

Barley β -glucan reduces blood cholesterol levels via interrupting bile acid metabolism

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Abstract

Underlying mechanisms responsible for the cholesterol-lowering effect of β -glucan have been proposed, yet have not been fully demonstrated. The primary aim of this study was to determine whether the consumption of barley β -glucan lowers cholesterol by affecting the cholesterol absorption, cholesterol synthesis or bile acid synthesis. In addition, this study was aimed to assess whether the underlying mechanisms are related to cholesterol 7 α hydroxylase (CYP7A1) SNP rs3808607 as proposed by us earlier. In a controlled, randomised, cross-over study, participants with mild hypercholesterolaemia (n 30) were randomly assigned to receive breakfast containing 3 g high-molecular weight (HMW), 5 g low-molecular weight (LMW), 3 g LMW barley β -glucan or a control diet, each for 5 weeks. Cholesterol absorption was determined by assessing the enrichment of circulating ¹³C-cholesterol over 96 h following oral administration; fractional rate of synthesis for cholesterol was assessed by measuring the incorporation rate of ²H derived from deuterium oxide within the body water pool into the erythrocyte cholesterol pool over 24 h; bile acid synthesis was determined by measuring serum 7 α -hydroxy-4-cholesten-3-one concentrations. Consumption of 3 g HMW β -glucan decreased total cholesterol (TC) levels ($P=0.029$), but did not affect cholesterol absorption ($P=0.25$) or cholesterol synthesis ($P=0.14$). Increased bile acid synthesis after consumption of 3 g HMW β -glucan was observed in all participants ($P=0.049$), and more pronounced in individuals carrying homozygous G of rs3808607 ($P=0.033$). In addition, a linear relationship between log (viscosity) of β -glucan and serum 7 α -HC concentration was observed in homozygous G allele carriers. Results indicate that increased bile acid synthesis rather than inhibition of cholesterol absorption or synthesis may be responsible for the cholesterol-lowering effect of barley β -glucan. The pronounced TC reduction in G allele carriers of rs3808607 observed in the previous study may be due to enhanced bile acid synthesis in response to high-viscosity β -glucan consumption in those individuals.

Key words: β -Glucan: CYP7A1: Molecular weight: Viscosity: Cholesterol: Bile acids: Mechanisms

Previous studies have demonstrated the critical roles of the high viscosity and molecular weight (MW) of β -glucan in its cholesterol-lowering effect^(1,2). Yet, to date the precise underlying mechanisms responsible for the action of β -glucan reducing blood cholesterol have not been well elucidated.

Cholesterol in humans is mainly determined by cholesterol *de novo* synthesis, dietary cholesterol intake and conversion to bile acid⁽³⁾. It has been proposed that β -glucan may reduce cholesterol via impacting any three of these pathways. β -Glucan is not absorbed by the small intestine, however, the physico-chemical properties of β -glucan may alter the luminal environment and lead to increased transit time and thickness of the unstirred water layer (UWL), consequently reducing cholesterol

absorption⁽⁴⁾. When β -glucan enters the large intestine, gut microbiota present in the colon are responsible for its degradation^(5,6). By-products resulting from β -glucan fermentation, SCFA, might then affect cholesterol synthesis^(7–9). In addition, cholesterol is the substrate for bile acid synthesis in the liver, thus β -glucan may affect cholesterol metabolism via interfering bile acid enterohepatic circulation. Approximately 95% of the bile acids are reabsorbed in the terminal ileum^(10,11) while a small amount of bile acids are excreted with the faeces⁽¹⁰⁾. The conversion of bile acid from cholesterol occurs at a rate that precisely corresponds to the loss of bile acids within the faeces⁽¹²⁾. β -Glucan has been thought to work as a bile acid sequestrant reducing cholesterol by decreasing bile acid

Abbreviations: 7 α -HC, 7 α -hydroxy-4-cholesten-3-one; CYP7A1, cholesterol 7 α hydroxylase; FRS, fractional rate of synthesis; HMW, high-molecular weight; LWM, low-molecular weight; MW, molecular weight; TC, total cholesterol.

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reabsorption and further up-regulating bile acid synthesis and thus consuming more cholesterol^(13,14). The interruption of bile acid enterohepatic circulation resulting from β -glucan consumption has been observed in ileostomy patients⁽¹⁵⁾, yet, evidence from healthy participants in supporting this proposed mechanism is limited.

