Original Research

Chitosan oligosaccharide decreases very-low-density lipoprotein triglyceride and increases high-density lipoprotein cholesterol in high-fat-diet-fed rats

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Abstract

It is well known that chitosan has beneficial lipid-regulating effects, but it remains unknown whether chitosan oligosaccharide (COS), the chitosan degradation product, has the same lipid benefits. High-fat-diet-fed Wistar rats were administrated with COS by gastric gavage for three weeks. The effects of COS on lipids, lipoprotein components and lipid metabolism related protein activities were investigated. Plasma lipids level assays by an enzyme method showed that COS decreased triglyceride (TG) by 29–31%, and increased high-density lipoprotein (HDL) cholesterol by 8–11%, but did not affect low-density lipoprotein (LDL) cholesterol. Lipid distribution analysis through fast protein liquid chromatography indicated that COS significantly decreased TG content distributed in very-low-density lipoprotein (VLDL)/LDL fractions but increased cholesterol content in HDL fractions. Apolipoprotein analysis through plasma ultracentrifugation and sodium dodecyl sulfate polyacrylamide gel electrophoresis displayed that COS decreased apolipoprotein B-100 of LDL and increased apolipoprotein E of LDL and apolipoprotein B-100 of VLDL, but did not change apoA-I content of HDL particles. Lipoprotein formation associated protein determination showed that COS also increased plasma activity of lecithin cholesterol acyl transferase but not phospholipid transfer protein. The present study suggests that COS may play a beneficial role in plasma lipid regulation of rats with dyslipidemia induced by high-fat diet. The COS-decreased VLDL/LDL TG and -enhanced HDL cholesterol may be related to the upregulated activity of lecithin cholesterol acyl transferase.

Keywords: chitosan oligosaccharide, hyperlipidemia, very-low-density lipoprotein triglyceride, high-density lipoprotein cholesterol, lecithin cholesterol acyl transferase

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Introduction

Dyslipidemia is one of the major risk factors for atherosclerosis, which can subsequently lead to life-threatening cardiovascular and cerebrovascular diseases.[1] Therapy of the lipid metabolism disorder, as an effective means of reducing risks of cardiovascular disease, has attracted increasing attention. Heightening antiatherogenic lipoprotein, such as high-density lipoprotein (HDL), and lowering pro-atherogenic lipoproteins, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), are major treatment strategies for dyslipidemia.[2] However, many chemical lipid-regulating medicines have some adverse reactions, and some of them can induce distinct injury to the liver and kidney.[3,4] Thus, much of the latest research is focusing on natural materials to explore better lipid-regulating compounds with less toxicity.[5]

Chitosan, the deacetylated form of chitin, primarily derived from the shells of crustaceans has been demonstrated to be a functional lipid-lowering material.[6,7] Chitosan oligosaccharide (COS), the soluble degradation product of chitosan, can be digested and absorbed more easily by animals and humans compared with chitosan.[8] A great line of research has shown that COS has similar properties to chitosan in improving immunity, antitumor and anti-infection.[9]

Some documents indicate that chitosan has beneficial lipid-regulating effects in animal and humans.[10–12] The mechanisms of chitosan on reducing blood lipids are not

clearly understood due to its complexity. When compared with chitosan, COS possesses more remarkable bioactivities; however, whether or not COS also has chitosan's lipid regulation function remains unknown. Moreover, the relevant mechanisms of COS are poorly understood. In order to know more about COS and its lipid regulation functions, in our research, we used high-fat-diet-induced hyperlipidemic rats to evaluate COS lipid-regulating effects.

Materials and methods

Materials

COS (pharmaceutical grade) with a molecular weight no more than 1000 Da was purchased from Shanghai U-sea Biotech Co Ltd (Shanghai, China). Sodium cholate, cholesterol and propylthiouracil were obtained from Shanghai Lanji Science and Technology Co Ltd (Shanghai, China). The high-fat diet was prepared by the laboratory animal center of Taishan Medical University according to the reported method by Srinivasan *et al.*[13] Assay kits for triglyceride (TG), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were from Biosino Bio-technology and Science Inc (Beijing, China). Lecithin cholesterol acyl transferase (LCAT) and phospholipid transfer protein (PLTP) activity assay kits were the products of Roar Biomedical (New York, NY, USA).

