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Gum arabic suppressed diet-induced obesity by alteration the expression of mRNA levels of genes involved in lipid metabolism in mouse liver



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ABSTRACT

Obesity is a global health concern associated with high morbidity and mortality. Therapeutic strategies include surgery and synthetic drugs; however, these may cause severe complications and high costs. The anti-obese effects of dietary fiber have widely been accepted in literature. Gum arabic (GA, *Acacia Senegal*) considered as a dietary fiber that could reduce the body fat deposition, nevertheless, its anti-obese effects remained unclear. In the present study, we fed mice either a normal diet (control), low fat diet (low), high-fat diet (high) or a high-fat diet supplemented with 10% w/w GA (High + gum) for 12 weeks. Body weights, visceral adipose tissue (VAT), plasma lipid profile, blood glucose and lipid metabolic genes expressions were measured. GA supplementation significantly decreased ($P < 0.01$) VAT, blood glucose, LDL, VLDL and total cholesterol, whereas, increased HDL concentrations. However, GA supplementation did not alter plasma triglycerides. Likewise, the supplementation of GA did not change lipogenic gene expression including fatty acid synthetase (FAS), stearoyl-coa desaturase (SCD) and acetyl-CoA carboxylase (ACC). Likewise, GA did not affect the expression of monoacylglycerol lipase (MGL), peroxisome proliferators activated receptor- γ (PPAR- γ) and HMG-CoA reductase (HMGR) gene expression. However, GA was significantly ($P < 0.05$) down-regulated super conserved receptor expressed in brain2 (SREB2) and adipose triglyceride lipase (ATGL) gene expression, in contrast GA was significantly ($P < 0.05$) up-regulated hormone sensitive lipase (HSL) and tumor necrosis factor- α (TNF- α) compared to the control groups in the liver. These findings conclude that GA has a potentiality to suppress obesity through alteration of lipid metabolic genes expression.

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1. Introduction

The prevalence of obesity is increasing in the population worldwide (Nguyen & El-Serag, 2010). Obesity is often part of the metabolic syndrome (Wahba & Mak, 2007), a condition which includes insulin resistance (Qatanani & Lazar, 2007), dyslipidaemia (Klop, Elte, & Cabezas, 2013), reduced HDL cholesterol (Rashid & Genest, 2007) and hypertension (Hosick et al., 2014). Metabolic syndrome increases the risk for development of cardiovascular

diseases such as coronary artery disease, stroke and end-stage renal disease (Hotamisligil, 2006). The obesity complications could not determine by absolute amount of fat in the body, however, it depend on distribution of fat (Jensen, 2008). The total body fat and distribution of adipose tissues were found to be associated with cardio-metabolic risk in adult females (Manson et al., 1990). The most important factor that determine fat deposition in adipose tissues include the rate of fatty acids uptake (Turcotte, Swenberger, Zavitz Tucker, & Yee, 2001), de novo fatty acid synthesis (Strable & Ntambi, 2010), triacylglycerols synthesis (Guo & Jensen, 2003), lipid degradation and transport of fatty acids (Hirsch & Han, 1969).

Obesity develops by the interaction of genetic components and certain environmental factors such as a high-fat diet. A recent prospective study showed that only subjects with a family history of obesity could gain weight by consuming high-fat diets

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(Heitmann, Lissner, Sorensen, & Bengtsson, 1995). Among genetic factors, low resting energy expenditure (Bogardus et al., 1986; Ravussin et al., 1988) and a low ability to oxidize fat (Zurlo et al., 1990) have been shown in prospective studies as risk factors for weight gain and obesity.

Several key enzymes were known to be committed in lipid metabolism in the target tissues (Zhao et al., 2010). Fatty acid synthase (FAS) (Smith, Witkowski, & Joshi, 2003), acetyl-CoA carboxylase (ACC) (Liu, Grant, Kim, & Mills, 1994), and glucose-6-phosphate dehydrogenase (G-6-PDH) (Young, Shrago, & Lardy, 1964) were considered the key lipogenic enzymes that could change the rate of biosynthesis of fatty acids through altering their activities. Furthermore, peroxisome proliferator-activated receptor γ (PPAR- γ) has been acknowledged as lipid metabolism regulator via regulation of lipid metabolic gene mRNA or protein expression in the adipose tissue, or by transporting fatty acids (Grindflek, Sundvold, Lien, & Rothschild, 2000). In contrast, hormone-sensitive lipase (HSL) (Kraemer & Shen, 2002) and monoacylglycerol lipase (MGL) (Taschler et al., 2011) are lipolytic enzymes which play a crucial role in lipid hydrolysis.

Gum arabic (GA) is an edible, dried sticky exudate from *Acacia seyal* and *Acacia senegal*, which is rich in non-viscous soluble fiber. It is commonly used in food industry and pharmaceutical field as an emulsifier and preservative (Ali, Ziada, & Blunden, 2009). In the North Africa and Middle East, it has been used as an oral hygiene material by various communities for centuries (Tyler et al., 1977). Previous studies have reported that, a high ingestion of dietary fiber, including GA were associated with beneficial effects on fat metabolism (Ali et al., 2009; Slavin, 2003). Dietary fiber was found to promote satiety, alter glycaemic index, affect gastric emptying and gut hormone secretions. Accordingly GA helps management of body weight (Chandalia et al., 2000).

