed rats were similar to those in HFCD-fed rats ($P > 0.05$). However, compared with CD and HFCD, CWD significantly lowered serum TC, TG and glucose concentration ($P < 0.05$).

Protein expression of SREBP-1c and PPAR γ

The regulation of CWD on SREBP-1c and PPAR γ protein expression in rat's liver and epididymal adipose by western blot is shown in Figure 2. The expression of

Table II.- Effect of compound whole-grain on body weight and organ/body weight ratios in rats fed high-fat/cholesterol diet for 8 weeks*.

	HFD	RCD	CD	CWD
Initial body weight (g)	265.73 ± 11.61	265.58 ± 10.27	266.54 ± 11.83	263.43 ± 18.42
Final body weight (g)	506.27 ± 16.14 ^b	471.29 ± 17.70 ^a	496.87 ± 20.55 ^b	462.19 ± 23.20 ^a
Liver index (g/g body weight)	0.0448 ± 0.0042 ^b	0.0256 ± 0.0029 ^a	0.0427 ± 0.0050 ^b	0.0328 ± 0.0028 ^a
Kidney index (g/g body weight)	0.0068 ± 0.0008	0.0068 ± 0.0006	0.0060 ± 0.0014	0.0063 ± 0.0003
Heart index (g/g body weight)	0.0031 ± 0.0004	0.0031 ± 0.0002	0.0031 ± 0.0003	0.0030 ± 0.0003
Spleen index (g/g body weight)	0.0024 ± 0.0005	0.0021 ± 0.0003	0.0021 ± 0.0004	0.0021 ± 0.0004
Fat mass (g/g body weight)	0.0306 ± 0.0063 ^b	0.0227 ± 0.0042 ^a	0.0302 ± 0.0072 ^b	0.0209 ± 0.0070 ^a

*Each value represents the means with their standard deviations for ten animals. Different superscript letters in each line indicate significant differences among groups ($P < 0.05$) ($n = 10$ for each group). For abbreviations, see Table I.

