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RESEARCH ARTICLE

Consumption of barley β-glucan ameliorates fatty liver and insulin resistance in mice fed a high-fat diet

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Consumption of a diet high in barley β-glucan (BG) has been shown to prevent insulin resistance. To investigate the mechanism for the effects of barley BG, three groups of male 7-wk-old C57BL/6J mice were fed high-fat diets containing 0, 2, or 4% of barley BG for 12 wk. The 2% BG and 4% BG groups had significantly lower body weights compared with the 0% BG group. The 4% BG group demonstrated improved glucose tolerance and lower levels of insulin-resistance index and glucose-dependent insulinotropic polypeptide. Consumption of the BG diet decreased hepatic lipid content. Mice on the BG diet also demonstrated decreased fatty acid synthase and increased cholesterol 7α-hydroxylase gene expression levels. The BG diet promoted hepatic insulin signaling by decreasing serine phosphorylation of insulin receptor substrate 1 and activating Akt, and it decreased mRNA levels of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. In summary, consumption of BG reduced weight gain, decreased hepatic lipid accumulation, and improved insulin sensitivity in mice fed a high-fat diet. Insulin signaling enhanced due to the expression changes of glucose and lipid metabolism genes by BG consumption. Consumption of barley BG could be an effective strategy for preventing obesity, insulin resistance, and the metabolic syndrome.

Keywords:
Barley β-glucan / Fatty liver / High-fat diet / Insulin resistance / Insulin signaling

1 Introduction

Metabolic syndrome is associated with a number of metabolic disorders such as hypertension, dyslipidemia, glucose intolerance, obesity, and hyperinsulinemia [1]. Insulin resistance and central obesity are predominant risk factors for the pathogenesis of metabolic syndrome [2, 3]. A highly specialized diet or dietary modification is one major strategy used to reduce body weight and insulin resistance.

Foods rich in whole grains, which are composed of germ, bran, and endosperm, improve insulin resistance and obesity and are inversely associated with metabolic syndrome in adults [4]. Although epidemiological studies concerning the effectiveness of whole-grain consumption on disease are questionable, dietary fiber is considered as a healthy component of the diet. Dietary fiber can be divided into soluble and insoluble fiber based on water solubility. Due to its viscosity, soluble fiber appears to have greater physiological effects than insoluble fiber for the management of intestinal disorders such as constipation [5]. Several studies have reported that meals containing soluble fiber reduce postprandial blood glucose and insulin concentrations [6, 7]. This is likely due to an increase in the viscosity of the contents of the stomach and small intestine, which reduces the gastric emptying rate and the rate of absorption and transport of digested nutrients [6, 7].

β-Glucan (BG) is found in cereal grains and comes primarily from the cell walls of oat and barley endosperms [8, 9]. It is a soluble fiber and is highly viscous in solution

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Abbreviations: BG, β-glucan; **Chol,** cholesterol; **GIP,** glucose-dependent insulinotropic polypeptide; **GLP-1,** glucagon-like peptide-1; **IRS,** insulin receptor substrates; **PEPCK,** phosphoenolpyruvate carboxykinase; **TG,** triglyceride

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[8, 9]. Studies using BG from barley or oats have had inconsistent results, with both positive [10–13] and negative [14, 15] effects on satiety, energy intake, weight loss, and glycemic control. The effects of cereal grains vary according to the supplemented amounts and sources of BG and the condition of the experimental subjects, *e.g.* non-diabetic or diabetic. A diet high in BG has beneficial effects on postprandial insulin and glucose responses and reduces plasma cholesterol (Chol) in human subjects [10, 11]. Nevertheless, the mechanisms by which BG exerts its beneficial effects are unclear. Therefore, we investigated the effects of consumption of barley whole-grain cereals containing varying amounts of BG in a high-fat-diet–induced mouse model of obesity.

