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Xylo-oligosaccharides regulated the key enzymes and receptors involved in cholesterol metabolism in hypercholesteremic hamsters

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Abstract
High levels of total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) in the blood are the major risk factors for coronary heart disease and atherosclerosis. In our previous study, we found that Xylo-oligosaccharides (XOS) were down-regulated plasma cholesterol and non-high-density lipoprotein-cholesterol (non-HDL-C) by the promotion of acidic sterols excretion. The underlying mechanism by how supplementation of XOS improved excretion of acidic sterols remains vague. Therefore, this study was designed to examine the role of xylooligosaccharides in the regulation of key enzymes and receptors in cholesterol metabolism including cholesterol 7α-hydroxylase (CYP7A1), liver X receptor α (LXRα), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and sterol regulatory element-binding protein-2 (SREBP-2). In this study, enzymes and receptors were measured in hamsters fed for six weeks one of three diets: low cholesterol diet (LCD), high cholesterol diet (HCD), and HCD plus 5% xylooligosaccharides (XOS).
The results of this study showed that protein levels of CYP7A1(The rate-limiting enzyme for bioconversion of cholesterol to acidic sterols in the liver) and LXRα (CYP7A1 regulator) were up-regulated by xylooligosaccharides supplementation. By contrast, HMG-CoA (The major regulatory enzyme in cholesterol biosynthesis), and SREBP-2 (SREBP-2 is a master nuclear transcription factor for regulation of HMGCR) in hepatic hamsters were down-regulated by xylooligosaccharides addition. Therefore, the present data indicate that XOS use as nutraceuticals or functional foods may be a strategy for the regulation of hypercholesteremia in humans.

Keywords: Xylo-oligosaccharides, cholesterol metabolism, enzymes, receptors, hypercholesteremic hamsters

Introduction
For several decades, heart disease remains the leading cause of human death (Mehra, 2007) [12]. Numerous experiments have shown that plasma TC and LDL-C elevation is considered a risk factor for heart disease (Wu *et al*., 2000) [16]. Whereas the elevation of high-density lipoprotein cholesterol (HDL-C) is linked with a low risk of cardiovascular disease. Plasma TC homeostasis is a function of excretion, bioconversion, absorption, and synthesis of cholesterol in the liver and intestine (Chen *et al*., 2008) [4]. Generally, cholesterol is needed for normal metabolism in humans' body, since it acts as a precursor for acidic sterols synthesis, a substrate steroid hormones synthesis (estrogen and androgen), an essential cell membrane modulator, and cholesterol derivative such as 7-dehydrocholesterol) the source for vitamin D synthesis*)* [Chen *et al*., 2008] [3]. However, the cholesterol sources in mammals are absorption from the diet and *in vivo* synthesis in the liver, in which 70% to 80% of cholesterol is synthesized by the liver *in vivo* (Xu *et al*.,2015) [17]. The synthesis of hepatic cholesterol is mainly governed by SREBP2 and its downstream enzyme including HMGCR, while the elimination of cholesterol is mainly controlled by liver X receptor α (LXRα) and its downstream enzyme CYP7A1(Zhao *et al*., 2018) [19]. In recent years, natural food components served as functional foods to lower blood cholesterol levels have attracted considerable interest due to their high efficiency without side effects (Lei *et al*., 2015) [8]. Xylo-oligosaccharides are on the list of potential cholesterol-lowering natural food components.
Xylo-oligosaccharides are a series of xylose polymers and have been widely applied in the food industry since their sweetness is approximately half of sucrose sweetness (Samanta *et al*., 2015; Lim *et al*., 2016; Ibrahim *et al*., 2018) [13, 10]. It was reported that *xylo*oligosaccharides supplementation could remarkably reduce plasma cholesterol levels in diabetic rats (Gobinath *et al*., 2010) [4].

A recent study had shown that dietary xylobiose, one of the main components in xylooligosaccharides, could significantly lower blood total cholesterol levels (TC) as well as low-density lipoprotein cholesterol levels (LDL-C) in high-fat diet-induced obese mice (Lim *et al*., 2016) [10]. Although the mechanism responsible for the cholesterol-lowering activity of xylooligosaccharides was not fully understood, some evidence suggested that xylooligosaccharides lower blood TC was possibly mediated by up-regulating the expression of acidic sterols -related enzymes via remodeling gut microbiota composition (Lim *et al*., 2016; Zhang *et al*., 2020) [10, 18]. The results of our previous report showed that XOS could successfully reduce TC in plasma of hypercholesteremic hamsters by the promotion of acidic sterols excretion (Abdo *et al*.,2021) [1]. However, it remains largely unknown how XOS improved the excretion of acidic sterols. Therefore, the present study was conducted to examine the effects of XOS on key enzymes and receptors associated with cholesterol metabolism in hamsters fed a high-cholesterol diet.

