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Gum Arabic Down-Regulate Cholesterol Biosynthesis Enzyme Gene mRNA Expression in Mice Muscle

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ABSTRACT

Gum Arabic (GA) is a complex polysaccharide used in the food manufacturing as a thickener and stabilizer. Our previous studies show that GA decreased body weight, visceral adipose tissue and plasma cholesterol level in mice. In addition, GA down regulated peroxisome proliferator-activated receptor gamma and stearoyl-CoA desaturase mRNA expression in mice liver. In the present study, we aimed to reveal the effect of GA on lipid biosynthesis gene mRNA expression in mice. 20 female CD-1 mice at 5 weeks age were divided into two groups, one served as control and the second received 10% of GA in drinking water for 6 weeks. Plasma glucose, total cholesterol, triglyceride, HDL-c and LDL-c were measured. Cholesterol biosynthesis gene mRNA expression of including down-regulated steroid 17-alpha-monooxygenase (CYP17) and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR) were measured by Q-PCR. Moreover, glucose 6-phosphatase and fatty acid synthase mRNA was measured. GA significantly ($P < 0.05$) reduced plasma glucose total cholesterol, HDL-c and LDL-c but not triglyceride when compared to the control group. GA significantly ($P<0.05$) down regulated CYP17 and HMGR mRNA expression in mice muscle when compared to the control group. Yet, GA did not affect G6P and FAS mRNA expression. Our data indicate that GA may be of useful in control of obesity.

KEYWORDS

Gum Arabic; Cholesterol Biosynthesis Gene; Mice.

INTRODUCTION

The incidence of obesity is increasing in the population worldwide [1, 2]. Obesity is frequently part of the metabolic disease, a situation which includes dyslipidaemia, decreased high density lipoprotein cholesterol and hypertension [3-5]. Metabolic syndrome increases the risk for development of cardiovascular disorders such as heart stroke, coronary artery disease, and chronic renal disease [6-8]. The complications of obesity are not only determined by absolute amount of fat in the body, nevertheless, it depends on distribution of fat [9]. The distribution of adipose tissues in the body was found to be linked with cardio-metabolic risk in many populations [9]. The most important factors that determine deposition of fat

in body include de novo fatty acid synthesis, triacylglycerols synthesis, the rate of fatty acids uptake, lipid degradation and synthesis of cholesterol [10-13].

A number of key enzymes are known to play a major role in lipid metabolism in the target tissues. 17-alpha-monooxygenase (CYP17), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR) and fatty acid synthase (FAS) are the key lipids biosynthesis enzymes that play a critical role in lipid biosynthesis [14-16]. HMGR catalyzes conversion of HMG CoA to mevalonate, a precursor of isoprenoid groups that are incorporated into many end-products including cholesterol [17]. HMGR has been well known as the rate-limiting enzyme in cholesterol biosynthesis. Inhibition of HMGR considers the key factor in controlling of hyperlipidemia which could be of clinical importance [18]. Several medicinal plants have been found to regulate HMGR such as *Emblica officinalis,* arabinoxylan and *Quercus mongolica* [19-21].

Gum arabic (GA) is an edible, dried sticky exudate from Acacia seyal and Acacia senegal is found to be rich in non-viscous soluble fiber. It has been commonly used in food manufacturing and pharmaceutical field as an emulsifier and preservative. In the Middle East and North Africa, it has been used as an oral hygiene material by various communities for centuries [22]. In our previous studies, we reported that GA suppressed diet-induced obesity by alteration the expression of mRNA levels of genes involved in lipid metabolism in mouse liver [23]. In both human and animal, the majority of studies have examined the anti-obese effects of GA on body mass index and fat deposition [24, 25]. However, the effects of GA on serum lipid profile and its association cholesterol biosynthesis enzyme gene mRNA expression remained unclear. Therefore, in the present study, we used experimental mice to investigate our hypotheses that serum lipid profile could be changed via administrated of GA in mice, and the changes in serum lipid profile may be associated with alterations of cholesterol biosynthesis enzyme gene mRNA expression in the muscle.

MATERIAL AND METHODS

Experimental animals

Twenty female CD-1 mice at 5 weeks age were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). The mice were housed under controlled lighting (12 h light, 12 h dark), temperature (21 8 C – 22 8 C) and humidity at 65% – 70%. The mice were allowed free access to a commercial pellet diet and drinking water throughout the experiment period. The experimental protocol involving mice was approved in accordance with the guide for the care and use of laboratory animals prepared by the Institutional Animal Care and Use Committee of Nanjing Agricultural University

Experimental design

After an acclimatization period of a week, mice were randomly divided into two equal groups. The first group continued to receive the same diet without treatment until the end of the study (control group). The second group was given normal food and received 0.5% of gum arabic aqueous solution as drinking water for 7 days, and then 10% solution for further 6 weeks. During the treatment period, the mice were weighed weekly. After 6 weeks, the mice were killed. Serum samples and muscle tissue were collected and stored at -80 ◦C.

