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Great Northern Beans (*Phaseolus vulgaris* L.) Lower Cholesterol in Hamsters Fed a High-Saturated-Fat Diet

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Abstract

Background: Dietary interventions for high cholesterol, a primary risk factor for cardiovascular disease, are generally considered before prescribing drugs.

Objective: This study investigated the effects of whole Great Northern beans (wGNBs) and their hull (hGNB) incorporated into a high-saturated-fat (HSF) diet on cholesterol markers and hepatic/small intestinal genes involved in cholesterol regulation.

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Methods: Each of the 4 groups of 11 male golden Syrian hamsters at 9 wk old were fed a normal-fat [NF; 5% (wt:wt) of soybean oil], HSF [5% (wt:wt) of soybean oil + 10% (wt:wt) of coconut oil], HSF+5% (wt:wt) wGNB, or HSF+0.5% (wt:wt) hGNB diet for 4 wk. Cholesterol markers and expression of genes involved in cholesterol metabolism and absorption were analyzed from plasma, liver, intestinal, and fecal samples. Data were analyzed by 1-factor ANOVA and Pearson correlations.

Results: Compared with the HSF group, the HSF+wGNB group had 62% and 85% lower plasma and liver cholesterol and 3.6-fold and 1.4-fold greater fecal excretion of neutral sterol and bile acid, respectively ($P \leq 0.05$). The HSF+hGNB group had 54% lower plasma triglycerides ($P < 0.001$) and 53% lower liver esterified cholesterol ($P = 0.0002$) than the HSF group. Compared with the HSF group, the expression of small intestinal Niemann-Pick C1 like 1 (*Npc1l1*), acylcoenzyme A:cholesterol acyltransferase 2 (*Acat2*), and ATP binding cassette transporter subfamily G member 5 (*Abcg5*) were 75%, 70%, and 49% lower, respectively, and expression of hepatic 3-hydroxy-3-methylglutaryl CoA reductase (*Hmgr*) was 11.5-fold greater in the HSF+wGNB group ($P \leq 0.05$).

Conclusions: Consumption of wGNBs resulted in lower cholesterol concentration in male hamsters fed an HSF diet by promoting fecal cholesterol excretion, most likely caused by *Npc1l1* and *Acat2* suppression. The hGNB may partially contribute to the cholesterol-lowering effect of the wGNBs.

Keywords: Great Northern bean, cholesterol, hamster, saturated fat, gene expression

Abbreviations:

Abcg5	ATP binding cassette transporters subfamily G member 5
Abcg8	ATP binding cassette transporters subfamily G member 8
Acat2	acyl-coenzyme A:cholesterol acyltransferase 2
CVD	cardiovascular disease
Cyp51	lanosterol 14α-demethylase
Cyp7a1	cholesterol-7a-hydroxylase
GNB	Great Northern bean
hGNB	Great Northern bean hull
Hmgr	hepatic 3- hydroxy-3-methylglutaryl CoA reductase
HSF	high-saturated-fat
HSF+hGNB	high-saturated-fat diet containing 0.5% hull
HSF+wGNB	high-saturated-fat diet containing 5% whole bean
Ldlr	LDL receptor
Mtp	microsomal triacylglycerol transport protein
NF	normal-fat
Npc1l1	Niemann-Pick C1-like 1
Srebp2	sterol regulatory element-binding protein 2
TC	total cholesterol
TG	triglyceride
wGNB	whole Great Northern bean

Introduction

in the United States, while nearly 85.6 million Americans present with a form of cardiovascular disease (CVD) (1). Atherosclerosis is linked to CVD, which is initiated by the accumulation of oxidized LDL cholesterol (2) in both the small and large arteries (3). Western diets contain saturated fats as the primary lipid source, which is a major factor for LDL-cholesterol elevation (4, 5). It is expected that the above mortality statistics will be maintained or even increase as dietary choices established during early ages are not easily altered (6). As such, several drugs have been developed to treat hypercholesterolemia, with statins being the most commonly prescribed. These drugs inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Hmgr*) (7), which is involved in de novo cholesterol synthesis. Due to potential side effects induced by these drugs (7, 8), diet modification might be considered before drug prescription.

Towards this goal, multiple studies have shown that phytosterols, dietary fiber, and polyphenols are potent cholesterol-lowering natural agents (9–11). The latter 2 compounds are present in dry edible beans at concentrations higher than most dietary sources, including fruits and vegetables (12, 13). Nonetheless, a critical gap in knowledge exists on dry beans as protectors against high cholesterol caused by a Western diet. Moreover, among the different market classes, the Great Northern bean (GNB; *Phaseolus vulgaris* L. var. Great Northern) is one of the least studied in terms of delivering any health benefit for reasons that remain unknown. As this white bean is chemically diverse and varies in composition compared with its colored counterparts, the objective of this study was to investigate the effects of the whole GNB (wGNB) and its hull (hGNB), both of which were thoroughly characterized, on high cholesterol induced by a high-saturated-fat (HSF) diet and their potential involvement in cholesterol regulation. To satisfy this objective, 4 hamster groups were provided 4 different diets, with 2 groups fed a normal-fat (NF) diet [containing 5% (wt:wt) of soybean oil] and HSF diet [containing 5% (wt:wt) of soybean oil + 10% (wt:wt) of coconut oil], which served as a negative and positive controls, respectively, and the other 2 groups were fed an HSF diet containing 5.0% wGNB or 0.5% hGNB. The working hypothesis was that wGNB added to an HSF diet reduces the plasma and liver cholesterol

concentrations compared with the HSF diet to a level comparable to an NF diet, while the hGNB contributes to this effect considering that the hull contains multiple heart health components (14). The 4 groups were monitored for plasma, liver, and fecal lipid markers and the expression of key small intestinal and hepatic genes involved in cholesterol regulation.