Cholesterol 7- α hydroxylase (CYP7A1) is the rate-limiting enzyme in the classical bile acid synthetic pathway in humans⁽¹⁶⁾. Its activity can be indicated by the circulating concentration of 7 α -hydroxy-4-cholesten-3-one (7 α -HC), a bile acid intermediate of the classical synthesis pathway^(16,17). Genetic variation of CYP7A1 SNP rs3808607 has been associated with different responses to high-viscosity β -glucan in lowering cholesterol levels in our previous study, where G allele carriers of CYP7A1 SNP rs3808607 showed more pronounced responses in reducing total cholesterol (TC) than T/T allele carriers⁽²⁾. The biological difference between G and T alleles in regulating bile acids and cholesterol metabolism is not entirely clear. Results from De Castro-Oros *et al.*'s⁽¹⁸⁾ study suggested that the T>G polymorphism might regulate cholesterol catabolism differently due to their varied ability in modulating transcriptional activity of CYP7A gene. G allele was speculated to be more responsive to dietary intervention in elevating the bile acid synthesis⁽¹⁸⁾. However, evidence from different phenotypes, in terms of bile acid synthesis at physiological level, is needed to confirm this speculation.

Collectively, our hypotheses are: (1) β -glucan lowers blood cholesterol levels via suppressing cholesterol absorption, decreasing cholesterol biosynthesis and /or interrupting the enterohepatic circulation of bile acids; and (2) G allele carriers of rs3808607 have a higher level of bile acid synthesis in response to β -glucan intervention. Accordingly, the primary objective of this study was to assess whether lowered serum cholesterol levels resulting from β -glucan consumption are due to inhibition of cholesterol absorption, cholesterol synthesis or increased bile acid synthesis. The additional objective was to assess the phenotype of CYP7A1 SNP rs3808607 in response to β -glucan intervention at a physiological level.

Methods

Diet and clinical trial procedure

A randomised, single-blinded, diet-controlled, cross-over trial was conducted at the Clinical Nutrition Research Unit at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN), University of Manitoba, Winnipeg. Participants with serum cholesterol between 5.0 and 8.0 mmol/l, LDL-cholesterol between 2.7 and 5.0 mmol/l (*n* 30) were randomly assigned to receive barley diets containing 3g high MW (HMW, 13.5 \times 10⁵ g/mol), 5g low MW (LMW, 2.9 \times 10⁵ g/mol), 3g LMW (2.9 \times 10⁵ g/mol) β -glucan or a control diet, each for 5 weeks. The washout period between the study phases was \geq 4 weeks. Breakfast foods in the format of crepes, tortillas, porridge and chips were formulated from barley to contain β -glucan varying in MW and dose or formulated from wheat and rice to substitute barley ingredients as a control. Lunch and dinner were provided to meet the energy needs of each subject. Overall, participants received study diets containing

Table 1. Nutrient content of the experimental diets

	WR control	3g LMW	5g LMW	3g HMW
Energy (kJ/d)	11 803	11 895	11 937	11 895
Energy (kcal/d)	2821	2843	2853	2843
Carbohydrate (% energy)	55.7	55.7	55.6	55.7
Fibre (g/d)	31.2	36.6	41.5	36.6
Soluble fibre (g/d)	5.7	8.7	10.7	8.7
β -Glucan (g/d)	<0.24	3.0	5.0	3.0
Protein (% energy)	15.9	15.9	15.9	15.9
Fat (% energy)	30.8	30.5	30.4	30.5
Saturated (% energy)	9.0	8.9	8.9	8.9
Cholesterol (mg/d)	312.7	312.7	312.7	312.7

WR, wheat and rice; LMW, low-molecular weight; HMW, high-molecular weight; fibre, total dietary fibre.

approximately 30% of energy as fat, 55% as carbohydrate and 15% as protein but with different levels of soluble fibre. Details of nutrition information for the study diets have been reported in a previous paper⁽²⁾ and are shown in Table 1. Participants consumed breakfast at the Clinical Nutrition Research Unit of RCFFN from Monday to Friday. Lunch and dinner for weekdays were picked up by participants and consumed at home. Weekend meals including breakfast were delivered to participant' home address. This study was conducted according to the principles expressed in the Declaration of Helsinki and all research procedures were approved by the University of Manitoba's Biomedical Research Ethics Board (Ethic reference no. B2010:057).

Serum lipid and genotype determination

Methods for determining blood lipid concentrations and the genotype of CYP7A1 SNP rs3808607 have been described in the previous study⁽²⁾. In brief, 12h fasted blood samples were collected on days 1, 2, 34 and 35. Blood samples were centrifuged at 2675 g for 20 min at 4°C, separated into serum, plasma, buffy coat and erythrocytes, and stored at -80°C until analysis. An average of values of days 34 and 35 were used for endpoint analysis for blood lipids. Serum TC, HDL-cholesterol and TAG were measured using the automated enzymatic methods on a Vitros-350 chemistry analyzer (Ortho Clinical Diagnostics). Serum LDL-cholesterol was estimated using the Friedewald equation⁽¹⁹⁾. DNA used for SNP determination were extracted from the buffy coat of heparinised blood samples following the instructions of the Dneasy[®] Blood & Tissue kit (QIAGEN). SNPs of rs3808607 for CYP7A1 were genotyped using TaqMan[®] GTExpress[™] Master Mix (Applied Biosystems, Inc.) with allele-specific probes on the StepOnePlus[™] Real-Time PCR System (Applied Biosystems, Inc.).