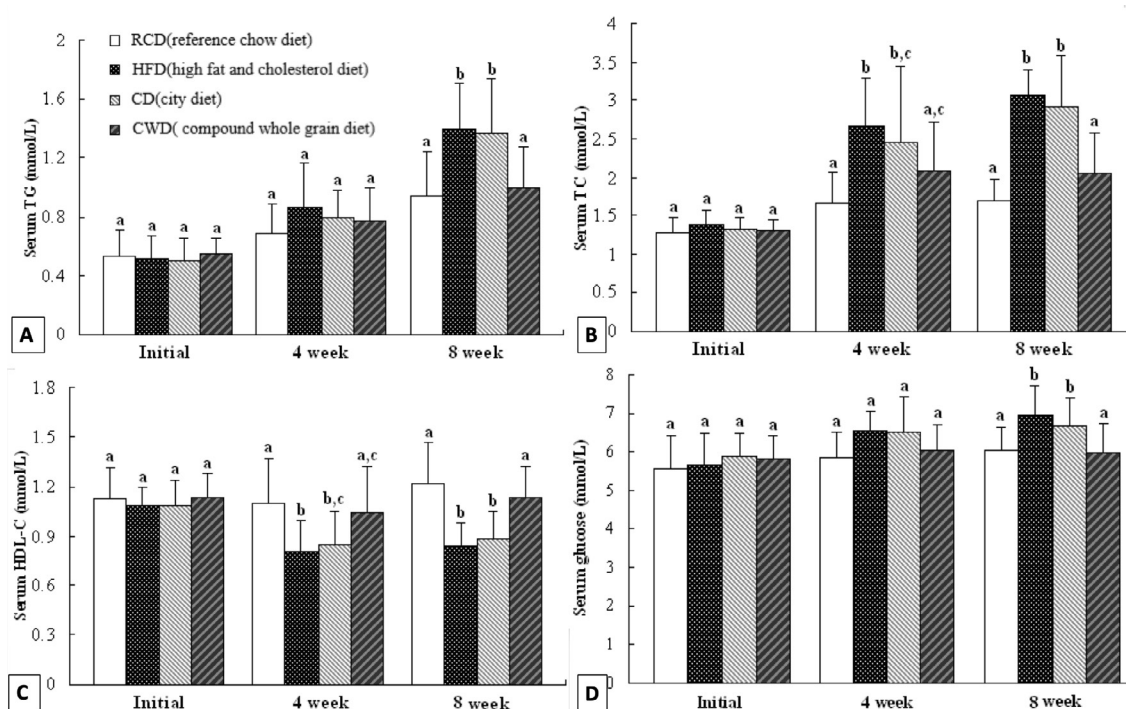


Fig. 1. Effect of compound whole-grain on serum lipid profiles and glucose levels in rats fed high-fat/cholesterol diet. Each value represents the means with their standard deviations for ten animals ($n = 10$ for each group). Bars without a common superscript letter indicate significant differences among groups ($P < 0.05$). (A), TG, triacylglycerol; (B), TC, total cholesterol; (C), HDL-C, high-density lipoprotein cholesterol; (D), glucose. For abbreviations, see Table I.

SREBP-1c and PPAR γ in liver and epididymal adipose tissue of rats in HFCD group was significantly upregulated than that in RCD group ($P<0.05$). However, compared with CD, CWD downregulated SREBP-1C protein expression ($P<0.05$). The relative level of SREBP-1c induced by CD was similar to that induced by HFD ($P>0.05$). The expression of PPAR γ in liver and epididymis adipose tissue in CWD group was significantly higher than that in HFCD and CD groups ($P<0.05$).

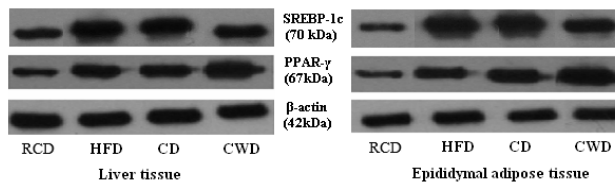


Fig. 2. Effect of compound whole-grain on protein expression of SREBP-1c and PPAR- γ in liver and epididymal adipose tissues of rats fed high-fat/cholesterol diet by western blot. The intensity of the bands was quantified by densitometric analysis and normalized with corresponding β -actin ($n = 5$ for each group). For abbreviations, see Table I.

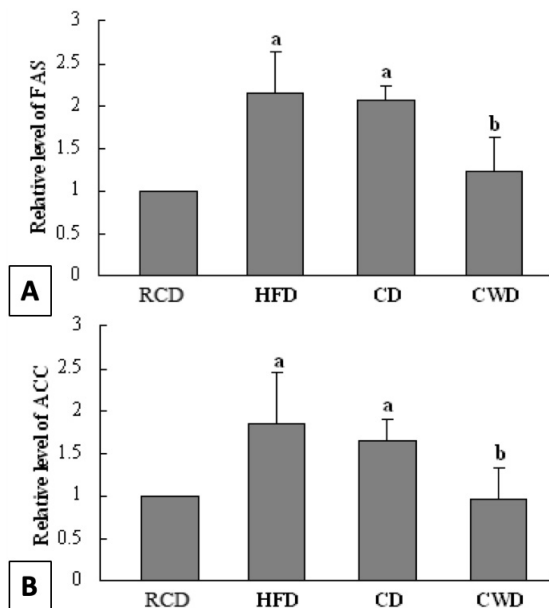


Fig. 3. Effect of compound whole-grain on relative mRNA abundance of FAS (A) and ACC (B) in liver tissue of rats fed high-fat/cholesterol diet. The steady-state mRNA concentrations of FAS and ACC were quantified with real-time PCR and $2^{-\Delta\Delta Ct}$ values were calculated to obtain fold expression levels. GAPDH mRNA levels were similarly measured and served as a reference control for mRNA quality and quantity. Each value represents the means with their standard deviations for five animals. Bars without a

common superscript letter indicate significant differences among groups ($P<0.05$). For abbreviations, see Table I.