Experimental animals

SPF Wistar rats (250 g) were purchased from Shandong Lukang (Jinan, Shandong, China). Thirty-two male rats were randomly allocated into four groups: regular chow diet group (Chow), high-fat-diet group (HF), high-fat-diet group with low dosage of COS (HF + COSL) and high-fat-diet group with high dosage of COS (HF + COSH). The latter two groups were administrated with COS dissolved in normal saline at a dose of 250 and 750 mg/kg by gastric gavage once a day for three consecutive weeks, respectively. The other two groups were treated with the same volume of normal saline also once a day for three consecutive weeks. At the end of the experiment, blood samples were collected from each rat's heart with heparin for anticoagulation. The separated plasma was then kept at -80°C until used for biochemical analysis. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guidelines for care and use of laboratory animals prepared by Taishan Medical University.

Morphological analysis

Common liver and kidney histology was performed in 3–4 μm sections stained with hematoxylin and eosin and photographed at $\times 200$ magnifications.

Plasma lipid measurement

Plasma TG, HDL-C and LDL-C were determined by an enzymatic method according to the assay kit instructions.

Lipid analysis of lipoprotein fractions

Lipoprotein fractions were obtained by fast protein liquid chromatography (FPLC) and then their lipid contents were assayed.[14] Briefly, 100 μL plasma was loaded onto a Superose 6 gel chromatography column connected with the FPLC on a ÄKTA purfier-900 system (Amersham Biosciences, Piscataway, NJ, USA) to separate the lipoprotein fractions, eluting with mobile phase (0.15 mol/L NaCl, 0.01 mol/L Na_2HPO_4 and 0.1 mol/L EDTA, pH 7.5) at a flow rate of 0.3 mL/min. Eighty tubes of eluate were collected and each contained 0.5 mL eluate. Cholesterol and TG content of each fraction were determined by enzymatic method.

Apolipoprotein analysis of lipoprotein particles

VLDL, LDL and HDL particles were separated by ultracentrifugation and the distribution and content of apolipoprotein were measured using a 5–15% gradient gel based on the method of Segrest.[15]

Lipoprotein formation associated protein activity measurement

The plasma activities of LCAT and PLTP were determined according to the instructions of the assay kits.

Statistical analysis

All the data were expressed as means $\pm$ standard deviation ($\bar{x} \pm$ SD). All parameters for inter-group differences were analyzed by one-way analysis of variance. $P < 0.05$ was considered significant.

Results

As shown in Figure 1, the levels of rat plasma TG, HDL-C and LDL-C of the three high-fat-diet groups were remarkably higher than that of the regular chow diet group, indicating that high-fat diet can induce hyperlipidemia in rats. The COS administration decreased the plasma level of TG by 29% and 31% as well as increased the plasma level of HDL-C by 8% and 11% in the low and high dosage of COS groups, respectively; however, COS had no significant influence on the plasma level of LDL-C when compared with the HF group.

The effects of COS on the distribution and content of the lipids contained in plasma lipoprotein fractions analyzed by FPLC are shown in Figure 2, which indicates that COS administration reduced the content of TG in plasma VLDL and LDL fractions and increased the content of cholesterol in plasma HDL fractions in rats.

The analysis of apolipoprotein content in lipoprotein particles by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after separation using ultracentrifugation (Figure 3) showed that COS significantly decreased the content of apoB100 and B48 in LDL particles. ApoB100 in VLDL particles and apoE in LDL particles were significantly increased with the treatment of COS. No content alternation of apoA-I in HDL particles was detected.