Cholesterol absorption

The cholesterol absorption in response to β -glucan intervention was determined by stable isotope method^(20,21). On day 30 of each study phase, participants ingested approximately 5g of margarine with 75 mg of [3, 4-¹³C] cholesterol (99% atom percentage excess; Cambridge Isotope Laboratories) spread on half of an English muffin. Fasted blood samples were obtained before oral administration of isotope tracers (0h) and also in the following 96h on day 31 (24h), day 32 (48h), day 33 (72h), and day 34 (96h). Free cholesterol was extracted from

erythrocytes by modified Folch procedure⁽²²⁾. The isotopic enrichment of [3,4-¹³C] cholesterol was determined using GC isotope ratio MS (GC-IRMS; Thermo Finnigan) where samples were run through a GC unit, a combustion reactor and a mass spectrometer^(20,21). The cholesterol extracts were combusted to ¹³C-enriched CO₂ and the combusted CO₂ gas was analysed for ¹³C enrichment against the international standard Vienna Pee Dee Belemnite limestone. The AUC of ¹³C enrichment from 0 to 96 h was calculated for determining cholesterol absorption.

Determination of cholesterol biosynthesis

The fractional cholesterol synthesis rate in response to the intervention diets was determined by stable isotope method using deuterated water⁽²³⁾. Specifically, cholesterol synthesis was assessed by measuring the rate of ²H derived from deuterium oxide (D₂O) within the body water pool into the erythrocyte cholesterol pool⁽²³⁾. On day 34 of each study phase, participants ingested 0.7 g of D₂O/kg estimated body water (60% of body weight). Fasting blood samples were collected before D₂O oral administration on day 34 (0 h) and day 35 after D₂O ingestion (24 h). Blood samples were centrifuged and separated into plasma, buffy coat and erythrocytes, and stored at -80°C until analysis. Total lipids were extracted from erythrocytes by a modified Folch procedure⁽²²⁾. The lipid extracts were separated using GC and isolated cholesterol was submitted into a pyrolysis reactor to release H₂ gas. Plasma water samples were run through a high temperature conversion elemental analyser. ²H enrichments for both erythrocytes and plasma water were measured by IRMS relative to the reference gas. Normalisation to Vienna standard mean ocean water was performed using a regression equation between the online and offline method with data from the offline method expressed relative to Vienna standard mean ocean water (V-SMOW). The fractional rate of synthesis (FSR) for cholesterol was calculated using the following equation:

$$\text{FSR (pools / d)} = \frac{\Delta \text{D-cholesterol}}{\Delta \text{D-PW} \times 0.478} \times 100 \%,$$

where $\Delta \text{D-cholesterol}$ is the difference in ²H enrichment between 0 and 24 h for cholesterol; $\Delta \text{D-PW}$ the difference in ²H enrichment between 0 h and 24 h for plasma water; and 0.478 the ratio of labelled H atoms replaced by ²H during *in vivo* biosynthesis⁽²⁰⁾.

Determination of bile acid biosynthesis

Blood samples for determining bile acid biosynthesis were collected on days 34 and 35. The mean of 7 α -HC levels on days 34 and 35 was used for indicating bile acid synthesis level. Bile acid biosynthesis was determined by measuring the serum concentration of 7 α -HC using the Ultra-Performance Liquid Chromatography (ACQUITY UPLC System; Waters) coupled with a tandem mass spectrometer (Quattro microTM API; Waters). 7 α -HC was extracted by C18-E solid phase extraction (SPE) (Phenomenex[®]) mounted on a pump (KNF lab pump; KNF Neuberger) connected SPE processor (Agilent Technologies) following the method modified from Burkard *et al.*⁽²⁴⁾. 7 α -hydroxy-4-cholesten-3-one-d₇ (Medical Isotopes, Inc.) was used as an internal standard. In brief, SPE cartridges were

