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Effects of yeast polysaccharide on biochemical indices, antioxidant status, histopathological lesions and genetic expressions related with lipid metabolism in mice fed with high fat diet

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ABSTRACT

The present study investigated the effects of yeast polysaccharide (YPS) on mice fed a high-fat diet (15%). Mice were fed either a normal control diet (ND) or an HFD supplemented without or with YPS (250 mg/kg and 450 mg/kg) respectively for 7 weeks. Mice were collected for blood samples and sacrificed for liver samples collection at the end of the experiment. Serum TC, TG, LDL, HDL, ALT and AST levels were measured using commercially available kits; hematoxylin and eosin stain was used to measure pathological changes of liver; real-time PCR was used to detect gene mRNA expressions related with lipid metabolism of liver. Results showed that HFD group significant increased the body weight, FER, TC, LDL, TG, ALT, AST, and MDA levels, and decreased HDL, GSH levels and SOD activities compared to ND group. The consumption of an HFD caused fatty liver deposits and steatosis and increased lipogenesis compared to ND. The consumption of an HFD up-regulated SREBP1, FAS and SCD1 mRNA levels and down-regulated PPARα and CPT2 mRNA levels compared to ND. Administration of YPS significantly decreased body weight gain, food intake, FER, TC, LDL, TG, ALT, AST, and MDA levels, increased SOD activity and GSH and HDL levels, alleviated fatty liver deposits and steatosis, down-regulated SREBP1, FAS and SCD1 mRNA levels and up-regulated PPARα and CPT2 mRNA levels, compared to HFD. Therefore, these results suggest that dietary supplementation with YPS may be useful to ameliorate high fat induced obesity.

1. Introduction

Obesity is a rapidly growing epidemic worldwide, presenting an increase in the risk of hypertension, hyperglycemia, hyperlipidemia, morbidity and mortality in many countries across the world (Wimalawansa, 2013). In general, it is accepted that obesity results from an imbalance between energy intake and expenditure, and is characterized by increased fat accumulation in adipose tissue and elevated lipid concentrations in the blood (Serra, Mera, Malandrino, & Mir, 2013). The amount of fat mass is increased when the number and size of adipocytes are increased by proliferation and differentiation (Asterholm, Tao, & Morley, 2014). Hyperlipidemia is known to enhance the risk of coronary heart disease and fatty liver disease which is associated with reactive oxygen species (ROS) formation due to reduction in antioxidant capacity and free radical load generated by high-fat diet (Savini, Catani, & Evangelista, 2013). However, it is also well reported that antioxidants can inactivate these ROS and thus prevent metabolic deregulation including metabolic syndrome (Araldi, Modolo, & de Sa Junior, 2016). According to the World Health Organization (WHO), obesity is one of the most preventable diseases (Vucenik & Stains, 2012).

Recently, because of dissatisfaction with the high costs resistance and potentially hazardous side-effects, the search for new drugs capable of reducing and regulating serum cholesterol and triglyceride levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents (Derosa, Romano, & Bianchi, 2014). Plant products are frequently considered to be less toxic and freer from side effects than synthetic agents (El-Sayyad, El-Sherbiny, & Sobh, 2011). These properties have led to the discovery of new therapeutic agents including antioxidants, hypoglycemic and hypolipidemics.

Yeast polysaccharide is a kind of water-soluble polysaccharides, extracted from yeast cell wall and contain soluble glucan and mannan (Schiavone, Vax, & Formosa, 2014). The glucan is beta-1,6-brangched

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beta-1,3-glucan, and the mannan is alpha-1,6-mannan and contain the peptide at the terminal (Free, 2013). The total polysaccharides of this product is 60% or higher (Schiavone et al., 2014). YPS has been reported to have many biological effects such as tumor suppression, antioxidant and antimutagenic activities (Kogan, Pajtinka, & Babincova, 2008). In a clinical trial, β-glucan itself had strong anticancer effects in mice and patients (Lee C.I., 2014; Vetvicka, Pinatto-Botelho, & Dos Santos, 2014). However, no studies have been performed to elucidate the anti-obesity properties of YPS.

The objectives of the present study are to investigate the effects of YPS on biochemical indices, antioxidant status, histopathological lesions and genetic expressions related with lipid metabolism in mice fed with HFD for 7 weeks via dietary integration supplementation.

2. Materials and method

2.1. Source of materials/animals

60 Female ICR 4-weeks-old mice (Average Weight 23.7 g) used for this study were obtained from Yangzhou Medical center (Yangzhou, China). The feed used (basal chow diet) were obtained from Laboratory Animal Center of Nanjing Medical University, China. High fat diets constitute of 15% lard, 1% cholesterol, 0.3% cholic acid, and 83.7% basal diet provided by the National Research Center (Jiangsu, China). Yeast polysaccharide mixed link b-1,3/1,6-glucans and mannan oligosaccharide was produced and supplied by Angel Yeast Co, LTD (Jiangsu, China). Yeast polysaccharide obtained from baker's yeast (S. cerevisiae) cell wall is a powder containing more than 25% b-1,3/1,6-glucans, more than 20% mannan oligosaccharide, more than 30% protein, and < 8% water.