2 Materials and methods

2.1 Animals and diets

After adaptation for 2 wk, 7-wk-old male C57BL/6J mice (Japan SLC, Hamamatsu, Shizuoka, Japan) were divided into three groups of 13 animals each. Mice were fed high-fat diets containing 0, 2, or 4% BG for 12 wk with water available *ad libitum*. Experimental diets were formulated and dietary ingredients were supplied by Dyets (Bethlehem, PA, USA) except for the dietary barley and wheat whole-grain cereals. We used the barley and wheat whole-grain cereals to test the biological effects of BG. Barley and wheat whole-grain cereals were generously provided from ConAgra Mills (Omaha, NE, USA). Barley whole-grain cereals are rich in soluble fiber BG, and wheat whole-grain cereals are predominantly high in insoluble fiber. Since barley can provide 12 g BG *per* 100 g grain, the test diets for the 0, 2, and 4% BG contained 320 g wheat (2.7 g BG), 160 g wheat and 160 g barley (20.5 g BG), and 320 g barley (38.4 g BG) *per* kilogram diet, respectively (Table 1). According to the macronutrients information provided by Dr. Elizabeth Arndt, ConAgra Mills, 11.8 g protein, 70.6 g carbohydrate, and 1.5 g fat can be provided by 100 g of whole wheat flakes (Shiloh Farms, PA, USA) and 15.0 g protein, 53.6 g carbohydrate, and 5.4 g fat can be provided by 100 g of Sustagrain barley (ConAgra Mills). By adding the different amounts of casein, corn starch, and beef tallow to three different diets, the final proportion of major macronutrients is the same across the experimental diets regardless of the type of cereal, wheat or barley whole grain. Thus, the experimental diets provided a total energy of 4.5 kcal/g, 19% from protein, 42% from carbohydrate, and 39% from fat (Table 1).

Mice were maintained on a 12:12-h light:dark cycle. Body weight and food intake were recorded weekly. This study was approved by the Experimental Animal Resources Laboratory of the Korean Food and Drug Agency.

2.2 Serum analysis and tissue collection

Mice were euthanized after 12 wk of treatment. Blood was obtained from the retroorbital sinus of the mice in the non-fasting state at 11 am to 12 pm. Tissues were collected, weighed, and immediately frozen in liquid nitrogen for further analysis. Concentrations of serum glucose (AM-201 K), triglycerides (TG, AM-157SK), Chol (AM-202 K), and HDL–Chol (AM203-3) were determined by an enzyme assay method (Asan Pharmaceutical, Yongin, Gyeonggido, Korea). LDL-Chol was calculated using the formula of Friedewald *et al.* [16]. Free fatty acids were determined using the non-esterified free fatty acid kit (Wako, Osaka, Japan). Serum

Table 1. Diet composition[a)]

Ingredient g/kg diet	0% BG	2% BG	4% BG
Casein	172.7	171.1	169.5
Corn starch	163.6	166.8	170.0
Wheat	320	160	0
Barley	0	160	320
Sucrose	100	100	100
Beef tallow	163.2	161.6	160
Corn oil	20	20	20
Cholesterol	10	10	10
L-cystein	3	3	3
AIN-93 mineral mixture[b)]	35	35	35
AIN-93VX vitamin mixture[c)]	10	10	10
Choline Bitartrate	2.5	2.5	2.5
TBHQ[d)]	0.014	0.014	0.014

a) The experimental diets provided a total energy of 4.5 kcal/g, 19% from protein, 42% from carbohydrate and 39% from fat. By adding the different amounts of casein, corn starch, beef tallow, wheat, and barley to three different diets, the final proportion of major macronutrients is the same across the experimental diets.
b) Mineral mixture: AIN-93M mineral mixture (ICN, CA, USA).
c) Vitamin mixture: AIN-93VX vitamin mixture (ICN, CA, USA).
d) TBHQ: *tert*-butylhydroquinone.

insulin levels were determined using an ELISA kit (Linco Research, St. Charles, MO, USA). Serum ghrelin (ALPCO Diagnostics, Salem, NH, USA), glucose-dependent insulinotropic peptide (GIP; Linco Research), and glucagon-like peptide 1 (GLP-1; Linco Research) were determined using an enzyme immunoassay kit. Insulin resistance indices were calculated using the homeostasis model assessment index as follows: (fasting serum insulin concentration, nmol/L) × (fasting serum glucose concentration, mmol/L)/22.5 [17]. A high homeostasis model assessment index denotes low insulin sensitivity and decreases in β-cell function.

2.3 Glucose tolerance tests

Glucose tolerance test (GTT) performed after 11 wk of treatment. After 5–7 h of fasting, mice were injected intraperitoneally with glucose (2 g *per* kg body weight). Blood glucose levels were determined from tail vein blood at 0, 15, 30, 60, and 120 min after glucose injection. Blood glucose was measured using the Super Glucocard II analyzer (ARKRAY, Kyoto, Japan). Area under the curve (AUC) values were calculated and normalized to baseline to measure glucose tolerance using SigmaPlot 8.0 (SPSS, Chicago, IL, USA).

2.4 Liver lipid content

Livers were homogenized, and tissue lipids were extracted in chloroform–methanol solution according to Bligh and Dyer method [18]. The solution was centrifuged after the addition of 0.9% NaCl, and the lower phase was collected for evaporation. Phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 was added to dissolve the remaining pellet [19]. Hepatic TG and total Chol concentrations were measured using the same commercial kits as those used for sera.