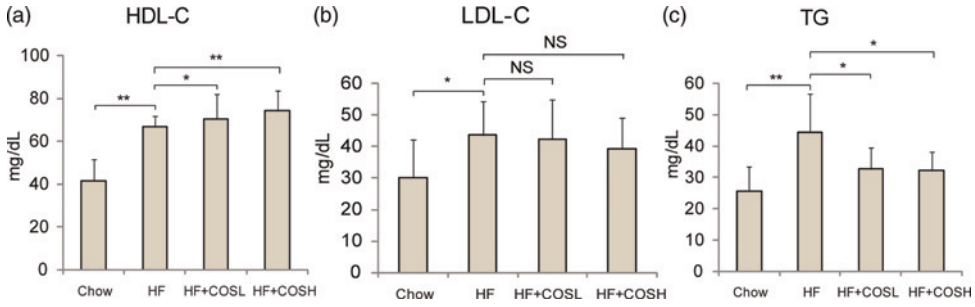

Figure 1 Effect of chitosan oligosaccharide (COS) on rat plasma lipid. Plasma high-density lipoprotein cholesterol (HDL-C, as shown in a), low-density lipoprotein cholesterol (LDL-C, as shown in b) and triglyceride (TG, as shown in c) were determined by an enzyme method. Data (means ± SD, $n = 8$) expressed as milligram per deciliter. Chow: general chow diet group; HF: high-fat-diet group; HF + COSL: high-fat-diet group with the treatment of 250 mg/kg COS; HF + COSH: high-fat-diet group with the treatment of 750 mg/kg COS. $^{*}P < 0.05$ versus HF; $^{**}P < 0.01$ versus HF. NS, no statistical significance (A color version of this figure is available in the online journal)

In order to investigate the effects of COS on lipids and apolipoproteins of lipoproteins in rats fed with a high-fat diet, we assayed activities of PLTP and LCAT, both of which were associated with lipoprotein formation (Figure 4). The results indicated that COS did not affect the enhanced plasma PLTP activity; however, COS could significantly restore the lowered plasma LCAT activity to some extent (from 68% to 86%) induced by a high-fat diet.

COS could ameliorate dyslipidemia in high-fat-diet rats; furthermore, the morphological analysis results showed no obvious morphological change in COS groups compared with the control group (data are shown in Supplementary material, which can be found at http://ebm.rsmjournals.com/cgi/content/full/ebm.2011.011032/DC1).

Discussion

The lipid-regulating effects of chitosan has been reported repeatedly;[8,10–12] however, it remains unknown whether COS, the degradation product of chitosan, has effects on lipid and lipoproteins.[16–18] The present results in hyperlipidemic rats showed that COS can decrease plasma TG and increase HDL-C, but not significantly affect the plasma LDL-C concentration. These results are consistent with the results reported by Zhou *et al.*[18] observed in broilers chickens. However, there is a slight difference with the results reported by Shen *et al.*[19] who demonstrated that COS can significantly reduce the level of LDL-C in mice. Such differences may have originated from the complexity of the component and metabolism of lipoprotein as well as species differences. It would have important clinical significance to the population with metabolic syndrome if COS was proved to decrease plasma or VLDL TG and increase HDL concentration in human. It is well known that high plasma TG and low HDL concentrations are characteristics for metabolic syndrome. TG as one of the important negative factors and HDL as the most important positive factors are documented for antiatherosclerosis. Therefore, the elucidation of the mechanism by which COS decreases TG-rich particles and improves HDL particles will provide solid evidence for COS application in vascular disease therapy. Plasma VLDL, LDL and HDL are particles composed of a variety of lipids and protein components; it is thus necessary to clarify what kinds of lipid and protein components of the lipoprotein could be affected by COS.