Methods

Chemical composition analysis of Great Northern beans and hull

The wGNB (Coyne cultivar) was obtained from Panhandle Research and Extension Center, University of Nebraska–Lincoln. The hull was separated from the beans with a dehulling machine (TADD, Venables Machine Works Ltd). The moisture, total ash, crude protein, lipid contents, and soluble and insoluble dietary fibers of wGNB and hGNB were analyzed using the Official Methods of Analysis of AOAC International 935.29, 985.01, 992.23, 2003.06, and 991.43, respectively (15).

The wGNB and hGNB samples were sequentially extracted with various solvents and the supernatant from each extraction was used to determine the total phenolic (16), flavonoid (17), and tannin (18) contents. The values from all assays were then added to obtain the final concentrations. Phenolic compounds were chromatographically determined as described previously (19).

The saponins from defatted samples were extracted twice with 10-mL aqueous methanol (80%) for 24 h and then combined. Total saponins were then determined using a colorimetric method as described by Tan et al. (20)

Diets and animals

The NF diet (5% fat) and the HSF diet (15% fat) were prepared based on the AIN-93 M purified rodent diet (21). The other 2 experimental diets consisted of an HSF diet containing either 5% wGNB (HSF+wGNB) or 0.5% hGNB (HSF+hGNB) at the expense of corn starch (**Supplemental Table 1**). The wGNB at 5% (wt:wt) dosage was selected to ensure the diet was palatable and did not harm the hamsters as

dry beans contain lectins (22), which might reach toxic concentrations in uncooked beans. A 0.5-g/kg diet of the hGNB was selected as it is proportional to the hull content of the wGNB. The ingredients of the diets were obtained from Dyets, Inc. (Bethlehem, PA, USA).

Four groups of 11 male golden Syrian hamsters (Charles River Laboratories, Wilmington, MA, USA) at 9 wk of age were fed 4 different diets for 4 wk. The individually housed hamsters were provided with water and food ad libitum during the 4-wk trial. Food intake was measured every day, while body weight was monitored on the first and last days of the feeding period. The climate of the room where hamsters were housed was a 12-h light/dark cycle, approximately 25°C, and 50% relative humidity. After 4 wk, the hamsters were feed-deprived for 16 h, killed by carbon dioxide suffocation, and plasma, liver, and small intestine were harvested. Fecal samples were collected during the last 12 h of the feeding period. All protocols for animal care were approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln (IUCAC no. 1204).

Plasma and liver lipids

The plasma total cholesterol (TC), HDL-cholesterol, and triglyceride (TGs) concentrations were measured enzymatically using kits (Total Cholesterol E, HDL Cholesterol E, and L-type Triglyceride M, respectively) purchased from Wako Diagnostics (Wako Life Sciences, Inc.).Non-HDL cholesterol was calculated from the difference between TC and HDL-cholesterol concentrations. Lipids from the livers were extracted following the Folch method (23). After eliminating the solvent under a nitrogen stream, lipids were dissolved into water with Triton X-100 as a surfactant. TC, free cholesterol, and TGs were quantified enzymatically using the Free Cholesterol E and the kits listed above. Liver esterified cholesterol was calculated by subtracting the free cholesterol from liver TC values.

Fecal sterols and bile acids

The fecal neutral sterol concentration was analyzed by a GC method (24), whereas fecal bile acids were determined using an enzymatic method (Diazyme total bile acid; Diazyme Laboratories, Inc.).

Gene expression analysis

Gene expression analysis was completed for 5 hepatic genes, including hepatic LDL receptor (*Ldlr*), *Hmgr*, sterol regulatory element-binding protein 2 (*Srebp2*), lanosterol 14α-demethylase (*Cyp51*), and cholesterol-7a-hydroxylase (*Cyp7a1*), and 5 small intestinal genes— namely, Niemann-Pick C1 like 1 protein (*Npc1l1*), acyl-coenzyme A:cholesterol acyltransferase 2 (*Acat2*), microsomal triacylglycerol transport protein (*Mtp*), and ATP binding cassette transporters subfamily G member 5 and 8 (*Abcg5* and *Abcg8*), following the protocol described previously (19). *Gapdh* was used as a reference, and expression of the hepatic and intestinal genes was normalized to the HSF group using the ΔΔCt method.

Statistical analysis

Power analysis for 1-factor ANOVA was conducted to estimate the sample size, with inputs being a type I error rate of 0.05, expected power of 0.8, the difference in the TC of 1.5 mmol/L, and SD of 1 mmol/L. Data from the animal study are presented as means ± SEMs, whereas data from the chemical characterization of beans are presented as means ± SDs. Data were subjected to 1-factor ANOVA, followed by Tukey's honestly significant difference (HSD) to determine differences between the group means. Pearson correlation analysis was performed to assess the correlations between fecal cholesterol and liver as well as plasma cholesterol concentrations, and between plasma, liver, and fecal cholesterol concentrations and gene expression. A difference or correlation with $P \leq 0.05$ was considered statistically significant. All statistics were analyzed using Minitab 18 (Minitab, Inc.).