Material and Methods

Materials and reagents

Liver samples were collected from hamsters fed one of three diets: low cholesterol diet (LCD), high cholesterol diet (HCD), and xylooligosaccharides experimental diet (XOS). Antibodies including SREBP2, HMG-CoA, CYP7A1, and LXRα were purchased from Santa Cruz Biotechnology (China). Other chemicals used in the present study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Protein Preparation

Samples of hamster's liver were weighted before being lysed in western homogenize buffer supplemented with protease inhibitor on ice and homogenized for few seconds using a glass homogenizer. After centrifuging at 13000 rpm for 10 min, the supernatant was collected. To collect the protein pellet, the supernatant again was centrifuged using ultra-centrifuge at 35000 rpm for 1 hour at 4 ℃. Thereafter, the protein concentrations were measured by BCA protein assay kit (He *et al*.,2019) [5].

Western Blot

Briefly, the protein was separated on the SDS page. After separation, the protein bands were transferred into the polyvinylidene fluoride (PVDF) membrane (0.45 μm, Millipore, Billerica, MA, USA), After blocking with 5% non-fat milk for 1h at room temperature, the membrane was respectively incubated with the following secondary antibodies: CYP7A1, SREBP2, LXRα, and HMG-CoA-R or beta-actin overnight at 4◦C. The m-1Ggkbp-HRP was used as a secondary antibody for incubation for one hour at room temperature. Thereafter, the membrane was washed three times in Tris-buffer saline containing 0.1% Tween 20 (TBST), the immunoreactive bands were detected with Immobilon Western Chemiluminescent HRP Substrate and exposed using Fluor Chem Q system (Shi *et al*., 2019) [14].

Statistical analysis

The experimental data were expressed as the means ± standard deviation (SD). Statistical differences were estimated by using the one-way analysis of variance (ANOVA) using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). The significant difference was defined as p-value was <0.05.

Results and Discussion

Protein abundance of LXRα, CYP7A1, SREBP2, and HMGCR the hamster's liver

Liver is the central organ for storage, de novo synthesis, and removal of cholesterol (Zhao *et al*.,2017) [20].