2.2. Treatment of animals

Mice were divided into 4 groups by a randomized complete block design containing woodchip bedding (15 per group). Mice in Group A were fed a basal diet and served as normal control diet (ND). Mice in group B fed a high fat diet (HFD), Mice in group C were fed the HFD supplemented with 250 mg/kg body weight YPS orally administered three times a week (HFD+YPS 250) group. Mice in group D were fed a high fat diet supplemented with 450 mg/kg body weight YPS orally administered three times a week (HFD+YPS 450). They were acclimatized at a normal standard temperature of 20–24 °C and a relative humidity of 50–70% with a 12 h light–dark cycle for 1 week on mice's chow diet only. The mice were given these diets for seven weeks along with water ad libitum. Weekly measurements of weight were recorded. All procedures using animals obtained the approval of the Animal Experiment Committee of China, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals at Nanjing Animal Hospital.

2.3. Collection of serum and organ tissues

At the end of the experiment, mice were fasted for 12 h, ether anaesthetized, and blood was collected from the orbital plexus into clean centrifuge tubes. Blood were allowed to clot at room temperature, centrifuged at 3500 rpm/min for 10 min at 4 °C and serum was separated and stored for measuring TC, TG, LDL, HDL, AST and ALT levels. All mice were sacrificed and Post-mortem examination was carried out immediately. Liver were dissected out, washed in saline water, blotted with filter paper and weighted. Portion liver samples were taken fixed in 10% neutral-buffered formalin for hematoxylin and eosin (H & E) stain determination. Portion liver samples were taken instantly into liquid Nitrogen and stored at −70 °C for determination of mRNA expressions by quantitative real-time PCR.

2.4. Serum Lipid measurements

Serum was obtained from blood by centrifugation at 5000*g*/min for 5 min at 4 °C. Serum TC, TG, LDL, and HDL were measured using commercially available diagnostic kits purchased from the Nanjing Jiancheng Bioengineering Institute (Jiangsu, China) according to the instructions.

2.5. Biochemical analysis of serum enzymes

Biochemical analysis were carried out to determine the activities of liver enzymes aspartate aminotransferase(AST) and alanine aminotransferase (ALT) using automated biochemistry analyzer BS-300 according to the manufacturers' instructions.

2.6. Measurement of GSH, SOD, and MDA in Liver Tissue

Measurement of glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) in liver tissue were weighted and homogenized with nine volumes of ice-cold buffer (1 mmol/L EDTA, 0.32 mol/L sucrose and 10 nmol/L Tris-HCl, pH =7.4). The homogenates were centrifuged at 5000gfor 5 min. The activities of SOD and the levels of GSH and MDA in liver tissues were determined using a commercial assay kit, according to the instructions.

2.7. Histopathological examination

Liver fixed in 10% neutral-buffered formalin were embedded in paraffin, sliced at a thickness of 5 μm and stained with H & E. The pathological changes were assessed and photographed under an Olympus BX-51 microscope connected to a digital camera (Olympus DP71, Tokyo, Japan).

2.8. RNA extraction and real time polymerase chain reaction (Real-Time PCR) Assay

The mRNA expression levels of Peroxisome proliferator-activated receptor alpha (PPARa), Carnitine palmitoyltransferase-1(CPT1), Carnitine palmitoyltransferase-2(CPT2), Acyl-coenzyme A oxidase(ACOX1), Sterol regulatory element-binding protein-1(SREBP1), Fatty acid synthase(FAS), Steaoryl-CoA desaturase(SCD1), Cholesterol 7α-hydroxylase gene(CYP7A1) and Low-density lipoprotein receptor (LDLR) were determined by real-time PCR. The primers of reference gene (β-actin) and target genes were designed by Primer 5.0 t1 online software and shown in Table 1. Total RNA was isolated from the frozen tissue samples using the RNAiso Plus (TaKaRa) reagent according to the manufacturer's protocol. Isolated RNA pellets were re-suspended in 30 μl of diethyl-pyrocarbonate-treated water, quantified by the measurement of the absorbamce at 260/280 nm and stored at −70 °C prior to cDNA synthesis. Quantitative real-time PCR was performed on an ABI Prism 7300 Detection System (Applied Biosystems, U.S.A.). All reactions were performed in duplicate.

The relative levels of mRNA were determined using the Δ cycle threshold (ΔΔCt) method with β-actin serving as a reference gene. For each of the target genes, the ΔΔCt values of all the samples were calculated by subtracting the average ΔCt of the ND group from the average △Ct of the HFD, 250 mg/kg YPS or 450 mg/kg YPS group. The ΔΔCt values were converted to fold differences by raising 2 to the power of −ΔΔCt (i.e., $2^{-\Delta\Delta Ct}$) (Gan, Chen, & Liao, 2014).

2.9. Statistical analysis

Data were analyzed statistically using SPSS 19.0 for Windows. Experimental results were expressed as mean ± SD. Statistical significance was tested with one-way ANOVA followed by the Student–Newman–Keuls post hoc test. P-values < 0.05 were considered statis-