Results

Chemical composition of Great Northern beans

Proximate analysis showed that protein in the wGNB was ~49% higher (per gram of wGNB and hGNB) than in the hGNB. However, hGNB

Table 1 Chemical composition of Great Northern beans and their hull*

Composition	*wGNB*	*hGNB*
Moisture, %	9.65 ± 0.05	9.30 ± 0.07
Ash, %	4.34 ± 0.04	6.11 ± 0.05
Lipids, %	1.10 ± 0.09	0.82 ± 0.09
Proteins, %	19.1 ± 0.30	12.8 ± 0.69
Carbohydrates, %	65.7 ± 0.22	70.9 ± 0.56
Dietary fibers, mg/g		
Soluble	42.4 ± 8.01	79.2 ± 4.40
Insoluble	142 ± 3.50	512 ± 20.5
Oligosaccharides, mg/g		
Raffinose	15.3 ± 0.39	15.6 ± 0.55
Stachyose	63.5 ± 2.57	40.8 ± 1.25
Total phenolic content, mg GAE/g	1.80 ± 0.02	0.86 ± 0.17
Total flavonoid content, mg CE/g	0.27 ± 0.05	0.15 ± 0.07
Phenolic acids, mg/100 g		
Cafeic acid	1.37 ± 0.05	3.04 ± 0.53
p-Coumaric acid	7.85 ± 0.35	18.3 ± 1.78
Ferulic acid	16.9 ± 0.50	50.9 ± 6.29
Sinapic acid	6.53 ± 0.38	16.1 ± 2.27
Condensed tannins, mg CE/g	6.70 ± 0.63	2.81 ± 0.16
Saponins, mg aescin/g	27.4 ± 0.64	103 ± 2.80

* Values are means ± SDs; $n = 3$.
CE, catechin equivalents; GAE, Gallic acid equivalents; hGNB, Great Northern bean hull; wGNB, whole Great Northern beans.

contained higher dietary fiber and saponins (by ~182 and ~276%, respectively), while wGNB contained almost 138% higher levels of tannins. The hGNB also contained markedly higher concentrations of phenolic acids, including caffeic, *p*-coumaric, ferulic, and sinapic acids (**Table 1**).

Growth parameters of the hamsters

Body weights of the 4 hamster groups did not differ at the beginning of the study (**Table 2**) but were significantly higher in the HSF group than in the other groups after a 4-wk trial ($P < 0.01$). No significant differences in food intake occurred between hamster groups, with no concomitant differences in body weight between HSF+wGNB or HSF+hGNB groups and the NF group, indicating that the wGNB and hGNB added to the diet at 5% (wt:wt) and 0.5% (wt:wt), respectively,

Table 2 Food intake, body and liver weights, and hepatic and fecal lipid profiles of male hamsters fed an NF diet or HSF diet alone or containing wGNB or hGNB for 4 wk*

	NF	HSF	HSF+wGNB	HSF+hGNB
Food intake, g/d	7.78 ± 0.11	7.87 ± 019	7.40 ± 0.14	7.48 ± 0.06
Initial body weight, g	112.9 ± 1.37	112.6 ± 1.80	113.9 ± 1.35	113.7 ± 1.41
Final body weight, g	113.7 ± 0.47b	123.6 ± 1.69a	111.7 ± 2.78b	114.3 ± 1.31b
Liver				
Relative weight, g/100 g BW	3.98 ± 0.11c	5.02 ± 0.11a	3.62 ± 0.07d	4.61 ± 0.07b
Free cholesterol, μmol/g	3.07 ± 0.12b	4.12 ± 0.21a	2.56 ± 0.09b	4.23 ± 0.14a
Esterified cholesterol, μmol/g	0.89 ± 0.19c	14.91 ± 2.01a	0.28 ± 0.20c	7.02 ± 1.28b
Triglycerides, μmol/g	2.64 ± 0.49ab	1.22 ± 0.18c	3.66 ± 0.49a	1.64 ± 0.20b,c
Fecal excretion, μmol · d^{-1} · 100 g BW^{-1}				
Neutral sterols	2.16 ± 0.13c	6.33 ± 0.35bc	28.84 ± 2.74a	9.81 ± 0.63b
Bile acids	1.27 ± 0.09c	2.02 ± 0.20bc	4.37 ± 0.28a	2.65 ± 0.20b

* Values are means ± SEMs, n = 11.
Labeled means in a row without a common letter differ, $P \leq 0.05$.
BW, body weight; HSF, high-saturated-fat; hGNB, Great Northern bean hull; NF, normal-fat; wGNB, whole Great Northern beans.

were palatable and did not result in any adverse effect on the growth of experimental animals. The liver weight of the HSF+wGNB group was significantly lower, whereas that of the HSF group was significantly greater than all other groups ($P \leq 0.05$; Table 2).

Plasma lipids response to diets

The HSF+wGNB group had 62%, 68%, and 52% lower plasma TC, HDL cholesterol, and non-HDL cholesterol, respectively, compared with the HSF group ($P \leq 0.05$; **Figure 1**A). The HSF+hGNB group presented with cholesterol concentrations comparable to the HSF group. TG concentrations were significantly lower in both HSF+wGNB and HSF+hGNB groups compared with the HSF group, to a degree similar to that exhibited by the NF group ($P < 0.01$). The plasma TC to HDL-cholesterol ratio was not significantly different between HSF+wGNB and NF groups ($P = 0.293$; Figure 1B), but was higher in the HSF+wGNB group than in the HSF group ($P = 0.008$).