In both human and animal, the majority of studies have investigated the anti-obese effects of GA on body mass index (Babiker et al., 2012) and fat deposition (Ushida, Hatanaka, Inoue, Tsukahara, & Phillips, 2011). However, the effects of GA on serum lipid profile, body mass index and its association with lipid metabolism genes expression in liver remained unclear. In the present study, we used experimental animals to investigate our hypotheses that serum lipid profile could be changed through administration of GA in mice, and those changes were associated with alterations of lipid metabolism genes expression in the liver.

2. Materials and methods

2.1. Animals

Eight-week-old male CD-1 mice were housed in a room at 23 ± 1 °C with a 12/12-h light–dark cycle. The animals had free access to water and standard mouse chow for an acclimatization period of 1 week. After that, animals weighing 23–24 g were randomly divided into four groups. The control group ($n = 20$) was fed standard mouse chow, low-fat diet (low, $n = 20$), high-fat diet (high, $n = 20$) and high-fat diet supplemented with gum arabic (GA) groups (high with gum, $n = 20$). The food was purchased from Jiangsu Province Cooperative Medical and Biological Engineering Co. Ltd (Table 1). Body weight was recorded throughout the study. At the end of 12 weeks, the whole blood was collected from the orbital fossa into EDTA-containing tubes, and plasma was separated by centrifugation at 3000 rpm for 15 min at 4 °C and stored at -80 °C until biochemical analysis. The mice then were killed by rapid decapitation. Liver and visceral adipose tissue (VAT) were dissected and weighed after washed shortly in cold PBS (pH 7.4). The tissue samples were stored at -80 °C until further analysis. The experimental procedures were approved by the Animal Ethics

Table 1

The effect of GA supplementation on organs weight, blood glucose and plasma lipid profile of mice fed with high-fat diet. The values are the means \pm SEM, $n=20$ /group. Different small letters in the columns indicate significantly different mean values at $P < 0.05$.

Nutrien	Low fat	High-fat	High-fat with Gum
Casein	19.0	25.8	25.8
L-Cystine	0.3	0.4	0.4
Cornstarch	56.8		
Maltodextrn	3.3	16.2	10.2
Sucrose	6.6	8.9	6.4
Cellulose	4.8	6.5	5.5
Soybean oil	2.4	3.2	3.2
Lard	1.9	31.17	31.17
Mineral mix1	1.0	1.3	1.3
Dicalcium phosphae	1.2	1.7	1.7
Calcium carbonate	0.5	0.7	0.7
Potassium citrate 1H ₂ O	1.6	2.1	2.1
Vitamin mix1	1.0	1.3	1.3
Choline bitartrate	0.2	0.3	0.3
Gum arabic			10
Total	100.5	100.0	100.5

Committee of Nanjing Agricultural University (Nanjing, China).

2.2. Plasma biochemical analysis

Blood glucose, plasma triglycerides, total cholesterol, LDL, VLDL and HDL were determined enzymatically using commercially kits (At Nanjing Military Hospital., Nanjing, China).

2.3. Quantitative real-time PCR

About 100 mg of liver was ground in liquid N₂, and a portion of about 50 mg was used for RNA extraction using the TRIzol total RNA kit (Invitrogen, Biotechnology Co, Ltd, Carlsbad, CA, USA) according to the manufacturer's instructions, and then total RNA was transcribed into cDNA using the Takara reverse transcription kit (Takara Biotechnology Dalian, Co. LTD). For establishing the effects of GA on lipid metabolism genes mRNA expression, real-time PCR was performed in an Mx3000 P (Stratagene, USA) according to our previous publication (Li et al., 2011). Mock RT and No Template Controls (NTC) were included to monitor the possible contamination of genomic and environmental DNA at the RT and PCR steps. A pooled sample made by mixing equal quantities of the RT products (cDNA) from all the samples was used for optimizing the PCR conditions and tailoring the standard curves for each target gene, and melting curves were performed to insure a single specific PCR product for each gene. The PCR products were sequenced to validate the identity of the amplicons. Primers specific for the PPAR- γ , ACC, FASN, HSL, ATGL, MGL, SREBP2, and TNF- α , G6P, HMGR, and SCD (Table 2) were synthesized by Geneary, Shanghai, China. A Mouse GAPDH was used as a reference gene for normalization purposes. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data (Livak & Schmittgen, 2001).

2.4. Statistical analysis

Descriptive statistics was performed to check the normality and homogeneity of variances before using parametric analyses. Body weight, organs weight, plasma lipids, plasma glucose, as well as the relative quantitative data of gene expression were analyzed by one-way ANOVA using SPSS 16.0 for Windows, followed by a least-significant difference (LSD) test for individual comparisons. A P -value ≤ 0.05 was considered significant.

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