pre-conditioned by 2 × 2 ml methanol, 2 × 2 ml water and 2 × 2 ml 100 mM ammonium carbonate buffer pH 9.3. Serum (750 μ l) mixed with ammonium carbonate buffer (1:1 v: v) and 150 μ l of internal standard (40 ng/ml) were applied to the activated SPE cartridge. Under a vacuum, the speed of the mixture passing the cartridge was controlled within 1 drop/s passing through the cartridge. Subsequently, the cartridges were washed with 2 × 2 ml of water and dried under N₂. Bile acids were desorbed with 3 ml of methanol with the speed of 1 drop/s for passing the cartridge. The eluted substances were dried under N₂ and dissolved in 150 μ l of methanol before injecting into the UPLC. A reserved phase C18 column (KinetexTM 1.7 μ m XB-C18 100 Å, LC Column 100 × 2.1 mm; Phenomenex[®]) with mobile phase water with 0.1% formic acid (A) and methanol (B) was used for the separation. The ions used for the tracing of unlabelled 7 α -HC were m/z 401 > 177 and the ions for tracing the 7 α -hydroxy-4-cholesten-3-one-d₇ were 407 > 177. The mass spectrometer was operated in the positive mode. Corona current was 3 μ A, cone voltage 25 V, source temperature 120°C, cone gas flow 62 litres/h, desolvation gas flow 400 litres/h, and collision energy 23 eV. The quantitation was performed with use of a standard curve, plotting the ratio between the response of 7 α -HC in the m/z 177 tracing generated from the mother ion m/z 401 (unlabelled) and the response in the m/z 177 tracing generated from the mother ion m/z 407 (labelled).

Statistical analyses

Cholesterol absorption indicated by AUC of ¹³C enrichment, cholesterol synthesis indicated by FSR and bile acid synthesis indicated by serum concentration of 7 α -HC were analysed using linear mixed-models (PROC MIXED, version 9.2; SAS Institute Inc.). Log transformations were used for data that were not normally distributed (7 α -HC concentrations). For detecting the diet and genotype effects, diet, genotype and genotype × diet were modelled as fixed factors and participants were modelled as the random factor in the mixed-model. Correlation between repeated measures of these individuals was modelled using the first-order autoregressive (AR (1)) option in the PROC MIXED procedure. Fisher's least significant difference tests were used for multiple comparisons. In the genotype sub-group analysis, each genotype group was analysed independently with diet as a fixed factor. Statistical analyses for blood lipids were described in the previous study⁽²⁾.

Linear regression of 7 α -HC concentration on log(viscosity) was performed using SAS (PROC REG, version 9.2; SAS Institute Inc.) and plotted with GraphPad Prism (GraphPad Prism version 6.00 for Windows; GraphPad Software). The non-parametric correlation was performed by using Spearman's rank correlation (JMP[®], version 10; SAS Institute Inc.). A value of $P < 0.05$ was considered to be significant.

Results

Drop-out rate and genotype distribution

In all, forty-five participants were recruited for the study. Eight participants dropped out from the study with reasons unrelated

to the study; seven participants were excluded from the study for reasons including low tolerance to the study diet, missing the study diet, later discovery of ineligibility (e.g. met inclusion criteria at screening but did not meet criteria when day 1 blood samples were analysed). A participant flow chart and baseline characteristics of participants were described in the previous published study⁽²⁾. In the thirty participants who completed four phases of the study, eleven were identified to be TT allele carriers, eleven were GT and eight were GG allele carriers of rs3808607⁽²⁾. The distribution of genotype of *CYP7A1* SNP rs3808607 among the participants in this study is similar to the minor genotype frequency reported in the general population⁽²⁵⁾.

Changes in serum cholesterol levels and body weight

Changes in blood lipid levels have been reported in the previous study⁽²⁾. For the convenience of the readers, these data are included in Table 2. Consumption of 3 g HMW/d β -glucan for 5 weeks lowered TC compared with the control ($P=0.029$) but the LMW β -glucan, at either 3 or 5 g/d, did not change serum cholesterol concentrations (Table 2). In addition, significant genotype-by-diet effect was observed in changing serum TC and LDL-cholesterol levels for 3 g HMW β -glucan (Table 2). Consumption of β -glucan did not change serum HDL-cholesterol or TAG levels (Table 2). Changes in body weight and waist circumference were not statistically different among treatments (data not shown).

When the participants were sub-grouped based on their genotypes of *CYP7A1* SNP rs3808607, consumption of 3 g/d HMW β -glucan resulted in lower TC levels in G allele carriers (G/T and G/G) and lower LDL-cholesterol in homozygous G allele carriers (G/G) (Table 3). T/T carriers did not respond to β -glucan in reducing serum TC or LDL-cholesterol levels. Serum HDL-cholesterol and TAG levels remained unchanged in all genotype groups.

Changes in cholesterol absorption, cholesterol synthesis and serum 7 α -hydroxy-4-cholesten-3-one

Cholesterol absorption was not affected by β -glucan consumption ($P=0.25$). The AUC of 96 h enrichment of ¹³C were 539.75, 542.08, 546.41 and 588.38 for the control, 3 g LMW,

5 g LMW and 3 g HMW β -glucan, respectively (Table 2). The FSR for cholesterol was also not affected by β -glucan ingestion ($P=0.14$). FSR resulting from the experimental diets assessed in a 24-h time window were 8.37, 9.25, 7.54 and 7.80 for control, 3 g LMW, 5 g LMW and 3 g HMW β -glucan, respectively (Table 2). Consumption of 3 g HMW β -glucan resulted in higher 7 α -HC levels compared with control (14.00 *v.* 11.05 ng/ml, $P=0.049$, Table 2). The 7 α -HC levels were not affected by LMW β -glucan, which is consistent with the results of cholesterol changes (Table 2).