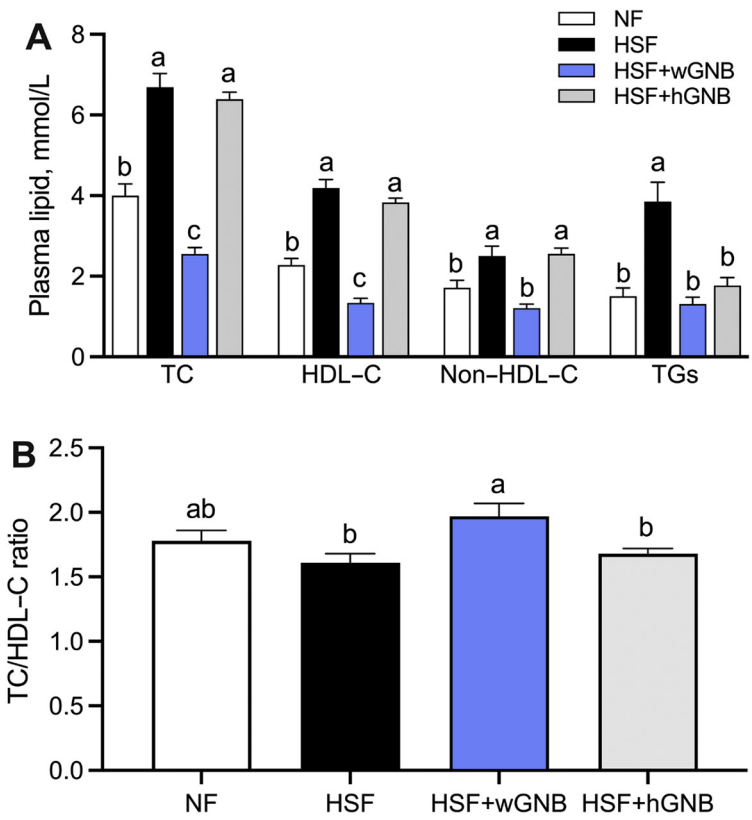

Figure 1 Plasma TC, HDL-C, non–HDL-C, and TG concentrations (A) and the TC to HDL-C ratio (B) of male hamsters fed an NF diet or an HSF diet alone or containing wGNB or hGNB for 4 wk. Values are means ± SEMs, n = 11. Labeled means in each category without a common letter differ, $P \leq 0.05$. hGNB, Great Northern bean hull; HDL-C, HDL cholesterol; HSF, high-saturated-fat; NF, normal-fat; non–HDL-C, non–HDL cholesterol; TC, total cholesterol; TG, triglyceride; wGNB, whole Great Northern beans.

Liver lipid response to diets

The NF and HSF+wGNB groups had comparable concentrations of liver free and esterified cholesterol, which were significantly lower than in the other 2 groups ($P \leq 0.05$; Table 2). Higher concentrations of liver esterified cholesterol occurred in the HSF group compared with the HSF+hGNB group, whereas free cholesterol did not differ between these 2 groups. A significantly higher TG concentration was detected in the HSF+wGNB group, whereas lower values were produced by the HSF and HSF+hGNB groups ($P \leq 0.05$). The HSF+wGNB and HSF+hGNB diets resulted in TG concentrations comparable to the NF diet ($P > 0.05$).

Fecal cholesterol and correlation with plasma and liver cholesterol

The HSF+wGNB diet resulted in a higher excretion of both neutral sterols and bile acids than the other diets ($P < 0.001$; Table 2). The HSF+hGNB and HSF groups excreted comparable concentrations of neutral sterols and bile acids. In addition, strong negative correlations existed between fecal cholesterol output and liver TC ($r = –0.774$, $P < 0.001$; **Figure 2**A) and plasma TC ($r = –0.891$, $P < 0.001$; Figure 2B) in all hamsters consuming the 3 diets containing saturated fat as the main lipid source.

Relative expression of small intestinal genes involved in cholesterol absorption

The HSF+wGNB diet reduced the expressions of *Npc1l1* and *Acat2* to levels comparable to the NF diet, whereas the HSF+hGNB did not result in a significant reduction in either gene expression compared with the HSF diet (**Figure 3**A). However, the expression of *Mtp* was lower in both HSF+wGNB and HSF+hGNB groups than in the HSF group ($P \leq 0.05$). Both HSF+wGNB and HSF+hGNB diets reduced the expression of *Abcg5* ($P \leq 0.05$) but did not affect the mRNA levels of *Abcg8* with respect to the HSF diet ($P > 0.05$; Figure 3A).

Relative expression of hepatic genes involved in cholesterol metabolism

Relative hepatic mRNA levels of *Hmgr* and *Cyp51* in response to the HSF+wGNB diet were higher than those in hamster groups consuming the other diets ($P \leq 0.05$; Figure 3B). Upregulation of *Srebp2* occurred in all groups fed the HSF diets compared with the NF group ($P \leq 0.05$). The opposite trend occurred with the *Cyp7a1* gene, as the expression of this gene was significantly higher in the NF group than in all other groups ($P \leq 0.05$). The HSF group presented with lower *Ldlr* expression than the remaining groups ($P \leq 0.05$).