Plasma lipid profile

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

RNA extraction and Real-time PCR

About 100 mg of muscle was ground in liquid N_2, and a portion of about 50 mg was used for RNA extraction using TRIzol total RNA kit (Invitrogen, Biotechnology Co, Ltd, Carlsbad, CA, USA) according to the manufacturer's instruction. Two approaches were taken to ensure that all the total RNA preparations are free of genomic DNA contamination. Firstly, total RNAs were treated with 10 U DNase I (Rnase Free, D2215, Takara, Japan) for 30 min at 37°C, and purified according to the manufacturer's protocol. Secondly, the primers for the reference gene were designed to span an intron, so any genomic DNA contamination can be reported easily with an extra product in the melting curves for real-time PCR. Real-time PCR was performed in Mx3000P (Stratagene, USA) according to the previous publication [23]. Mock RT and No Template Controls were included to monitor the possible contamination of genomic and environmental DNA at both RT and PCR steps. The pooled sample made by mixing equal quantity of RT products (cDNA) from all samples was used for optimizing the PCR condition 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 CYP17, HMGR, G6P and FAS (Table 1) were synthesized by Geneary (Shanghai, China). Mice GAPDH were used as a reference gene for normalization purposes. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data [26]. The mRNA abundances were presented as the fold change relative to the average level of the control group.

Group	Glucose	Triglyceride	Total cholesterol	HDL	LDL
	(mmol/L)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)
Control	8.01±.51a	49.1±3.08a	82.7±3.4a	55.82±2.32a	75.36±5.51a
Gum	4.06±0.70b	45.4±2.4a	63.7±3.15 b	67.56±3.14b	45.61±3.31b

Statistical Analysis

Data are expressed as the mean ± SEM and compared by one way analysis of variance and Student's T test. P < 0.05 as considered significant [27]. All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA).

RESULTS

Plasma lipid profile and blood glucose

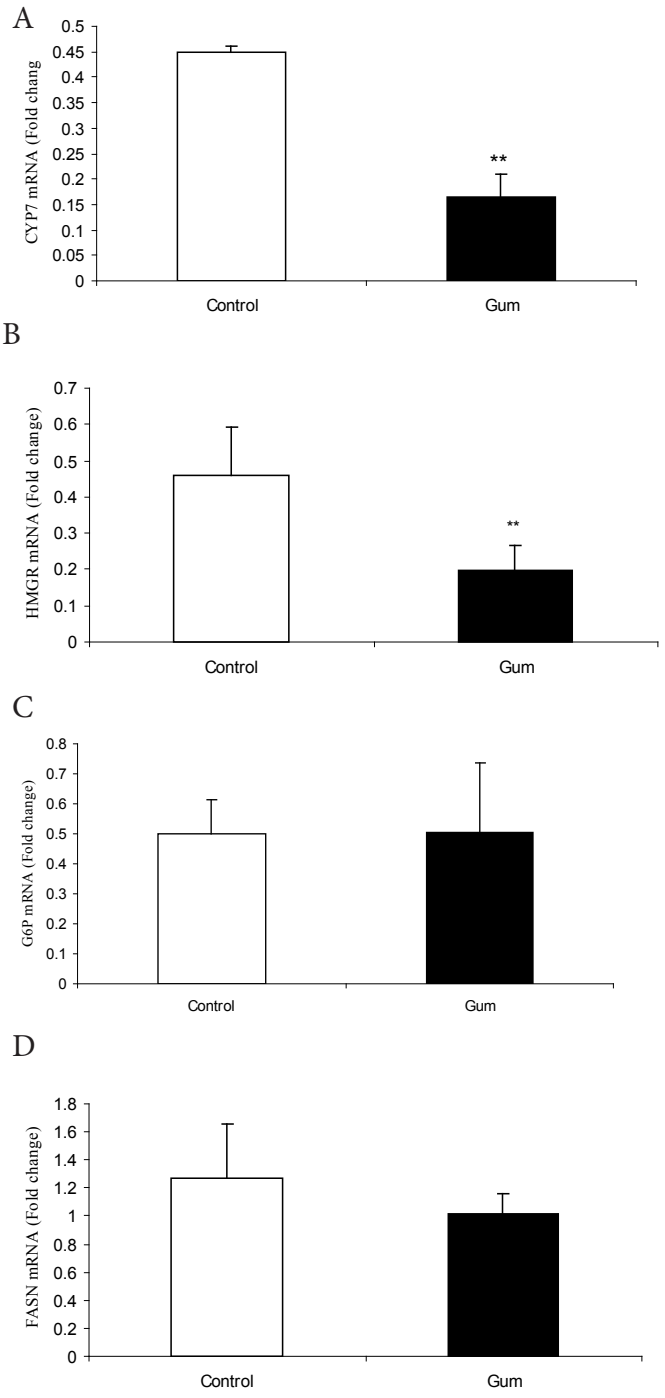
GA treatment significantly (P < 0.05) decreased plasma glucose total cholesterol, HDL-c and LDL-c compared to the control group (Table 2). No changes were observed in triglycerides concentration regarding GA supplementation.