2.5 Liver histology

Livers were removed following euthanasia and fixed overnight in 4% paraformaldehyde in phosphate-buffered saline. The tissues were then transferred to 70% ethanol and embedded in paraffin. Samples were cut into 5-μm sections, and hematoxylin-eosin staining was performed.

2.6 Total pancreatic insulin content

The pancreas was homogenized in 10 mL acid ethanol (165 mM HCl in 75% ethanol) *per* gram of pancreas [20]. The homogenate was incubated overnight at 4°C and then centrifuged at 2000 × *g* for 5 min. The supernatant was collected for measurement of insulin content. Pancreatic insulin content was measured from pancreatic extracts using a radioimmunoassay kit (Linco Research, St. Charles, MO, USA).

2.7 RNA extraction and analysis of mRNA expression

Total RNA was extracted from tissues using TRI reagent (Molecular Research, Cincinnati, OH, USA) according to the manufacturer's instructions. RNA expression was quantified by real-time quantitative PCR using SYBR green PCR reagents (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 7900 HT sequence detection system (Applied Biosystems). The relative quantitation value was calculated by analyzing changes in SYBR green fluorescence during PCR according to the manufacturer's instructions. Data were expressed as $2^{-\Delta\Delta C_T}$ values obtained by normalizing to 18S rRNA and mean ΔC_T values normalized to the 0% BG group. Mouse-specific gene primers were used for fatty acid synthase (FAS) forward 5′-tgcaactgtgcgttagccacc-3′, reverse 5′-tgtttcaggggagaagagacc-3′; peroxisomal acyl CoA oxidase (AOX) forward 5′-cacaatcgccatacgataca-3′, reverse 5′-ctcaggcagttcactcaggt-3′; HMG CoA reductase (HMGCoAR) forward 5′-ggtggtgggaaccttct-3′, reverse 5′-cacgccccttgaacaccta-3′; Chol 7α-hydroxylase (CYP7AI) forward 5′-caagtgtccccctctaga-3′, reverse 5′-actcaatatcatgtagtggtggcaaa-3′; LDL receptor (LDL-R) forward 5′-caggccgatgcattcctgact-3′, reverse 5′-agttcatccgagccattttca-3′; glucokinase (GK) forward 5′-agcagatccacaacatcctaagc-3′, reverse 5′-tcctgcggagcacatatggc-3′; glucose-6-phosphatase (G6P) forward 5′-gaaggccaagagatggtgtga-3′, reverse 5′-tgcagctcttgcggtacatg-3′; phosphoenolpyruvate carboxykinase (PEPCK), forward 5′-cccaggaagtgaggaagtttgt-3′, reverse 5′-ggagccgtcgcagatgtg-3′; 18S rRNA, forward 5′-gtcgtaccactggcattgtg-3′, reverse 5′-ctctcagctgtggtggtgaa-3′.

2.8 Immunoblot analysis

Equal amounts of liver whole lysate protein were separated by 6–10% SDS-polyacrylamide gel electrophoresis, transferred to PVDF membranes, incubated in blocking buffer, and treated with primary antibodies. Rabbit polyclonal antibodies against actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal antibodies against phospho-IRS-1-ser307, insulin receptor substrate (IRS-1), Akt, and phospho-Akt-thr308 were obtained from Cell Signaling Technology (Danvers, MA, USA). Appropriate secondary antibodies were used, and the bands were visualized using ECL Western Blotting Detection Reagents (RPN2106, Amersham, Buckinghamshire, UK) and X-ray film (AGFA, Mortsel, Belgium). Tina 2.0 software (Silk Scientific, Orem, UT, USA) was used for densitometric analysis of immunoreactive bands. Actin was determined for each blot to verify equal protein loading.

2.9 Statistical analysis

All data are expressed as means ± SE. Differences between groups were analyzed by one-way analysis of variance using the SAS statistical analysis program (SAS Institute, Cary, NC, USA). Duncan's multiple range tests were used to determine the significance of differences in group means. Differences between means were considered statistically significant at $p < 0.05$.

3 Results

3.1 Effects of barley containing BG on body weight

Consumption of 2 and 4% BG for 12 wk resulted in 6.4 and 4.5% reductions in body weight, respectively, compared with 0% BG group ($p < 0.05$; Fig. 1A). No significant differences in food intake between the treatment groups were observed (Fig. 1B). Liver weights were reduced by 19 and 18.6% in response to the 2 and 4% BG diets, respectively ($p < 0.05$; Table 2). The combined weights of the epididymal and inguinal adipose tissues tended to be lower in mice-fed BG, but this did not reach statistical significance (Table 2). There were no significant reductions in other organ weights after BG consumption (data not shown).

3.2 Effects of barley containing BG on serum lipid composition

Consumption of BG did not affect levels of serum TG, free fatty acids, total Chol, HDL–Chol, and LDL–Chol after 12 wk of treatment (Table 3).