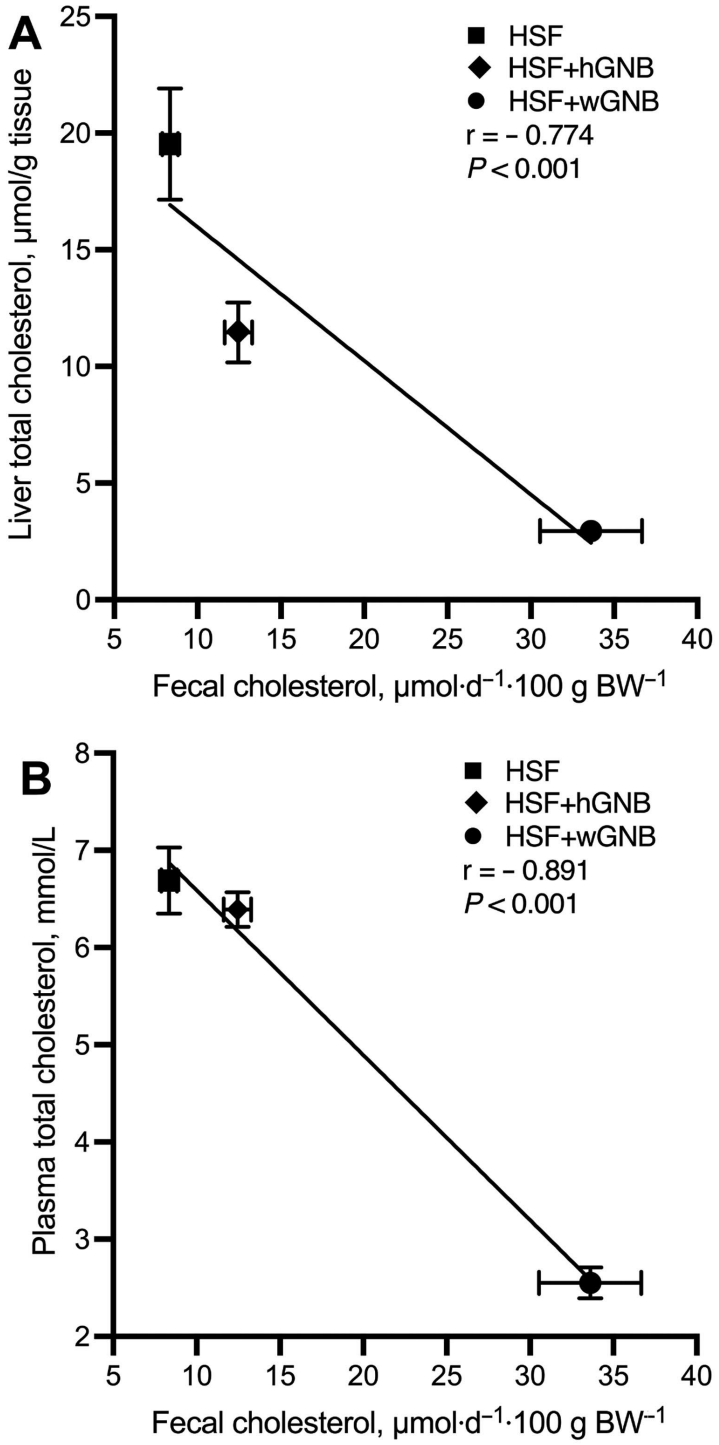

Figure 2 Pearson correlations between fecal cholesterol and liver total cholesterol (A) and plasma total cholesterol (B) of male hamsters fed an HSF diet alone or containing wGNB or hGNB for 4 wk. Values are means ± SEMs, n = 11. The correlation was considered to be significant if $P \leq 0.05$. BW, body weight; hGNB, Great Northern bean hull; HSF, high-saturated-fat; wGNB, whole Great Northern beans.