In the sub-group analysis, cholesterol absorption and FSR for cholesterol remained unchanged for all genotypes of *CYP7A1* SNP rs3808607. Although genotype \times diet effect was not statistically significant for 7 α -HC concentrations (Table 2), the three genotypes of *CYP7A1* showed inconsistent results of 7 α -HC concentrations (Table 3). G/G group responded to 3 g /d HMW β -glucan in increasing 7 α -HC concentrations compared with control ($P=0.033$); yet serum 7 α -HC levels were not affected by β -glucan consumption in G/T and T/T groups. This observation is consistent with the changes in circulating cholesterol levels that individuals carrying homozygous G alleles responded to 3 g HMW β -glucan in reducing TC and LDL-cholesterol (Table 3).

Linear relationship between serum 7 α -hydroxy-4-cholesten-3-one concentrations and viscosity of β -glucan

Viscosity of β -glucan is believed to be the determinant for its cholesterol-lowering effect. Our previous study has shown a linear relationship between viscosity of β -glucan and cholesterol changes in participants that responded to β -glucan in reducing their circulating cholesterol levels⁽²⁾. In this study, results consistently support the critical role of viscosity of β -glucan in mediating cholesterol metabolism. A linear relationship between viscosity of β -glucan and 7 α -HC concentrations was observed in homozygous G allele carriers ($P=0.025$, r^2 0.95, Fig. 1).

Correlation between cholesterol and bile acid kinetic parameters

In the non-parametric correlation (Table 4), AUC and FSR were negatively related with each other, although the correlation

Table 2. Changes in serum lipids, ¹³C-labelled cholesterol enrichment in erythrocytes, fractional synthesis rate (FSR) for cholesterol and serum 7 α -hydroxy-4-cholesten-3-one (7 α -HC) in response to β -glucan intervention for 5 weeks in mildly hypercholesterolemic adults (Least squares means (LSM) with their standard errors)

	Diet				SEM	P		
	WR control	3 g LMW	5 g LMW	3 g HMW		Diet	Genotype	Genotype \times diet
Changes in blood lipids								
Total cholesterol (mmol/l)	5.49 ^a	5.44 ^{a,b}	5.54 ^a	5.37 ^b	0.096	0.029	0.9	0.013
LDL-cholesterol (mmol/l)	3.40	3.39	3.46	3.31	0.078	0.077	0.93	0.045
HDL-cholesterol (mmol/l)	1.36	1.31	1.33	1.31	0.02	0.19	0.98	0.73
TAG (mmol/l)	1.43	1.41	1.45	1.44	0.07	0.95	0.19	0.19
Changes in cholesterol and bile acid metabolism								
AUC- ¹³ C enrichment	539.75	542.08	546.41	588.38	29.80	0.25	0.87	0.084
FSR for cholesterol (%)	8.37	9.25	7.54	7.80	0.76	0.14	0.63	0.23
7 α -HC (ng/ml)	11.05 ^a	12.89 ^{a,b}	11.97 ^{a,b}	14.00 ^b	1.15	0.049	0.31	0.26

WR, wheat and rice; LMW, low-molecular weight; HMW, high-molecular weight.

^{a,b} LSM in the same row with unlike superscript letters are significantly different among treatments (Fisher's least significant difference test for multiple comparison, $P < 0.05$).

Table 3. Changes in serum lipids, ¹³C-labelled cholesterol enrichment in erythrocytes, fractional synthesis rate (FSR) for cholesterol and serum 7 α -hydroxy-4-cholesten-3-one (7 α -HC) in response to β -glucan intervention for 5 weeks in three genotype groups of cholesterol 7 α hydroxylase SNP rs3808607 (Least squares means (LSM) with their standard errors)