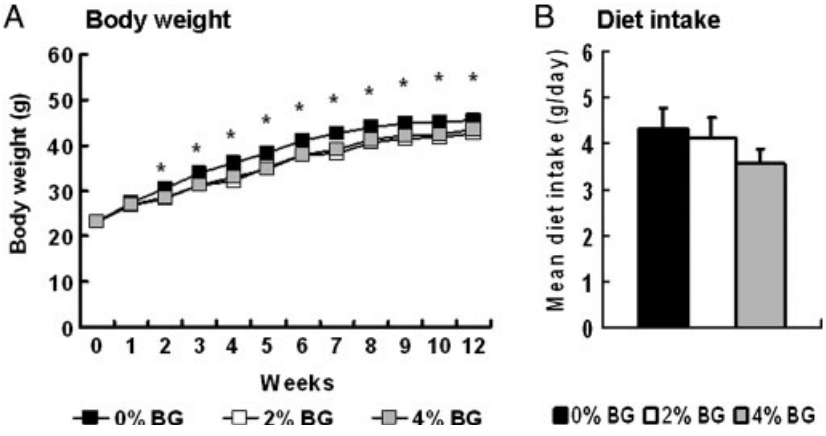

Figure 1. Effect of BG on body weight (A) and food intake (B) in high-fat-fed mice. Seven-week-old mice were provided diets containing 0, 2, or 4% BG for 12 wk. Data are expressed as means ± SE; $n = 12$–13/group. $^{*}p < 0.05$ *versus* 0% BG group.

Table 2. Effect of BG on liver and adipose tissue weight

Group	0% BG	2% BG	4% BG
Liver weight (g)	3.61 ± 0.13[a]	2.91 ± 0.16[b]	2.94 ± 0.16[b]
Liver (% total body weight)	7.93 ± 0.29[a]	6.80 ± 0.29[b]	6.74 ± 0.35[b]
Adipose tissue weight (g)	4.03 ± 0.09	3.83 ± 0.18	3.77 ± 0.09
Adiposity (% total body weight)	8.89 ± 0.29	8.89 ± 0.41	8.65 ± 0.21

Data are expressed as means ± SE; $n = 12$–13/group. Different letters indicate significant difference at $p < 0.05$.

Table 3. Effect of BG on serum lipid composition

Group	0% BG	2% BG	4% BG
TG (mmol/L)	0.57 ± 0.02	0.56 ± 0.05	0.57 ± 0.03
Total Chol (mmol/L)	1.95 ± 0.18	1.84 ± 0.19	1.87 ± 0.21
HDL-C (mmol/L)	0.53 ± 0.05	0.47 ± 0.05	0.47 ± 0.05
LDL-C (mmol/L)	1.27 ± 0.16	1.11 ± 0.19	1.14 ± 0.19
Free fatty acid (mmol/L)	46.1 ± 2.14	49.8 ± 5.88	49.1 ± 3.79

Data are expressed as means ± SE; $n = 12$–13/group.

3.3 Effects of barley containing BG on hepatic lipid content

The content of hepatic TG and Chol in animals fed 2 and 4% BG was significantly decreased compared with in the 0% BG group (Fig. 2). Hematoxylin-eosin staining showed an apparent reduction of lipid vacuoles in the 2 and 4% BG groups compared with the 0% BG group (Fig. 2).

3.4 Effect of barley containing BG on glucose tolerance

Glucose tolerance tests were performed after 11 wk of BG consumption. Consumption of 4% BG significantly increased the glucose response during 2-h glucose tolerance tests compared with the 0% BG group (Fig. 3A). Area under the curve values were significantly reduced in the 2 and 4% BG group (Fig. 3B), which is an indicative of enhanced insulin sensitivity.

3.5 Effect of barley containing BG on serum insulin, the insulin resistance index, GIP, GLP-1, and ghrelin

Consumption of 4% BG markedly reduced serum insulin compared with 0% BG (from 550.1 ± 65.4 to 340.1 ± 43.1 pmol/L, respectively), even though insulin content in the pancreas was not significantly different (Table 4). Consumption of 4% BG significantly reduced serum GIP compared with 0% BG (Table 4), but did not