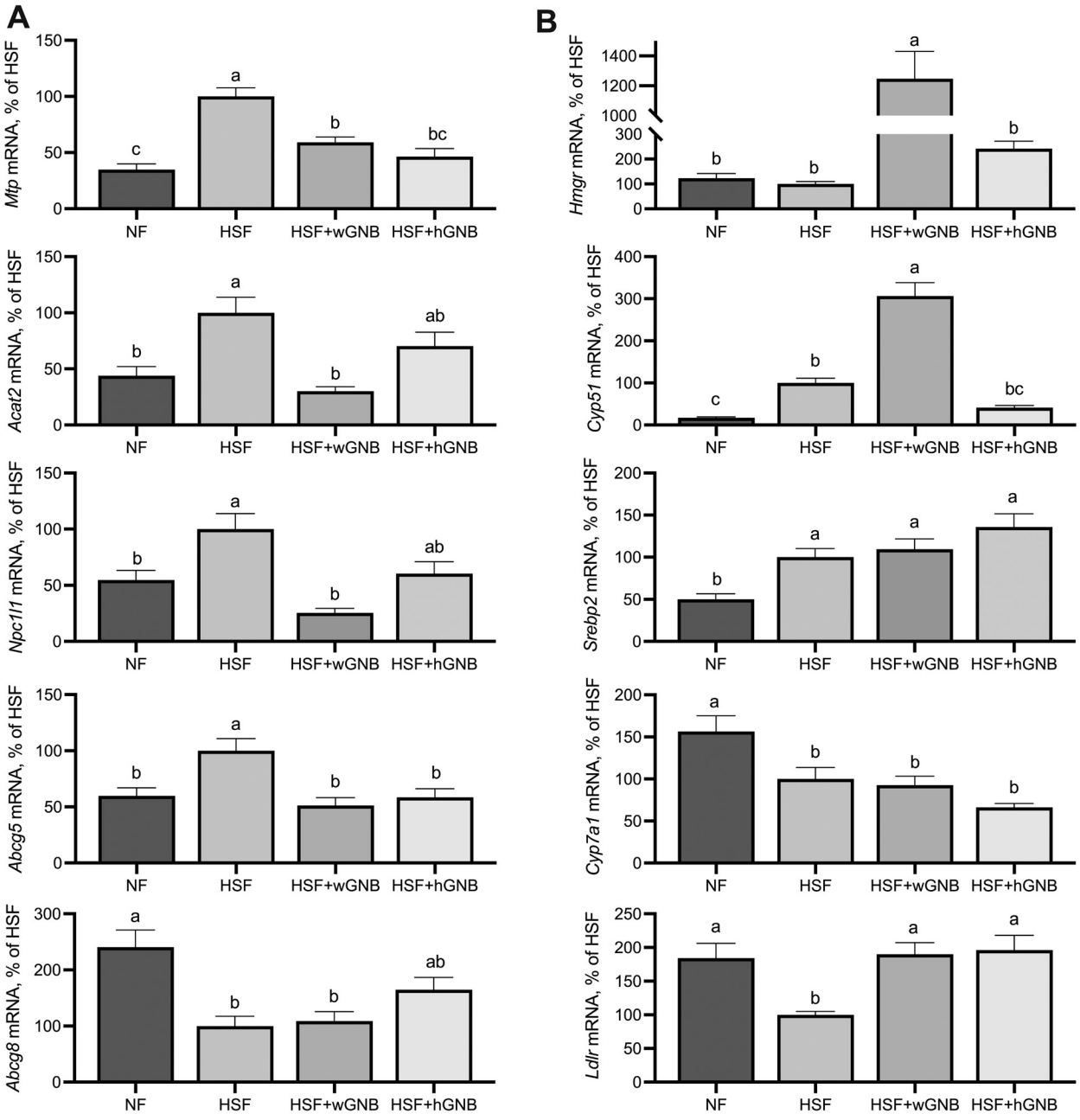

Figure 3 Relative expression of small intestinal (A) and hepatic (B) genes of male hamsters fed an NF diet or HSF diet alone or containing wGNB or hGNB for 4 wk. Values are means ±SEMs, n=11. Labeled means without a common letter differ, $P \leq 0.05$. *Abcg*5, ATP binding cassette transporters subfamily G member 5; *Abcg8*, ATP binding cassette transporters subfamily G member 8; *Acat2*, acyl-coenzyme A:cholesterol acyltransferase 2; *Cyp51*, lanosterol 14α-demethylase; *Cyp7a1*, cholesterol-7a-hydroxylase; hGNB, Great Northern bean hull; *Hmgr*, hepatic 3-hydroxy-3-methylglutaryl CoA reductase; HSF, high-saturated-fat; HSF+hGNB, high-saturated-fat diet containing 0.5% Great Northern bean hull; HSF+wGNB, high-saturated-fat diet containing 5% whole Great Northern beans; *Ldlr*, LDL receptor; *Mtp*, microsomal triacylglycerol transport protein; NF, normal-fat; *Npc1l1*, Niemann-Pick C1-like 1; *Srebp2*, sterol regulatory element-binding protein 2; wGNB, whole Great Northern beans.

Correlations between plasma, hepatic, and fecal cholesterol concentrations and gene expression

For the hepatic cholesterol synthesis pathway, the expression of *Hmgr* and *Cyp51* strongly and negatively correlated with both plasma and hepatic cholesterol concentrations, while positively correlated with fecal excretion of cholesterol (**Table 3**). In the case of the cholesterol absorption pathway, *Npc1l1* and *Acat2* expression levels were positively correlated with both plasma and liver cholesterol concentrations but negatively correlated with fecal cholesterol excretion. A low positive correlation existed between the mRNA levels of *Abcg5* and liver cholesterol concentration, whereas a low negative correlation occurred between the expression of this gene and fecal cholesterol concentrations. Expression of the other analyzed genes did not show a significant correlation with any other cholesterol markers.

Table 3 Pearson correlations between plasma, hepatic, and fecal cholesterol concentrations and hepatic and intestinal gene expression of male hamsters fed an NF diet or HSF diet alone or containing wGNB or hGNB for 4 wk[a]

	Plasma cholesterol	*Hepatic cholesterol*	*Fecal cholesterol*
Hmgr	–0.842∗∗∗	–0.700∗∗∗	0.786∗∗∗
Cyp51	–0.834∗∗∗	–0.670∗∗	0.666∗∗∗
Ldlr	–0.259	–0.402	0.344
Mtp	0.187	0.316	–0.370
Acat2	0.544∗∗	0.712∗∗∗	–0.667∗∗∗
Npc1l1	0.479∗	0.642∗∗	–0.626∗∗
Abcg5	0.349	0.427∗	–0.490∗∗

a. Values are Pearson correlation coefficients, $n = 33$.
∗,∗∗,∗∗∗ Significant correlation: ∗ $P \leq 0.05$; ∗∗ $P \leq 0.01$; ∗∗∗ $P \leq 0.001$.
*Abcg*5, ATP binding cassette transporters subfamily G member 5; *Acat2*, acyl-coenzyme A:cholesterol acyltransferase 2; *Cyp51*, lanosterol 14α-demethylase; hGNB, Great Northern bean hull; *Hmgr*, hepatic 3-hydroxy-3-methylglutaryl CoA reductase; HSF, high-saturated-fat; *Lldr*, LDL receptor; *Mtp*, microsomal triacylglycerol transport protein; NF, normal-fat; *Npc1l1*, Niemann-Pick C1-like 1; wGNB, whole Great Northern beans.