Table 2: Real-Time PCR Primers.

Target genes	Gene bank number	Product Size	Primer
FASN	NM_007988.3	129	F: 5'- GATATTGTCGCTCTGAGGCTGTTG -3'
			R: 5'- GGAATGTTACACCTTGCTCCTTGC -3'
HMGR	NM_008255.2	83	F: 5'- TGACCTTTCTAGAGCGAGTGCAT -3'
			R: 5'- CACGAGCTATATTTTCCCTTACTTCA -3"
CYP17	NM_001291508.1	2	F: 5'- CAACTCAGCGGGTGGATACC-3"
			R: 5'- GGACCGGGCGTCTATAACAG-3"
GAPDH	NM_008084.2	141	F: 5'- ACATGGTCTACATGTTCCAGTA -3' R: 5'- GGAGTCTACTGGTGTCTTCA-3'

Liver and Muscle 11β-HSD 1 mRNA expression

The supplementation of GA significantly (P<0.05) down regulated CYP17 (Figure. A) and HMGR (Figure. B) mRNA expression in mice muscle compared to the control group. However, supplementation of GA did not change G6P (Figure. C) and FASN (Figure. D) mRNA expression in mice muscle.



DISCUSSION

Obesity is a risk factor for several metabolic diseases such as diabetes, coronary heart disease, heart stroke, nonalcoholic fatty liver disease and many other disorders including cancer [28-30]. In the present study, gum arabic (GA) supplementation significantly reduced blood glucose. Our findings are in line with pervious studies that dietary fibre reduced blood glucose either in normal or fed high fat diet mice [23, 31]. Recent studies reported that GA treatment decreased plasma total cholesterol, triglyceride and low density lipoprotein (LDL) concentrations in human and mice [31, 32]. In agreement with these findings, we reported that treatment of GA decreased

plasma total cholesterol, HDL-c and LDL-c. Hyperlipidemia is well known as risk factor for the development of atherosclerosis [33]. Hyperlipidemia can significantly increase the risk of developing cardiovascular disease, including coronary artery disease, diabetes mellitus, hypertension, cerebrovascular disease, and limbs peripheral vascular disease. These conditions can in turn cause heart strokes, heart attacks, and metabolic syndrome [34-40]. The reduction of plasma lipid profile by GA may contribute in the decreasing the metabolic syndrome. Therefore, GA may be promising to be used as Antiobesity choice but requires further studies.

Several mechanisms have been projected to disclose the hypocholesterolemic effects of dietary fibre [41]. One of the potential justification is that dietary fibre increases the intestinal contents viscosity, therefore interfering with micelle formation and absorption of nutrient, which, in turn, decreases intestinal lipid absorption [20]. An additional mechanisms proposed that soluble dietary fibers decreases the enterohepatic circulation of bile acids, resultant in increases of bile acid excretion and subsequently decreases plasma cholesterol concentrations [42, 43]. Moreover, the viscosity of fermentable dietary fibers is reported to contribute in lipid lowering in rat [44].

Obesity is frequently associated with type 2 diabetes, hypertension and hypercholesterolemia, which consequently result in coronary heart disease [45]. Based on World Health Organization (WHO) report, risks for type 2 diabetes and hypercholesterolemia are greatly increased in obese patients. In the present study, the reduction of plasma total cholesterol in GA supplemented mice associated with alterations in the expression of cholesterol biosynthesis enzyme gene. Supplementation of GA significantly down-regulated CYP17 and HMGR mRNA expression in mice muscle. Our results are agreed with previous report that supplementation dietary fiber reduced liver HMGR mRNA expression in mice [46, 47]. Inhibition of the key enzyme of cholesterol biosynthesis may prevent hypercholesterolemia in obesity patient and thus it may reduce the obesity complications such as diabetes, coronary artery disease, heart stroke and hypertension [48-50]. Moreover, total ablation of androgen production by potent CYP17 inhibitors may offer effective treatment of prostate cancer patients [51, 52]. However, the supplementation of GA did not change G6P and FASN mRNA levels. Our results are mismatch with previous finding that the consumption of dietary fibre reduced FASN mRNA expression in obese human [53]. The inconsistent of these results may be due to the species differences or experimental design. FAS mRNA levels were unchanged, whereas SREBP-1c increased. The obesity subjects have found to an increased hepatic lipogenesis that may contribute to their excessive fat mass but no evidence for an increased lipogenic capacity of adipose tissue. Therefore, GA may contain principle substance that could contribute in reducing obesity and its complications.

CONCLUSION

The data concludes that gum arabic can reduced blood glucose, plasma total cholesterol associated with down-regulation of cholesterol biosynthesis gene in mice muscle, thus GA is may be of useful in the control of hyperlipidemia in obese patient.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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