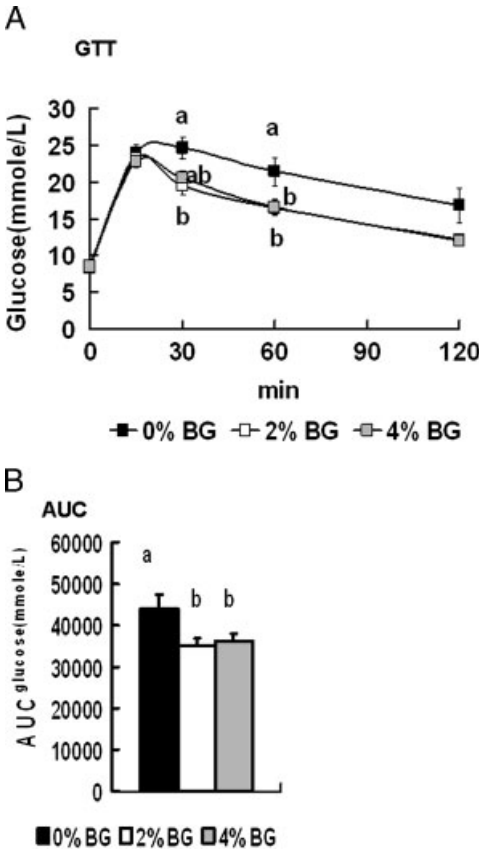

Figure 3. Effect of BG on glucose tolerance in high-fat-fed mice. (A) Glucose tolerance tests were performed in the fasting state. (B) Area under the curve values. Values are expressed as means $\pm$ SE; $n = 12$–13/group.

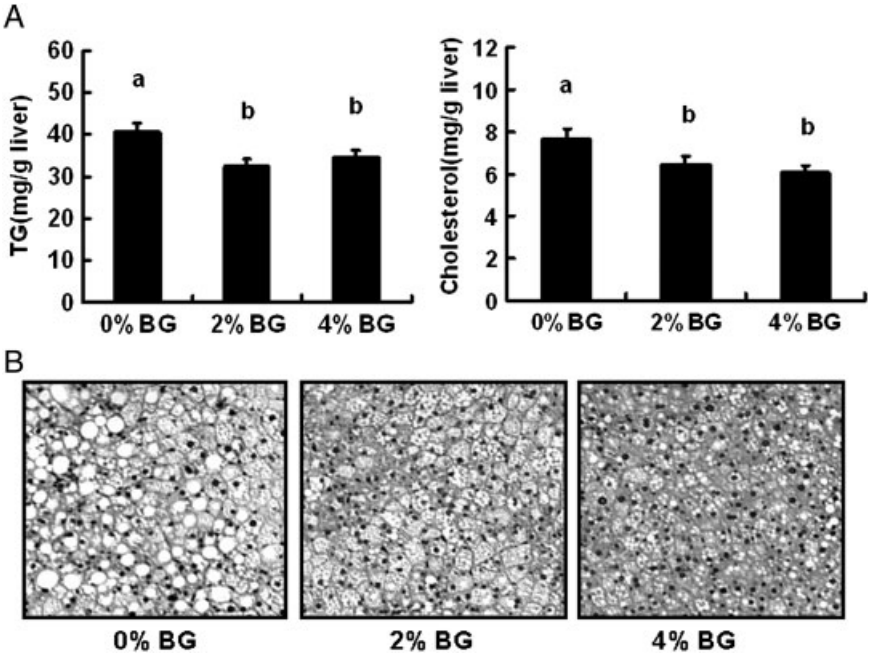

Figure 2. Effect of BG on hepatic lipid content (A) and histology (B) in high-fat-fed mice. Seven-week-old mice were provided diets containing 0, 2, or 4% BG for 12 wk, and livers were removed for hepatic histological examination. (A) 12–13/group; (B) $n = 7$–8/group. The different letters indicate significant differences at $p < 0.05$.

Table 4. Effect of BG on the serum levels of glucose and hormones

Group	0% BG	2% BG	4% BG
Total pancreatic insulin (U/g)	4.07±0.55	4.20±0.47	3.22±0.27
Serum glucose (mmol/L)	13.9±0.68	15.3±0.50	14.5±0.81
Serum insulin (pmol/L)	550.1±65.4[a]	446.1±63.3[ab]	340.1±43.1[b]
HOMA index	48.37±5.39[a]	43.39±6.11[ab]	32.70±4.67[b]
GIP (pg/mL)	140.34±19.99[a]	132.63±15.17[a]	96.90±5.50[b]
GLP-1 (pg/mL)	13.29±2.59	16.90±2.99	13.48±3.21
Ghrelin (pg/mL)	42.47±2.42	49.96±3.37	46.34±3.26

Data are expressed as means±SE; $n = 12$–13/group. Different letters indicate significant difference at $p < 0.05$. HOMA: Homeostasis model assessment insulin resistance index.