Discussion

The hypocholesterolemic effect of colored dry beans has been reported elsewhere (19, 25). However, the role of wGNB, a white bean commonly produced in the United States as well as other regions of the world, in cholesterol regulation is nonexistent to our knowledge. Therefore, this study is the first to address the effect of wGNB on cholesterol homeostasis via the investigation of multiple metabolic pathways. A hamster model was chosen for this purpose as hamsters were reported to have similar lipid metabolism to that in humans (26), although HDL cholesterol is present in greater quantity than non-HDL cholesterol in hamsters, while the opposite is normally observed in humans (27). As evidenced by this study, wGNB included at 5% (wt:wt) in an HSF diet that contained coconut oil as a main source of SFA substantially reduced both plasma and hepatic cholesterol, while significantly improving the excretion of cholesterol through feces compared with the HSF diet alone. However, the reduction in plasma cholesterol was more pronounced in the HDL fraction than in the non- HDL counterpart, which resulted in a higher TC to HDL-cholesterol ratio in hamsters fed HSF+wGNB than those fed the HSF diet. These results may be due to the fact that hamsters have higher HDL-cholesterol concentrations than non–HDL-cholesterol concentrations (27). As an outcome, wGNB may affect the HDL assembly to a greater degree than its non-HDL counterpart. Nevertheless, this higher cholesterol ratio may compensate for the heart health benefit exerted by the cholesterol-lowering effect of wGNB.

It was shown in this study that plasma TC, non-HDL cholesterol, and body weight increased in hamsters fed the HSF diet compared with the NF- and HSF+wGNB-fed counterparts, while the 3 groups consumed an equivalent amount of carbohydrates. These results may suggest that the HSF group absorbed dietary fat at a higher rate, which led to higher plasma TG concentration. As liver TGs and esterified cholesterol are components for the assembly of VLDLs, which then deposit TGs in peripheral tissues to form LDL (28), the concentrations of plasma and liver TGs may be associated with one another. As such, the higher concentration of plasma non-HDL cholesterol and TGs in HSF-fed hamsters may suggest that VLDL production by these hamsters was higher, consequently resulting in lower liver TG concentrations

than the NF- and HSF+wGNB-fed hamsters. In contrast, the low liver cholesterol concentrations in the HSF+wGNB-fed hamsters may link to their higher liver TG concentrations, as liver cholesterol shortages, especially esterified cholesterol, may lead to lower VLDL assembly and excretion of TGs into the circulation. Lower concentrations of plasma HDL cholesterol and liver cholesterol exhibited by the HSF+wGNB-fed hamsters compared with those consuming the HSF or HSF+hGNB diet may be due to the higher elimination rate of cholesterol via feces. This hypothesis was supported by a high negative correlation between fecal TC excretion and liver TC or plasma TC concentrations. Although HDL is involved in the removal of cholesterol from peripheral tissues, it is not the only marker used to determine cardiovascular health. Dorfman et al. (29) indicated that the lower HDL-cholesterol concentration might not be detrimental if components involved in the reverse cholesterol transport process, such as apoA-I and scavenger receptor B class 1 increase, but for this study, these markers were not analyzed. Moreover, Bartlett et al. (30) demonstrated that the risks of CVD were lower in human subjects with low HDL-cholesterol concentrations in conjunction with low concentrations of both blood TGs and LDL cholesterol (≤100 mg/dL) compared with individuals presenting with higher concentrations of these lipids.

Gene expression analysis was conducted on several key genes involved in cholesterol absorption to elucidate the mechanism underlying the cholesterol-lowering effect of the wGNB. The results showed that the wGNB lowered cholesterol concentrations compared with hamsters fed the HSF diet by preventing cholesterol absorption from the small intestine, which was supported by the downregulation of intestinal *Npc1l1* and *Acat2* combined with the significant correlations of *Npc1l1* and *Acat2* expression levels with plasma, liver, and fecal cholesterol concentrations. Among the components identified in the beans, saponins have been reported to downregulate *Acat2* (31), while polyphenols suppress *Npc1l1* expression (32). Additionally, the *Acat2* mRNA level may have also been reduced due to insufficient enterocyte cholesterol in the HSF+wGNB diet–fed hamsters, as this gene is regulated by cholesterol (33). Although the intake of both wGNB and hGNB led to the downregulation of *Mtp*, the expression of this gene did not correlate with any cholesterol markers. Further studies are needed to investigate the involvement of this protein in the

cholesterol-lowering effect of the wGNB. As the *Abcg5* transporter is responsible for refluxing excessive cholesterol and other sterols from enterocytes back to the intestinal lumen (34), its downregulation in the wGNB-containing diet group may be due to lower enterocyte cholesterol availability caused by a lack of *Npc1l1* transporters.