Gene expression of FAS and ACC

The levels of ACC and FAS that are two key factors in adipogenesis were significantly up-regulated in the liver of HFCD and CD rats ($P<0.05$), the data was showed in Fig. 3. However, compared with CD and HFCD groups, the gene expression of ACC and FAS in CWD group were significantly lower ($P<0.05$).

DISCUSSION

Whole grain products can prevent the development of chronic diseases has been confirmed by more and more studies (Giacco *et al.*, 2011; Foerster *et al.*, 2014; Seal and Brownlee, 2015; Aune *et al.*, 2016). At the same time, our daily life style, especially the unbalanced energy-rich diet lacking in fibers and protective compounds (such as micronutrients and phytochemicals), is one of the reasons for the high incidence of chronic metabolic diseases. To data, such studies and intervention experiments have mainly focused on isolated free bioactive compounds in vitro studies or in animals. However, it is generally agreed that the health effects of whole grains come from the synergistic effects of active substances in whole grains, which mainly come from the bran and germ parts of grains (Liu, 2007). Based on the hypothesis, we examined the protective effects of compound whole-grain including bran and germ in a rat model of high fat and cholesterol feed-induced obesity and hyperlipidaemia. Our results indicate that CWD play a beneficial role in lipid metabolism by preventing the elevation of serum TG and TC and the decrease of HDL-C levels in rats even fed with high fat and cholesterol diet. CWD also decreased the fasting glucose level. In addition, CWD also inhibited excessive weight gain and visceral fat content caused by high fat and cholesterol diets. However, CD used in this study can lead to obesity and hyperlipidemia, which are manifested by increased obesity index and serum levels of TG and TC. This is consistent with previous study (Zhang, 2009). The composition of CD was based on the dietary habits of modern Chinese urban residents, rich in saturated fat and cholesterol. The sources of dietary carbohydrates are processed white rice and wheat starch, which lose most of the health compounds with removal of bran and germ during grinding and processing (EUFIC, 2015). For example, processed wheat starch is loss of about 79% vitamin E and 58% fiber (Truswell, 2002). The processed white rice and wheat starch digestibility is higher than whole grains, resulting in increased compensatory blood sugar overload and plasma insulin concentration

(Chatenoud *et al.*, 1999). Therefore, they cannot provide a rich balance of nutrients roughly the same as those contained in the seeds of original cereals.

The observed health benefits of CWD might be accounted for the synergistic effects of the package of nutrients available from compound whole grain, rather than the individual components. Compound whole-grain has richer dietary fiber (13.7g/100g) than processed white rice (0.4g/100g) and wheat starch (2.5g/100g), and its glycemic index (GI = 52.6) is very low (Jiang *et al.*, 2007). Epidemiological studies have shown that high dietary fiber diet and low GI food can not only improve blood glucose control, but also benefit weight management (Kendall *et al.*, 2010). Supplementation involving dietary fiber can result in health-promoting foods, lower food energy density and the postprandial glucose response that is thought to increase satiety, as well as a reduction in cholesterol and fat (Elleuch *et al.*, 2011). However, the benefits of whole grains lie not only in dietary fiber, but also in the synergistic effects of other bioactive compounds and micronutrients in whole grains. Except for dietary fiber, its bran and germ were found to contain other beneficial bioactive compounds including micronutrients, antioxidant properties and phytochemicals (Liu, 2007; Okarter and Liu, 2010) are recognized as sources of several physiologically activity components and health promoters (Hirawan *et al.*, 2010). The influence of compound whole-grain consumption on obesity and possibly other metabolic diseases may well depend on the presence or absence of many constituents and their interactions. Study has shown that a combination of phytochemicals in food has better health benefits than single phytochemicals by combining additions and/or synergistic effects (Eberhardt, 2000). Different kinds of cereals have different compositions of bioactive compounds, so they have different health effects (Adom and Liu, 2002). Among these compositions, millet and sorghum are rich in phenolic compounds, which strongly correlates with high antioxidant activity, such as reducing oxidative stress and lipid peroxidation (Dykes and Rooney, 2006). As an important part of a healthy diet, soy contains low or no starch, about 20% oil and 40% high quality protein. In addition, it contains several important biologically active compounds, including laurel, trypsin inhibitors, isoflavones and saponins. In place of the mixture of bioactive components present in the compound whole-grain, it is possible that more than one mechanism underlying this reduction in lipid accumulation is involved. However, the component analysis of phytochemicals was only from relevant literature review, the precise compositions of compound whole-grain should be investigated in further studies.