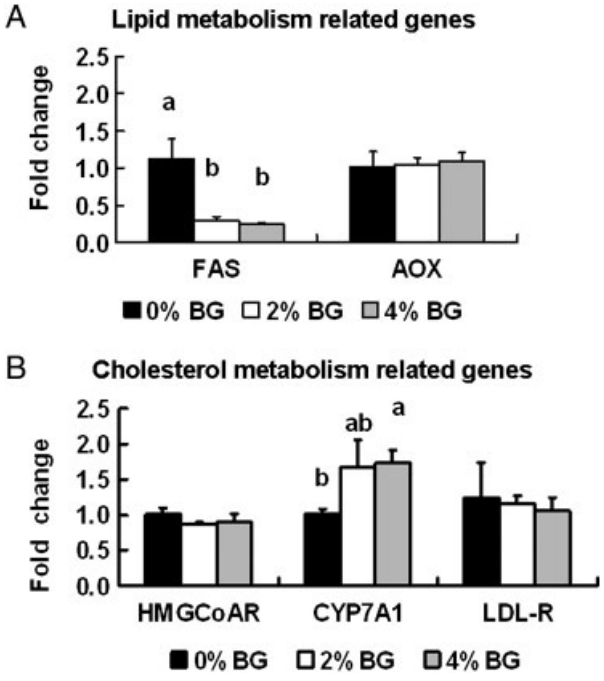

Figure 4. Effect of BG on the hepatic expression of genes related to lipid metabolism. mRNA data are expressed as means±SE; $n = 12$–13/group. The different letters indicate significant differences at $p < 0.05$.

affect serum GLP-1 levels (Table 4). There was no significant effect of BG on serum ghrelin (Table 4).

3.6 Effect of barley-containing BG on hepatic levels of genes related to lipid metabolism

As hepatic fat content was significantly reduced by BG intake, we measured the hepatic mRNA levels of several representative lipid metabolism-related genes. Consumption of 2 and 4% BG significantly decreased fatty acid synthase mRNA, and 4% BG significantly increased the mRNA level of cholesterol-7α-hydroxylase (Fig. 4). However, the mRNA levels of peroxisomal acyl coenzyme A oxidase, HMG CoA reductase, and LDL receptor were not changed by BG consumption.

3.7 Effect of barley-containing BG on the insulin-signaling pathway and genes related to gluconeogenesis

Increased serine phosphorylation of IRS-1 blocks IRS-1 tyrosine phosphorylation by the insulin receptor, which in turn inhibits the activity of the phosphatidylinositol 3-kinase/Akt pathway. The expression of key enzymes of gluconeogenesis, such as glucose-6-phosphatase and PEPCK, could be inhibited by Akt-mediated phosphorylation and nuclear exclusion of the forkhead transcription factor FOXO1 [21]. Consumption of 2 and 4% BG significantly inhibited serine phosphorylation of IRS-1, increased tyrosine IRS-1 phosphorylation, and activated Akt (Figs. 5A and B) in the liver. The mRNA levels of glucose-6-phosphatase and PEPCK were significantly reduced by consumption of 2 and 4% BG, suggesting BG also inhibited liver gluconeogenesis (Fig. 5C).

4 Discussion

In this study, we investigated the effects of barley BG on high-fat-induced body weight gain, insulin resistance, and hepatic glucose and lipid metabolism in mice. It has been hypothesized that the soluble fiber BG may regulate satiety, food intake, and body weight by increasing intestinal viscosity and short-chain fatty acids; however, data concerning these phenomena are limited. In overweight women, satiety was not induced after consumption of barley cereal containing 2 g BG compared with wheat cereal containing 0 g BG [22]. In the present animal study, BG at 2 or 4% concentrations did not significantly decrease food intake or serum ghrelin compared with the 0% BG diet. This implies that replacement of wheat containing insoluble fiber with barley containing soluble BG does not change food consumption or control satiety. However, groups fed 2 or 4% BG demonstrated lower weight gains than the group fed 0%