Gene expression analysis was also conducted for selected genes involved in cholesterol metabolism in the liver. The relative mRNA levels of *Hmgr* and *Cyp51*, which are involved in the de novo cholesterol biosynthesis pathway, were significantly higher in HSF+wGNB-fed hamsters, indicating that hepatic cholesterol synthesis increased with wGNB intake. This result is in accordance with the results reported by Bartley et al. (35), who showed that *Cyp51* was significantly upregulated in hamsters presenting with lower circulating cholesterol concentrations but high concentrations of fecal cholesterol. The increase in *Hmgr* and *Cyp51* expression in hamsters fed the diet containing wGNB was likely a compensatory mechanism that does not fully counteract the beneficial effect of the decreased cholesterol absorption, resulting in an overall reduction in plasma cholesterol concentration. This effect of wGNB is likely to link with its protein component, as Macarulla et al. (36) reported that the cholesterol-lowering effect of the faba bean protein was not caused by a reduction in *Hmgr* activity but by fecal cholesterol excretion, which is in accordance with the results reported in the current study. For both the wGNB- and hGNB-fed hamsters, upregulation of *Ldlr* may be a response to decreased liver cholesterol, leading to the clearance of LDL cholesterol from the plasma, as expression was similar to those of the hamsters fed the NF diet. It must be noted, however, that this hypothesis was not supported by the correlation between *Ldlr* mRNA levels and plasma TC as well as liver TC concentrations. Moreover, the expression of *Srebp2*, a transcription factor of *Hmgr*, was not impacted by the wGNB and hGNB, suggesting that the beans may affect the expression of other *Srebp* isoforms or act by another mechanism that is beyond the scope of this study. Last, the increase in fecal bile acids excreted by HSF+wGNB-fed hamsters did not coincide with the expression of *Cyp7a1*, a gene involved in bile acid synthesis. Different enzymes, genes, or transcription factors involved in bile acid synthesis may have been mediated by wGNB, thus requiring further investigation to elucidate this response.

Interestingly, the hGNB diet contributed similarly or trended accordingly, albeit insignificantly, to the expression of several intestinal/hepatic genes with respect to the NF and/or the wGNB treatments. However, the cholesterol markers, such as plasma TGs and hepatic esterified cholesterol concentrations, were significantly different from the HSF-fed groups. As such, the hGNB may contribute to the overall effect of wGNB at the phenotype level. Additional studies are needed that include a thorough characterization of GNBs without the hulls (cotyledon) to fully understand the components responsible for the cholesterol-lowering effects. Also, the roles of individual components from the beans in cholesterol regulation need to be investigated to better understand their cholesterol-lowering effect. For example, dietary fibers from wGNB may increase the viscosity of intestinal contents, reducing the absorption of bile salts (37), or physically bind to bile acids, thereby promoting cholesterol excretion via feces (38).

In conclusion, the wGNB incorporated in the HSF diet substantially reduced circulation and hepatic cholesterol concentrations by eliminating cholesterol from the body in the form of neutral sterols and bile acids. Based on the data, a possible mechanism responsible for this outcome is the downregulation of the small intestinal *Npc1l1* and *Acat2*. Cholesterol concentrations were maintained in the hamsters fed the wGNB-containing diet via the upregulation of the hepatic *Hmgr* and *Cyp51* and the small intestinal *Abcg5*. The hull may partially contribute to the overall cholesterol-lowering effect of the whole beans. As this study has elucidated, further research is needed to investigate the effect of GNBs on transcription factors of *Hmgr* and *Ldlr* and elements involved in bile acid synthesis. Yet, this study is significant as it is the first to evaluate the possible benefits of GNBs on mitigating cholesterol concentrations as a supplement to high-cholesterol-inducing diets commonly consumed by Western societies.

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Supplemental Table 1 follows the **References**.

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Supplementary Table 1 Composition of treatment diets[1]

Ingredient	NF	HSF	HSF+wGNB	HSF+hGNB
	g/kg diet			
Corn starch	455.7	353.7	313.7	348.7
Dextrinized cornstarch	155	155	155	155
Casein	140	140	130	140
Sucrose	100	100	100	100
Coconut oil	---	100	100	100
Soybean oil	50	50	50	50
Insoluble fiber (cellulose)	40	40	40	40
Soluble fiber (guar gum)	10	10	10	10
Great northern bean	---	---	50	---
Great northern bean hull	---	---	---	5
Cholesterol	---	2	2	2
AIN-93 mineral mix[2]	35	35	35	35
AIN-93 vitamin mix[3]	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5

1. HSF, high saturated fat diet; HSF+hGNB, high saturated fat diet containing 0.5% (w/w) great northern bean hull; HSF+wGNB, high saturated fat diet containing 5% (w/w) whole great northern beans; NF, normal fat diet.
2. AIN-93 mineral mix ingredient (per kg mix): calcium carbonate anhydrous (357 g), potassium phosphate monobasic (250 g), potassium citrate-H_2O (28 g), sodium chloride (74 g), potassium sulfate (46.60 g), magnesium oxide (24 g), ferric citrate (6.06 g), zinc carbonate (1.65 g), sodium metasilicate-$9H_2O$ (1.45 g), manganous carbonate (0.63 g), cupric carbonate (0.30 g), chromium potassium sulfate-$12H_2O$ (0.28 g), boric acid (81.5 mg), sodium fluoride (63.5 mg), nickel carbonate (31.8 mg), lithium chloride (17.4 mg), sodium selenate anhydrous (10.25 mg), potassium iodate (10 mg), ammonium paramolybdate-$4H_2O$ (7.95 mg), and ammonium vanadate (6.6 mg).
3. AIN-93 vitamin mix ingredient (per kg mix): Vitamin E as all-rac-α-tocopheryl acetate (7.50 g), nicotinic acid (3 g), calcium pantothenate (1.60 g), pyridoxine-HCl (0.70 g), thiamin-HCl (0.60 g), riboflavin (0.60 g), vitamin A as all-trans-retinyl palmitate (0.22 g), folic acid (0.20 g), vitamin K as phylloquinone (0.08 g), biotin (0.02 g), cyanocobalamin (2.50 mg), vitamin D as cholecalciferol (2.50 mg).