The alteration of plasma apoA-I, a major apolipoprotein of HDL, can affect the component and metabolism of HDL, and influence the distribution of HDL subclasses;[20] therefore, the lipids and protein components of lipoprotein were analyzed in this study. We found that in COS-administrated rats, the content of cholesterol increased while apoA-I was not altered, which suggests that COS may stimulate the maturation of HDL particles with more cholesterol accumulation or limit catabolism of HDL by blocking

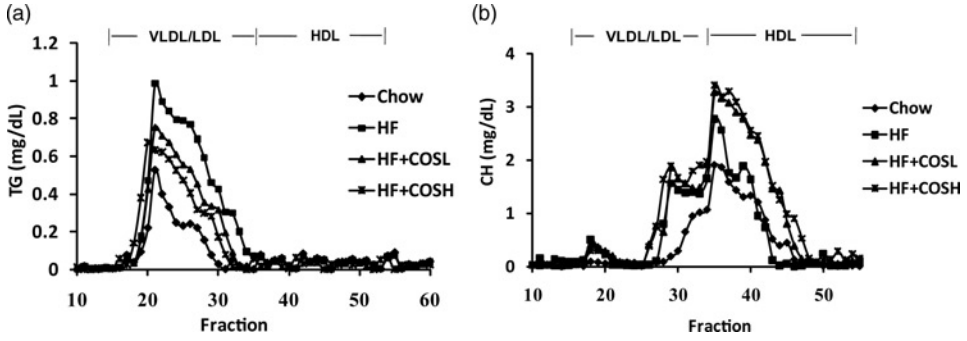

Figure 2 Effects of chitosan oligosaccharide (COS) on the distribution and content of the plasma lipoprotein lipids after separation by fast protein liquid chromatography. An aliquot of 80 μL from the corresponding fraction was used for the determination of triglyceride (TG, a) and cholesterol (CH, b), and the data expressed as mg per deciliter. Chow: general chow diet group; HF: high-fat-diet group; HF + COSL: high-fat-diet group with the treatment of 250 mg/kg COS; HF + COSH: high-fat-diet group with the treatment of 750 mg/kg COS