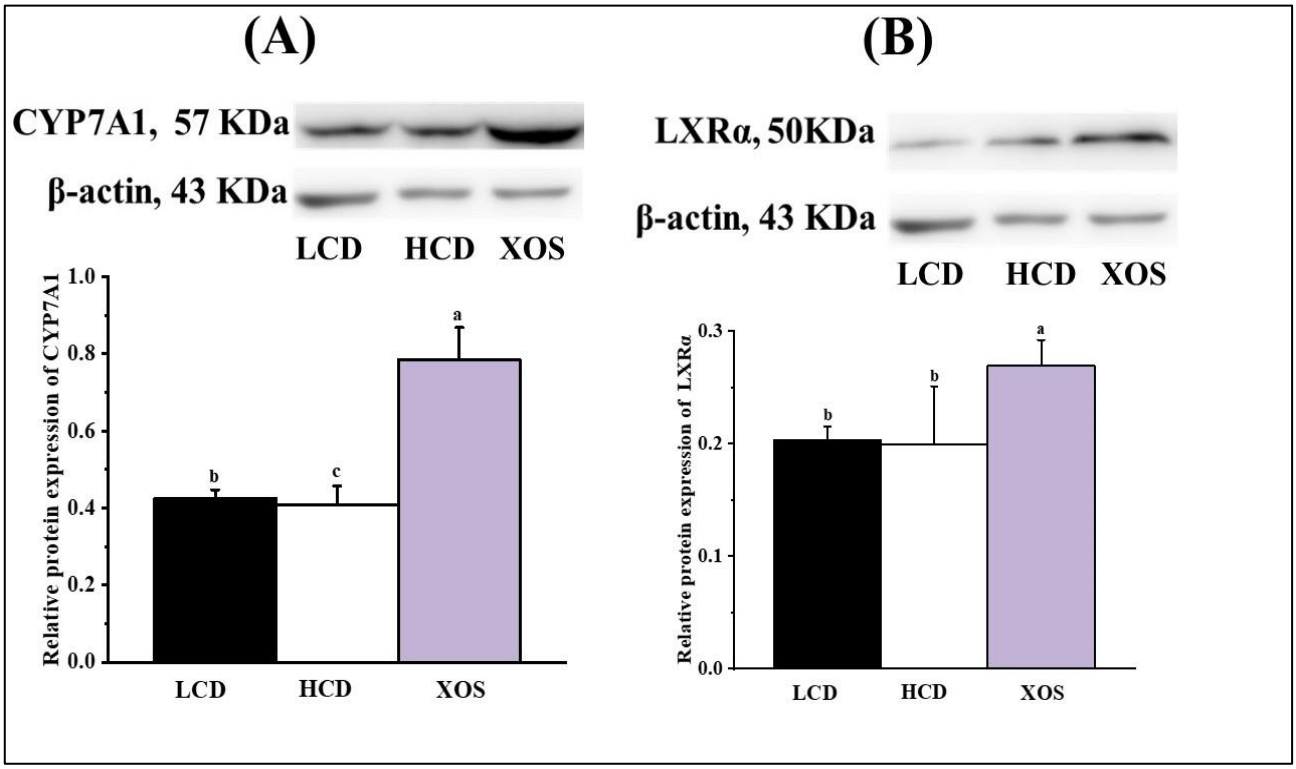

Fig 1: Relative changes in protein expression of hepatic liver X receptor α (LXRα), and cholesterol 7α hydroxylase (CYP7A1) in hamsters fed the Low-cholesterol diet (LCD), high-cholesterol diet (HCD), and HCD diet + 5% xylooligosaccharides (XOS).

In your previous study, we found that the XOS diet could remarkably decrease levels of plasma TC and non-HDL-C by increased fecal acidic sterols excretion in hypercholesterolemic hamsters, indicating that XOS was potent in modulating hypercholesterolemia (Abdo *et al*.,2021) [1]. To verify the underlying mechanism by how supplementation of XOS improved excretion of acidic sterols, this study was designed to examine the interaction of XOS with protein expression of the key enzymes and nuclear receptors involved in cholesterol metabolism and synthesis of acidic sterols in hamster's liver. CYP7A1 is the first rate-limiting enzyme in acidic sterols synthesis. Accompanied by the increase in fecal acidic sterols excretions, Since, conversion of excessive cholesterol in the liver to acidic sterols is mediated by CYP7A1. In our study, XOS significantly up-regulated protein expressions of CYP7A1 (Fig.1A). These results were in agreement with the study of Lim *et al*., (2016) [10]; Lim *et al*., (2018) [9], who presented that dietary xylobiose (A major component of xylooligosaccharides) reduced TC by activating CYP7A1 in diabetic mic. Furthermore, the nuclear receptor LXRα was responsible for regulating CYP7A1 transcription (Calkin Tontonoz, 2012) [2]. In this study, XOS remarkably increased LXRα protein expressions compared with HCD (Fig. 1B).