	Diet				SEM	P
	WR control	3g LMW	5g LMW	3g HMW		
Total cholesterol (mmol/l)						
T/T (n 11)	5.40 ^a	5.47 ^{a,b}	5.66 ^b	5.59 ^{a,b}	0.22	0.11
G/T (n 11)	5.53 ^a	5.44 ^a	5.52 ^a	5.16 ^b	0.14	0.0093
G/G (n 8)	5.56 ^a	5.43 ^{a,b}	5.52 ^{a,b}	5.24 ^b	0.20	0.036
LDL-cholesterol (mmol/l)						
T/T (n 11)	3.32	3.22	3.33	3.40	0.085	0.39
G/T (n 11)	3.59	3.47	3.42	3.27	0.13	0.25
G/G (n 8)	3.55	3.49	3.58	3.32	0.17	0.0033
HDL-cholesterol (mmol/l)						
T/T (n 11)	1.59 ^a	1.47 ^b	1.51 ^{a,b}	1.55 ^{a,b}	0.030	0.053
G/T (n 11)	1.31	1.21	1.26	1.22	0.037	0.18
G/G (n 8)	1.33	1.36	1.32	1.31	0.031	0.72
TAG (mmol/l)						
T/T (n 11)	1.28	1.57	1.60	1.64	0.13	0.066
G/T (n 11)	1.78	1.66	1.80	1.54	0.12	0.39
G/G (n 8)	1.45	1.32	1.31	1.34	0.11	0.77
AUC-¹³C enrichment in erythrocytes						
T/T (n 11)	581.65	541.96	558.85	556.92	47.02	0.50
G/T (n 11)	493.25	563.53	554.82	533.48	40.09	0.39
G/G (n 8)	544.57	519.55	523.8	673.75	72.12	0.070
FSR for cholesterol (%)						
T/T (n 11)	6.08	7.96	8.63	9.18	1.10	0.12
G/T (n 11)	8.90	8.70	7.92	10.40	1.21	0.24
G/G (n 8)	8.23	6.13	8.54	8.07	1.78	0.49
7α-HC (ng/ml)						
T/T (n 11)	10.23	12.88	9.77	11.75	1.26	0.27
G/T (n 11)	16.60	15.49	15.85	18.62	1.26	0.57
G/G (n 8)	8.13 ^a	10.00 ^{a,b}	10.96 ^{a,b}	12.59 ^b	1.32	0.033

WR, wheat and rice; LMW, low-molecular weight; HMW, high-molecular weight.
^{a,b} LSM in the same row with unlike superscript letters are significantly different among treatments (Fisher's least significant difference test for multiple comparison, $P < 0.05$).

strength is weak ($P = 0.0008$, Spearman's $\rho = -0.30$). Serum 7 α -HC concentrations were not related to either AUC of ¹³C enrichment in erythrocytes or FSR for cholesterol.

Discussion

Consistent with the previous findings that high-viscosity β -glucan with HMW is effective in reducing circulating cholesterol levels⁽²⁾, we found that consumption of 3g/d HMW β -glucan resulted in higher serum 7 α -HC levels compared with the control, but not either of the LMW β -glucan diets. However, cholesterol absorption or cholesterol synthesis was not changed by any of the β -glucan diets. These results indicate the importance of the physiochemical characteristics of β -glucan in its cholesterol-lowering action and suggest that increasing bile acid synthesis rather than inhibition of cholesterol synthesis or absorption is the mechanism responsible for the cholesterol-lowering effect of β -glucan.

Inhibition of cholesterol absorption has been speculated widely as one of the mechanisms responsible for the cholesterol-lowering effect of β -glucan⁽¹³⁾. Hypothetically, with the viscous property, β -glucan might impact lipid absorption by altering the luminal environment such as slowing the transit time and