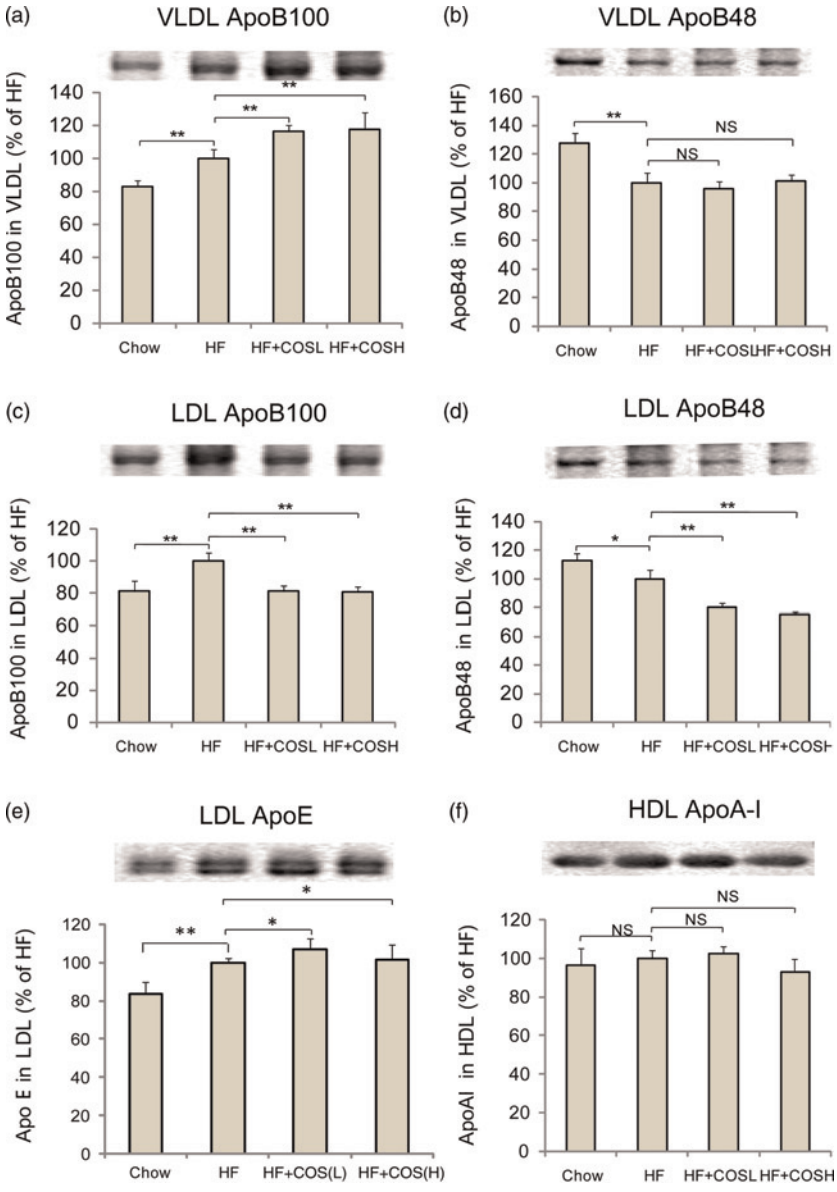

Figure 3 Effect of (COS) on rat plasma lipoprotein. Rat plasma lipoproteins were isolated by sequential ultracentrifugation: very-low-density lipoprotein (VLDL), $d < 1.006$ g/mL; low-density lipoprotein cholesterol (LDL), d 1.006–1.063 g/mL; high-density lipoprotein cholesterol (HDL), d 1.063–1.210 g/mL. Samples containing 2 μg protein from VLDL, LDL and HDL of each group were conducted by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and quantified by densitometry with Image-pro Plus (Media Cybernetics, Bethesda, MD, USA). A representative SDS-PAGE result from four independent experiments is shown (upper panel) and the quantification of these bands is shown in the lower panel. (a) apoB100 in VLDL; (b) apoB48 in VLDL; (c) apoB100 in LDL; (d) apoB48 in LDL; (e) apoE in LDL; (f) apoAI in HDL. Chow: general chow diet group; HF: high-fat-diet group; HF + COSL: high-fat-diet group with the treatment of 250 mg/kg COS; HF + COSH: high-fat-diet group with the treatment of 750 mg/kg COS. All values presented as mean ± SD of four independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ versus HF. NS, no statistical significance (A color version of this figure is available in the online journal)

hepatic uptake of the particles. Further analysis by FPLC confirmed that COS can increase the cholesterol content of HDL fractions while decreasing TG content of VLDL/LDL fractions, all of which suggest that COS may have certain impacts on some lipoprotein metabolism associated proteins.

It is known that the recombination and metabolism of VLDL and HDL in plasma can be affected by PLTP which transfers lipids between VLDL and HDL and LCAT which promotes esterification of cholesterol and maturation of HDL. Therefore we measured the activities of plasma PLTP and LCAT. The results indicated that COS has no effect on PLTP activity but can stimulate LCAT activity significantly, which at least can partially account for the increase of cholesterol content in HDL particles. HDL particles have antagonistic action on the formation of atherosclerosis by clearing cholesterol in peripheral tissues, transporting them into blood and then into the liver conversely to have the cholesterol transformed and excreted. ApoA-I located at the surface of HDL promotes the transformation of cholesterol to cholesterol ester by activating LCAT, and cholesterol ester can be further transformed to bile acids or excreted out of body by bile directly.[21] Unfortunately, we did not find that COS administration increased apoA-I in HDL particles, which may need further study applying to more animal models.