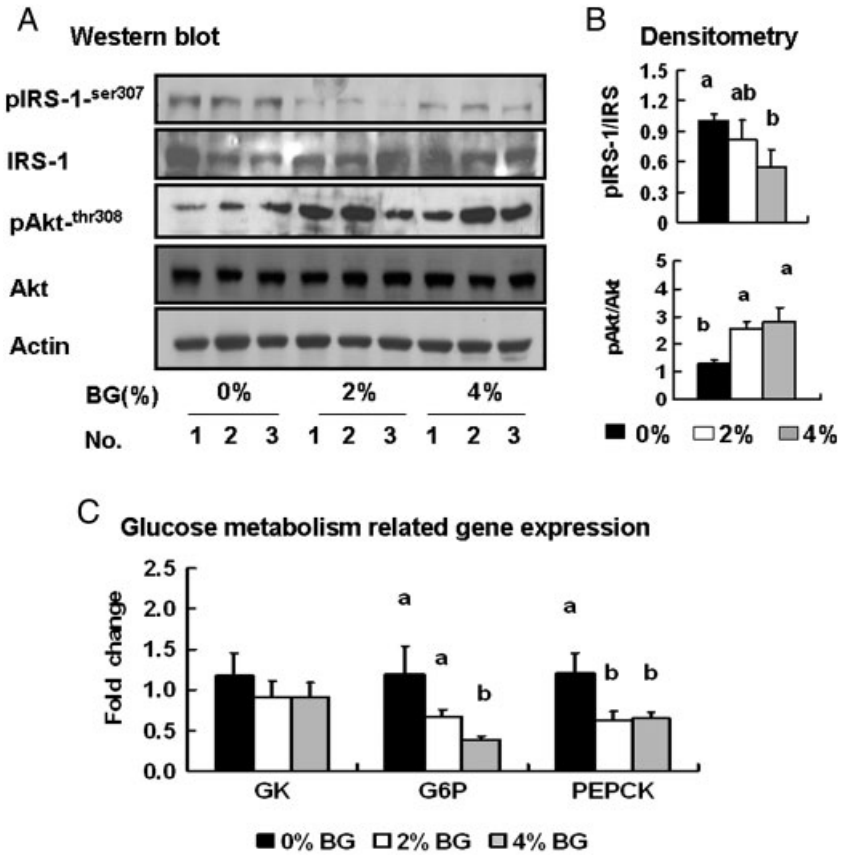

Figure 5. Effect of BG on the insulin-signaling pathway and mRNA levels of genes related to gluconeogenesis in the liver. (A) Representative immunoblots of p-IRS-1 (Ser 307), total IRS-1, p-Akt (thr308), total Akt, and actin. (B) Relative densities of p-IRS-1 (Ser 307), total IRS-1, p-Akt (thr308), total Akt, and actin. (C) mRNA levels of glucose metabolism-related genes. Data are expressed as means $\pm$ SE; $n = 12$–13/group. The different letters indicate significant differences at $p < 0.05$.

BG. We assumed that BG could change the energy absorption and metabolism and then reduced the weight gains.

There were no significant differences in serum postprandial levels of total Chol, LDL-Chol, and HDL–Chol between the whole-grain wheat consumption group (0% BG) and the barley cereal groups (2 or 4% BG) after 12 wk. Nonetheless, some studies have reported that BG has plasma lipid lowering effects [23, 24]. Although those studies suggested that water-soluble fiber effectively lowers serum total and LDL-Chol, no such effects were observed in our study. The reason for this remains unclear. Chronic consumption of a high-Chol diet (*e.g.* 1% Chol) may increase serum LDL-Chol to a level that cannot be ameliorated by BG. Another possibility is that measurement of non-fasting serum lipids may obscure differences between groups. Lastly, changes in serum lipids by increasing BG consumption might be relatively subtle when the same levels of calorie and fiber intake were maintained.

However, in our study, BG consumption significantly improved glucose tolerance and decreased risk factors for metabolic syndrome such as hyperinsulinemia and insulin resistance. Area under the curve values were significantly reduced in the 2 and 4% BG group, which is an indicative of enhanced insulin sensitivity. Furthermore serum insulin levels were markedly reduced in the 4% BG group compared with the 0% BG group. Consequently HOMA index was also reduced in the 4% BG group. GIP and GLP-1 are hormones released by nutrients from the gastrointestinal tract that amplify glucose-induced insulin release. The levels and effects of these incretin hormones are reduced in the diabetic condition [25–27]. The 4% BG diet significantly reduced GIP levels but did not affect GLP-1 levels compared with the 0% BG diet, indicating that GIP levels are related to decreased serum insulin levels. Discordant responses of GIP and GLP-1 to fiber have been reported in both human and animal subjects [28–30]. One reported no effect on GIP by fiber consumption [28]. Postprandial GIP levels were differently responded to the types of fiber. Insoluble fiber rich cellulose-supplemented meal did not affect circulating GIP levels but soluble fiber rich in guar gum reduced serum GIP level [29]. Whereas, the other reported lower levels of insulin and GIP after consumption of the rye bread containing BG than white wheat bread [30]. Similarly to the latter study, we also observed the intake of BG might help mice maintain lower serum postprandial levels of insulin and GIP. BG at 2% concentrations appeared to lower the risk factors for metabolic syndrome, even though the differences were not statistically significant. Taken together, these results suggest that consumption of barley cereals containing more than 2% BG may prevent weight gain and decrease insulin resistance, thereby reducing the probability of acquiring metabolic syndrome.