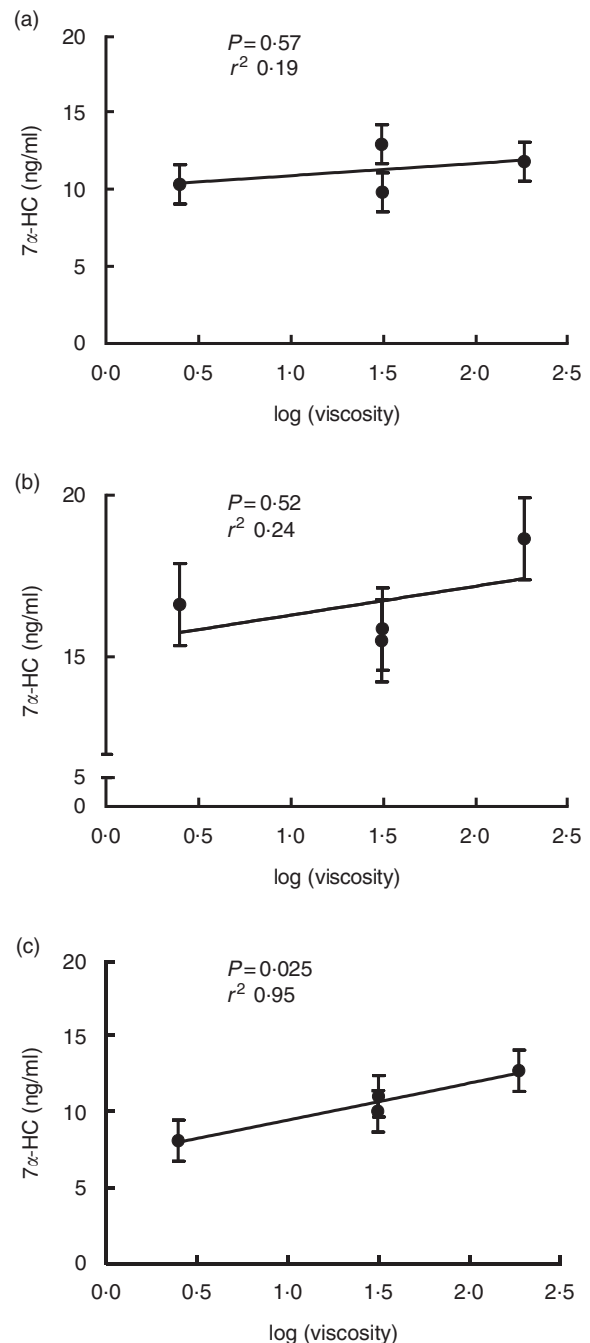


Fig. 1. Linear relationship between 7 α -hydroxy-4-cholesten-3-one (7 α -HC) concentration and log (viscosity) for the three genotype carriers of cholesterol 7 α hydroxylase (CYP7A1) SNP rs3808607. (a) T/T carriers; (b) G/T carriers; (c) G/G carriers. Values are least squares means with their standard errors for the four treatments following the order of wheat and rice control, 3g low-molecular weight (LMW), 5g LMW and 3g high-molecular weight from left to right.

increasing the thickness of UWL, or affecting activities of digestive enzymes and the formation of micelles^(4,26). Naumann *et al.* reported that sitosterol as a cholesterol absorption marker was decreased in a fruit drink containing β -glucan⁽²⁷⁾; however, in an ileostomy study, Lia *et al.*⁽²⁸⁾ found no changes in the absorption of dietary cholesterol after an oat bran meal containing β -glucan using a radioisotope method with [β -4-¹⁴C]-sitosterol and

Table 4. Non-parametric correlation between cholesterol absorption, synthesis and bile acid synthesis

Variables	By variable	Spearman's ρ	P
FSR	AUC	-0.30	0.0008
	7 α -HC	0.15	0.13
AUC	FSR	-0.30	0.0008
	7 α -HC	-0.074	0.44
7 α -HC	FSR	0.15	0.13
	AUC	-0.074	0.44

Spearman's ρ , Spearman's correlation coefficient; FSR, fractional rate of synthesis for cholesterol; 7 α -HC, 7 α -hydroxy-4-cholesten-3-one.

[1 α , 2 α -n-³H]-cholesterol as tracers. This discrepancy may be due to the different methodologies used for measuring cholesterol absorption. As an inhibitor of intestinal cholesterol uptake, the use of sitosterol may lead to an underestimation of the actual capacity of absorption⁽²⁹⁾. In agreement with Lia *et al.*'s finding, our study using a single isotope method showed that cholesterol absorption after consuming a β -glucan diet did not differ from the control diet ($P=0.25$). Therefore, our results provide more evidence suggesting that the cholesterol-lowering effect of β -glucan is not mediated through the inhibition of cholesterol absorption but through alternative mechanisms.

The ²H incorporation method utilised in this study is a reliable means to assess cholesterol synthesis⁽³⁰⁾. Ellegard *et al.*⁽³¹⁾ reported in ileostomy patients that within 24 h consumption of native β -glucan increased bile acid excretion and also increased serum lathosterol concentration, a marker for cholesterol synthesis. Cholesterol homeostasis is regulated by both cholesterol input and output. The loss of bile acid in excretion and demand for cholesterol to replenish the bile acid pool can possibly up-regulate the synthesis of cholesterol⁽¹³⁾. However, in our study, changes in cholesterol FSR did not reach statistical significance ($P=0.14$). Despite the discrepancy in increasing and not changing cholesterol synthesis, results of both Ellegard *et al.*'s and our present study do not support the hypothesis that consumption of β -glucan inhibits the cholesterol synthesis.

Our results demonstrated that consumption of HMW β -glucan is able to increase bile acid synthesis. Moreover, our data also revealed that the high viscosity resulting from HMW of β -glucan is the factor that drove this mechanism: serum 7 α -HC concentrations showed a linear relationship with the viscosity of the treatment diets (Fig. 1). After ingestion, high-viscosity β -glucan enters the lumen of the small intestine, where it may act similarly as a bile acid sequestering agent to interact with bile acids and lead to bile acid being entrapped instead of being reabsorbed. The expected consequences of these actions would increase faecal bile acid loss and enhance bile acid synthesis from cholesterol to replenish the bile acid pool⁽¹³⁾. The interruption of enterohepatic circulation of bile acid caused by β -glucan has been reported in ileostomy patients^(28,31-34). In a short-term interventional cross-over study, nine participants with conventional ileostomies received a diet containing either native or hydrolysed β -glucan (11.6 g), each for 3 d. The native β -glucan consumption resulted in 40% more ileal bile acid excretion compared with consuming the hydrolysed β -glucan diet. Moreover, native β -glucan in this study also increased