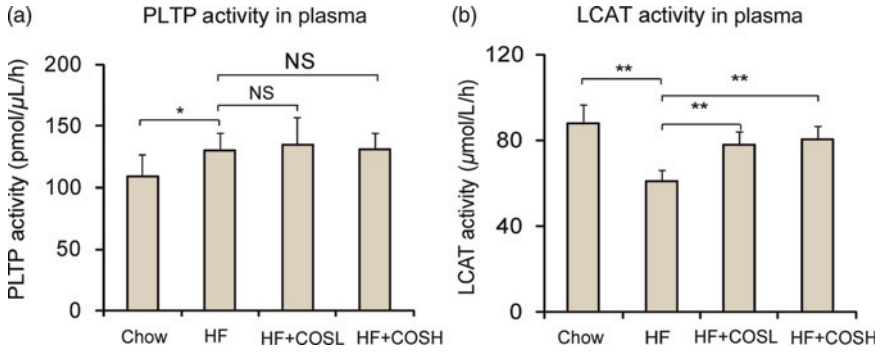

Figure 4 Effect of chitosan oligosaccharide (COS) on rat plasma phospholipid transfer protein (PLTP) and lecithin cholesterol acyl transferase (LCAT) activities. (a) Plasma PLTP activity; (b) plasma LCAT activity. Data (means ± SD, $n = 8$) were expressed as pmol per micro liter per hour and μmol per micro liter per hour for PLTP activity and LCAT activity, respectively. Chow: general chow diet group; HF: high-fat-diet group; HF + COSL: high-fat-diet group with the treatment of 250 mg/kg COS; HF + COSH: high-fat-diet group with the treatment of 750 mg/kg COS. All values were presented as mean ± SD of four independent experiments. $^{*}P < 0.05$ versus HF; $^{**}P < 0.01$ versus HF. NS, no statistical significance (A color version of this figure is available in the online journal)

As we know, LDL is bad cholesterol particles in plasma leading to atherogenesis. Two main apolipoproteins are present in LDL particles, apoB and apoE. In this study, we found that apoB48 and B100 were surprisingly lowered while apoE content increased remarkably in the LDL particles, all of which may contribute to the unchanged LDL-C.

Presently, many lipid-regulating drugs can have adverse effects on liver and kidney functions. However, this study has demonstrated that COS has no obvious effect on liver and kidney functions and presents no morphological abnormity (data are shown in Supplementary material, which can be found at http://ebm.rsmjournals.com/cgi/content/full/ebm.2011.011032/DC1) in rats, all of which highlight that COS may possess special strengths when compared with the general lipid-regulating drugs.

In summary, the present study indicated that COS has beneficial regulating effects on high-fat-diet-induced lipid abnormality in rats. This effect is partially related with its regulation of lipid and protein contents of lipoprotein particles as well as the regulation of important enzymes such as LCAT. Although the mechanisms by which COS improves plasma lipoprotein profiles by reducing VLDL TG, increasing HDL-C and decreasing apoB-containing particles are not clearly understood, its potential for anti-atherosclerosis is worthy of further study.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. DW was responsible for the funding and study design. JH and XL were responsible for animal maintenance, killing, blood and tissue collection, acquisition of data, analysis and manuscript preparation. YY and SJ were responsible for lipoprotein separation by ultracentrifugation and protein analysis, acquisition of data, analysis and manuscript preparation. YW and HT assisted in animal gastric gavage, tissue collection and the LCAT activity assay. TL assisted in plasma FPLC-TG analysis and the PLTP activity assay. SG was responsible for interpretation and manuscript preparation. SQ was responsible for the funding, study design, data acquisition and analysis, interpretation and manuscript preparation. DW, JH and YY contributed equally to this study.

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REFERENCES

1 Assmann G, Cullen P, Jossa F, Lewis B, Mancini M. Coronary heart disease: reducing the risk: the scientific background to primary and secondary prevention of coronary heart disease. A worldwide view. International Task force for the Prevention of Coronary Heart disease. *Arterioscler Thromb Vasc Biol* 1999;**19**:1819–24

2 Krauss RM. Dietary and genetic probes of atherogenic dyslipidemia. *Arterioscler Thromb Vasc Biol* 2005;**25**:2265–72

3 Negishi K, Noiri E, Maeda R, Portilla D, Sugaya T, Fujita T. Renal L-type fatty acid-binding protein mediates the bezafibrate reduction of cisplatin-induced acute kidney injury. *Kidney Int* 2008;**73**:1374–84

4 Kostapanos MS, Milionis HJ, Elisaf MS. Rosuvastatin-associated adverse effects and drug-drug interactions in the clinical setting of dyslipidemia. *Am J Cardiovasc Drugs* 2010;**10**:11–28