Impaired insulin clearance is associated with fatty liver [31]. Reductions in weight gain and hepatic lipid levels are associated with enhanced insulin sensitivity [32, 33]. Despite its effects on the levels of serum lipid, high-fat diets containing 2 and 4% barley BG decreased the occurrence of fatty liver, lowered TG, and decreased Chol accumulation in hepatic tissue. The significant reduction in hepatic lipid content after BG consumption possibly contributed to the observed and the reduction in serum insulin. The significant reduction in serum insulin levels by BG consumption could be involved in the regulation genes related to hepatic lipid accumulation. Therefore, we measured mRNA levels of several fatty acid and Chol metabolizing genes. We observed no significant changes in the expression of hepatic genes such as peroxisomal acyl coenzyme A oxidase, HMG CoA reductase, and the LDL-Chol receptor between the control and BG-fed groups. However, barley BG significantly decreased mRNA levels of fatty acid synthase and increased gene expression of cholesterol 7α-hydroxylase in hepatic tissue. The observed decrease in fat accumulation in the liver could be related to these changes. Lowered hepatic TG accumulation is expected likely to the study that suggested the decrease in hepatic TG synthesis by a reduction of fatty acid synthase [34]. Furthermore the concurrent increase of cholesterol 7α-hydroxylase expression by barley BG may shift the liver from Chol synthesis to bile acids synthesis, and reduce the hepatic pool of free Chol. After the excretion of this bile acid to the intestinal lumen, ingested BG binds to bile acids during formation of lipid micelles in the intestinal lumen and thus leads to an increased fecal output of fat and bile acids [9, 35, 36]. Thus, increased hepatic conversion of Chol into bile acids in the liver and the increased excretion of bile acids in the intestine may speed up the decrease in hepatic Chol. Less absorption of fat into the body and increased excretion of bile acids to the lumen could lead to less occurrence of fatty liver. If further study regarding the measurement of fat, and bile acids content and composition in the feces were followed, the beneficiary effect of BG could be strongly verified. Some investigators [37–39] suggested that water-soluble fibers inhibited hepatic Chol synthesis by fermentation products and delayed the absorption of macronutrients leading to increased insulin sensitivity. Water-soluble fibers are fermented in the large bowel and resulted in the production of short-chain fatty acids. Lowered postprandial insulin levels after BG consumption also decreased insulin-stimulated HMG CoA reductase activity and hence Chol synthesis [39]. Although we did not measure the effect of BG on short-chain fatty acids production in the lumen and activity of enzyme related to Chol synthesis, evidences from the reported studies and our findings suggested that BG could decrease hepatic lipid deposit and increase insulin sensitivity *via* modulating many steps between liver and intestine, especially the increases in the process of bile acids metabolism and the decreases in fatty acids synthesis.

The observed reduction in hyperinsulinemia, insulin resistance, and fatty liver in mice-fed barley BG suggests that the existence of mechanisms act directly on insulin signaling. Barley BG feeding significantly reduced serine phosphorylation of IRS-1 and increased phosphorylation of Akt, which would result in increased insulin signaling and likely suppress glucose production by suppressing FOXO-1 function [21]. Moreover, activation of Akt activates glycogen synthase kinase-3 by decreasing the rate of phosphorylation of glycogen synthase; this increases glycogen synthesis and suppresses hepatic glycogenolysis [40, 41]. It is interesting that barley BG significantly decreased the mRNA levels of the glucose-6-phosphatase and PEPCK genes, implying reduced hepatic gluconeogenesis and improved insulin sensitivity. The beneficial effects of barley BG consumption on insulin sensitivity and glucose tolerance could be achieved by reducing *de novo* lipid synthesis, decreasing lipid storage, and improving insulin signaling in hepatic tissues. These data suggest that barley cereals containing greater than 2% BG are required to prevent insulin resistance and weight gain induced by a high-fat diet.

In this study, to mimic human casual dietary intake barley grain and wheat flake were used. With the limited information regarding content of micronutrients such as vitamins, minerals, antioxidants, and other phytonutrients in wheat and barley, there could be some confounding effects due to some variations in the amounts of micronutrients. Further studies with purified BG could help to explore more specific effects BG regarding the lipid metabolism and insulin action in the body.

In summary, consumption of whole-grain barley cereal rich in BG suppressed weight gain and fatty liver in mice fed a high-fat diet. Furthermore, BG consumption improved insulin sensitivity and markedly enhanced glucose tolerance compared with animals fed a high-fat diet supplemented with wheat cereal containing 0% BG. These effects appear to be independent of changes in serum TG and Chol. A significant decrease in GIP was associated with insulin levels, possibly *via* decreased absorption of glucose after barley cereal consumption. The reduction in serum insulin may be related to the decreased hepatic lipid content and the improved hepatic insulin clearance. These findings support a potential link between consumption of whole-grain barley cereals and a reduction in risk factors for metabolic syndrome.

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The authors have declared no conflict of interest.

5 References

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