serum 7 α -HC levels by 57% within 24 h of consumption⁽³¹⁾. A study from Marlett *et al.* investigated whether oat β -glucan lowers cholesterol levels by decreasing bile acid and fat absorption and increasing bile acid synthesis in nine normolipidaemic men. Participants consumed a low-fibre diet for 28 d followed by an oat bran diet containing 5.4 g of soluble β -glucan for another 28 d without an interval⁽¹⁴⁾. The oat bran diet resulted in increased synthesis and fractional turnover rates of two primary bile acid cholic acid and chenodeoxycholic acid, along with decreased serum cholesterol levels compared with a low-fibre diet without β -glucan⁽¹⁴⁾. These previous studies observed bile acid metabolism being interrupted by β -glucan but possess limitations such as small sample size ($n<10$). Moreover, the ileostomy studies used short intervention periods of just a few days. Marlett *et al.* had a longer term for intervention but lacked a washout period. Therefore, results from the present study using thirty healthy participants with a controlled, randomised cross-over design provide stronger evidence to support that β -glucan reduces circulating cholesterol concentration through increasing bile acid synthesis.

The cholesterol-lowering effect of β -glucan was associated with the genetic variation of *CYP7A1* rs3808607. Specifically, individuals carrying SNP rs3808607-G allele *CYP7A1* were more responsive to HMW β -glucan in lowering LDL-cholesterol levels. In the present study, the participants were sub-grouped based on genotypes of *CYP7A1* SNP rs3808607, and we found that individuals carrying homozygote for G allele (G/G) showed significant response to 3 g/d HMW β -glucan in increasing 7 α -HC concentrations compared with control, but not for participants who carry T/T and G/T genotype of *CYP7A1* SNP rs3808607. This result reveals the important role of *CYP7A1* in the bile acid interruption mechanism and the influence of the genetic variation in this action. As reported previously, G-allele mediates greater gene expression than homozygote T based on increased transcriptional activity, which was suggested to increase bile acid synthesis in the liver as *CYP7A1* is the key enzyme in the classical pathway⁽¹⁸⁾. These results confirm that rs3808607-G allele carriers are more responsive to elimination of bile acids from the enterohepatic circulation caused by β -glucan^(14,28,31-33). As a consequence, under the stimulation of 3 g HMW β -glucan that 'bound' or 'entrapped' the greatest amount of bile acid, G/G carriers responded more to the 'elimination signal' given by the decreased level of primary bile acid in the liver, to produce more bile acid to restore the bile acid pool.

A negative correlation between AUC of ¹³C enrichment and FSR was observed, which agrees with the theory that when more free cholesterol enters the liver from the diet, synthesis of cholesterol will be suppressed, and vice versa. Yet, neither of these pathways was influenced by β -glucan intervention. The concentration of 7 α -HC increased by 3 g/d HMW β -glucan but was corrected with neither cholesterol absorption nor cholesterol synthesis. Accordingly, it is reasonable to speculate that interrupting bile acid enterohepatic circulation might be the sole mechanism for the cholesterol-lowering effect of β -glucan. This notion is further supported by the observation that log(viscosity) of β -glucan has a linear relationship with 7 α -HC levels but not with cholesterol absorption or synthesis parameters.

This study is an extension of a previous published study with serum cholesterol changes as the primary outcome⁽²⁾. It should be noted that sample size of this study was not determined for detecting changes in cholesterol and bile acid metabolism following β -glucan consumption or the interaction between genetic variation of *CYP7A1* SNP rs3808607 and β -glucan intervention. Although the distribution of genotype of *CYP7A1* SNP rs3808607 among the participants in this study is similar to the minor genotype frequency reported in the general population⁽²⁵⁾, we suggest a future study with appropriate sample size to confirm our current findings.

Overall, results in the current study showed that consumption of 3 g HMW β -glucan increased serum 7 α -HC but did not change the AUC of ¹³C-cholesterol enrichment in erythrocytes and FSR for cholesterol. Secondly, aligning with our previous findings, homozygous rs3808607-G allele carriers resulted in greater responses in lowering cholesterol levels and a higher level of bile acid synthesis after ingesting 3 g HMW β -glucan. In conclusion, our results suggest that interrupting enterohepatic circulation of bile acids rather than inhibiting cholesterol absorption and synthesis might be the mechanism responsible for the cholesterol-lowering effect of β -glucan; the different responsiveness of individuals to the cholesterol-lowering effect of β -glucan might be attributed to the different levels of bile acid synthesis of the three genotypes, G/G, G/T and T/T following β -glucan consumption.

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None of the authors has any conflicts of interest to declare.

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