5 Okuda H. Hints of western medicine from Chinese medicine. *Exp Biol Med (Maywood)* 2003;**228**:1250–5

6 Xu G, Huang X, Qiu L, Wu J, Hu Y. Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. *Asia Pac J Clin Nutr* 2007;**16**(Suppl. 1):313–7

7 Aam BB, Heggset EB, Norberg AL, Sorlie M, Varum KM, Eijsink VG. Production of chitooligosaccharides and their potential applications in medicine. *Mar Drugs* 2010;**8**:1482–517

8 Hossain S, Rahman A, Kabir Y, Shams AA, Afros F, Hashimoto M. Effects of shrimp (*Macrobracium rosenbergii*)-derived chitosan on plasma lipid profile and liver lipid peroxide levels in normo- and hypercholesterolaemic rats. *Clin Exp Pharmacol Physiol* 2007;**34**:170–6

9 Nam KS, Shon YH. Suppression of metastasis of human breast cancer cells by chitosan oligosaccharides. *J Microbiol Biotechnol* 2009;**19**:629–33

10 Ausar SF, Morcillo M, Leon AE, Ribotta PD, Masih R, Vilaro Mainero M, Amigone JL, Rubin G, Lescano C, Castagna LF, Beltramo DM, Diaz G, Bianco ID. Improvement of HDL- and LDL-cholesterol levels in diabetic subjects by feeding bread containing chitosan. *J Med Food* 2003;**6**:397–9

11 Zhang J, Liu J, Li L, Xia W. Dietary chitosan improves hypercholesterolemia in rats fed high-fat diets. *Nutr Res* 2008;**28**:383–90

12 Sumiyoshi M, Kimura Y. Low molecular weight chitosan inhibits obesity induced by feeding a high-fat diet long-term in mice. *J Pharm Pharmacol* 2006;**58**:201–7

13 Srinivasan K, Patole PS, Kaul CL, Ramarao P. Reversal of glucose intolerance by by pioglitazone in high fat diet-fed rats. *Methods Find Exp Clin Pharmacol* 2004;**26**:327–33

14 Qin S, Kawano K, Bruce C, Lin M, Bisgaier C, Tall AR, Jiang X. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J Lipid Res* 2000;**41**:269–76
15 Segrest JP, Albers JJ. Plasma lipoproteins. Part A: Preparation, structure, and molecular biology. *Methods Enzymol* 1986;**128**:1–992
16 Liu B, Liu WS, Han BQ, Sun YY. Antidiabetic effects of chitooligosaccharides on pancreatic islet cells in streptozotocin-induced diabetic rats. *World J Gastroenterol* 2007;**13**:725–31
17 Choi YJ, Kim EJ, Piao Z, Yun YC, Shin YC. Purification and characterization of chitosanase from *Bacillus* sp. strain KCTC 0377BP and its application for the production of chitosan oligosaccharides. *Appl Environ Microbiol* 2004;**70**:4522–31
18 Zhou TX, Chen YJ, Yoo JS, Huang Y, Lee JH, Jang HD, Shin SO, Kim HJ, Cho JH, Kim IH. Effects of chitooligosaccharide supplementation on performance, blood characteristics, relative organ weight, and meat quality in broiler chickens. *Poult Sci* 2009;**88**:593–600
19 Shen J, Ye X, Shen J, Jin B, Wang Y. Studies on the effect of chito oligosaccharide on reducing lipid and protecting liver of chito oligosaccharide in hyperlipidemia mice. *J Northwest A F Univ (Nat Sci Edn)* 2007;**35**:4
20 Muth N, Laughlin G, Von MD, Smith S, Barrett-Connor E. High-density lipoprotein subclasses are a potential intermediary between alcohol intake and reduced risk of cardiovascular disease: the Rancho Bernardo Study. *Br J Nutr* 2010;**29**:9
21 Calabresi L, Franceschini G. Lecithin:cholesterol acyltransferase, high-density lipoproteins, and atheroprotection in humans. *Trends Cardiovasc Med* 2010;**20**:50–3

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