In liver and adipose tissue, a serious of fatty acids

stimulate PPAR γ , which is required for differentiation of preadipocytes to mature adipocytes and plays a key role in lipid metabolism. Studies showed that high-fat diet could result in liver steatosis through up-regulation of PPAR γ levels (Okumura Kohgo, 2006). In the present study, we also found that the protein expression of PPAR γ is upregulated in the liver and epididymal adipose tissue of rats fed with high fat and cholesterol diet. Interestingly, the protein expression of PPAR γ in CWD group was significantly higher than that in CD group and HFCD group. Whole-grain foods contained a large proportion of n-3 polyunsaturated fatty acids and their metabolites, which have been identified as natural ligands of the nuclear receptor PPAR γ (Lehrke and Lazar, 2005). Hence, we deduced that compound whole-grain activated PPAR γ and then resulted in changes a series gene related to lipid metabolism to accelerate fatty acid β -oxidation and lipid clearance. The activation of PPAR γ was also effective in improving low-grade inflammation and insulin signal transduction.

Obesity is due to the imbalance between adipose synthesis (adipogenesis) and lipolysis (adipolysis), both of which are regulated by the molecular level and activity involved in lipid metabolism. Cholesterol and fatty acid biosynthesis are regulated by the family of membrane-bound transcription factors (SREBP) in human cells (Foufelle and Ferre, 2002). SREBP-1c can be regulated by nutritional environment. Studies have demonstrated that SREBP-1c mediates the transcriptional effects of gene-coding enzymes related to lipogenesis, glycolysis and gluconeogenesis (Foufelle and Ferre, 2002). At the transcriptional level, SREBP-1c regulates the expression of several lipase-producing enzymes, such as FAS and ACC (Sato, 2010). Inhibiting FAS and ACC can reduce body fat accumulation (Strable and Ntambi, 2010; Okamoto, 2011). According to our findings, CD increased SREBP-1c protein expression of, while CWD decreased the relative level of SREBP-1c protein in liver and epididymal adipose tissue of rats fed high fat and cholesterol diet. Therefore, in the CWD group, the mRNA level of lipid-producing enzymes including FAS and ACC was also significantly decreased. Overall, our results suggest that compound whole grain inhibits fat production by downregulating SREBP-1c expression, which leads to a decrease in fat and fat accumulation. These findings provide important insights and indicate that the potential of compound whole-grain on preventing obesity and hyperlipidaemia in rats. Furthermore, our results provide a basis for promoting the development and utilization of whole grain agricultural products to prevent and treat metabolic diseases.

CONCLUSION

In conclusion, the present study provides new information about molecular mechanism of action that could be involved in the protective effects of compound whole-grain to revert and/or improve obesity and lipid accumulation induced by high fat and cholesterol diet in rats. The possible mechanism was that compound whole-grain can activate the nuclear transcription factors PPAR γ and down-regulate the expression of SREBP-1c involved in lipid mechanism. It is thought that the synergistic action of the package of nutrients available from compound whole-grain may be greater than the sum of the individual components. Future studies should investigate the effectiveness of compound whole-grain in attenuating undesirable metabolic diseases associated eastern-type diets.

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Conflicts of interest

There is no conflict of interest that needs to be disclosed.

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