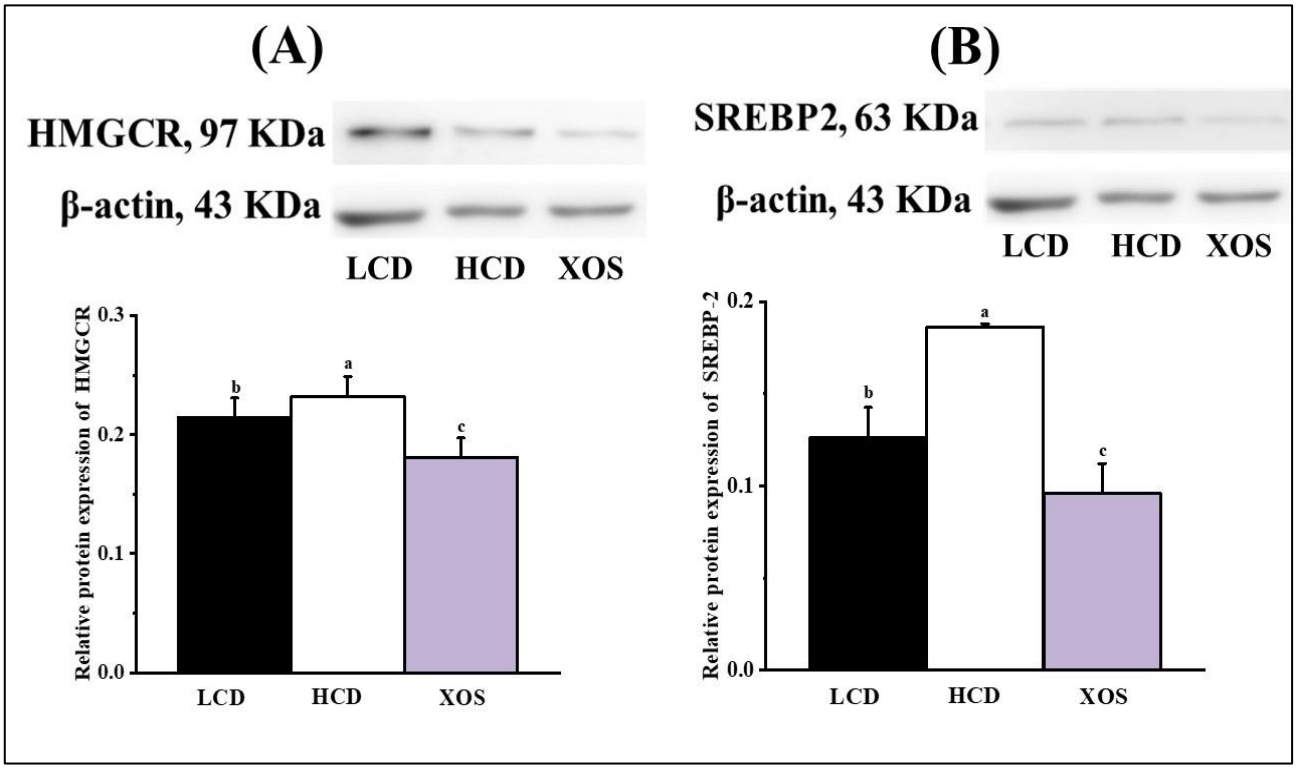

Fig 2: Relative changes in protein expression of hepatic sterol regulatory element-binding protein 2 (SREBP2), 3-hydroxy-3-methylglutaryl- CoA reductase (HMGCR) in hamsters fed the Low-cholesterol diet (LCD), high-cholesterol diet (HCD), and HCD diet + 5% xylooligosaccharides (XOS).

Another approach to treat hypercholesterolemia is by inhibiting the synthesis of cholesterol (Hunter, Hegele, 2017) [6]. HMGCR is a rate-limiting enzyme involved in the synthesis of liver cholesterol (Thomas *et al*., 2008) [15]. Supplementation of XOS in diets could down-regulate the protein expression of HMGCR (Fig. 2A). suggesting that XOS are able to reduce plasma TC via inhibiting synthesis and conversion of hepatic cholesterol. SREBP2 is a key regulator in the metabolism of cholesterol by activation of gene expression of HMGCR (Zhao *et al*., 2017) [20]. In line with the down-regulation of HMGCR, we found that XOS supplementation significantly decreased SREBP2 protein expressions (Fig. 2B), suggesting that XOS induced reduction in plasma TC was also mediated by down-regulation of SREBP2. This result was consistent with the previous study of Lim *et al*., (2016) [10], who claimed that xylobiose (A major component of xylooligosaccharides) have cholesterol-lowering activity in diabetic mice by inhibiting mRNA expression of HMGCR. What is more, SREBP-1C was mediated by xylose (The monosaccharide of XOS) supplementation into the diet of obese mice (Lim *et al*., 2015) [11]. The modulation of liver proteins by XOS feeding was maybe due to the ability of XOS to increase short-chain fatty acids, since the addition of 5% xylooligosaccharides into the HCD diet could significantly increase fecal acetate, propionate, and butyrate (Abdo *et al*., 2021) [1].

Conclusion

In this study, we studied the effects of dietary xylooligosaccharides on the regulation of key enzymes and receptors involved in cholesterol metabolism in hamsters fed high cholesterol diets. XOS may lower plasma TC by regulating the expression of key enzymes and receptors in the liver. Thus, the results of this work provide valuable insight into the probable application of XOS as a dietary supplement for the prevention of hypercholesterolemia.

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