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Original Article

Bifidobacterium breve strain IDCC 4401 improves dyslipidemia in rat model

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Abstract: Background: Dyslipidemia is a metabolic disease caused by an imbalance in cholesterol metabolism and is a major risk factor for cardiovascular disease. *Bifidobacterium* plays an important role in metabolic diseases such as obesity and diabetes. In most studies aimed at improving the lipid metabolism effects of probiotics, end products related to lipid metabolism were examined, whereas factors related to metabolic processes were not evaluated. Method: We prepared a rat model in which dyslipidemia was induced by feeding of a high-fat diet (HFD) and 25% aqueous fructose solution for 14 days. Compared to the normal diet groups, the HFD and 25% fructose solution significantly increased the levels of cholesterol metabolism-related factors. Result: *Bifidobacterium breve* strain ID-BBR4401 was orally administered for 4 weeks, which increased the production of bile acid (+34.5%, $P < 0.001$) in the feces and low-density lipoprotein-receptor (+44.9%, $P = 0.006$), bile acyl-CoA synthetase (+43.8%, $P = 0.008$), and CYP7α1 (+48.0%, $P = 0.003$) in the liver. These effects promoted cholesterol metabolism and consequently reduced total cholesterol (-35.8%, $P < 0.001$), low-density lipoprotein-cholesterol (-32.7%, $P < 0.001$), triglyceride (-58.3%, $P < 0.001$), very low-density lipoprotein-cholesterol (-33.6%, $P < 0.001$), and ApoB (-55.2%, $P < 0.001$) levels in the serum. Conclusion: Our results suggest that ID-BBR4401 can be used as a probiotic agent to improve dyslipidemia by stimulating cholesterol metabolism in the body.

Keywords: *Bifidobacterium*, cholesterol, dyslipidemia, metabolic disease, probiotic

Introduction

Atherosclerotic cardiovascular disease is among the major causes of death worldwide. Dyslipidemia is one cause of atherosclerotic cardiovascular disease [1]. Cholesterol levels in the blood have very important effects on the pathogenesis of atherosclerosis, and understanding cholesterol metabolism will enable the development of drugs and diets to reduce the risk of cardiovascular disease [2]. Dyslipidemia is generally characterized by increased total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol, as well as decreased high-density lipoprotein (HDL) cholesterol. Dyslipidemia is caused by genetic factors (primary dyslipidemia) or unhealthy lifestyle and metabolic diseases (secondary dyslipidemia) [3]. ApoB is an apoprotein of LDL-cholesterol and very low-density lipoprotein (VLDL) cholesterol which carries fat particles. ApoB is an important risk factor in coronary disease [3, 4].

In the treatment of dyslipidemia, altering lifestyle factors such as diet, exercise, and smoking cessation are important; after these interventions, the risk of cardiovascular disease and level of LDL-cholesterol in the patient are checked to decide whether drug administration is necessary. Statins lower LDL/VLDL-cholesterol levels by inhibiting hepatic synthesis of ApoB-100. Statins are the most widely used drugs for primary treatment and are highly effective for lowering total and LDL-cholesterol [5, 6]. The side effects of statins, although rare, include myopathy and hepatotoxicity, resulting in abdominal pain and digestive disturbances;

some patients can also develop diabetes [6, 7]. Thus, studies are being actively conducted to identify substances to replace or supplement existing drug therapies.

Probiotics are strains that are beneficial to the intestinal environment when they reach the intestines after ingestion. *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* have been identified as probiotics, among which *Bifidobacterium* is the most abundant in the mammalian gut [8, 9]. They are known to play an important role in obesity, diabetes, and metabolic diseases [10]. Interest in *Bifidobacterium* has steadily increased in the food sector, as well as in the medical field as this bacterium has been shown to be effective for treating various diseases. Previous studies reported that *Bifidobacterium* spp improved lipid metabolism [11-14]. However, these studies only identified end-products related to lipid metabolism but did not evaluate factors related to metabolic processes. Therefore, we aimed to examine the lipid metabolism-improving effect of *Bifidobacterium breve* strain ID-BBR4401. This strain was developed in our laboratory and its effects have not been evaluated.

In this study, we prepared a rat model of dyslipidemia by feeding of a high-fat diet (HFD) and 25% aqueous fructose solution for 14 weeks. *Bifidobacterium breve* strain ID-BBR4401 improved the dyslipidemia of lipid metabolism based on biomarker analysis in the dyslipidemia model. The efficacy of *B. breve* strain ID-BBR4401 in improving lipid metabolism associated with bile acids was also confirmed.

Methods

Preparation of ID-BBR4401

ID-BBR4401 was provided by the Ildong Pharmaceutical R&D Center, Ildong Pharmaceutical Co., (Hwa-seong, Korea). The probiotic *B. breve* strain IDCC 4401 (GenBank accession number KP325411) was from our own collection (Ildong Pharmaceutical Co., Ltd., Korea). This strain was anaerobically incubated at 37°C for 16 h. Cultured organisms were heat-killed at 80°C for 1 h and centrifuged (5,000×g, 10 min). The pellets were suspended in distilled water at a 2:1 ratio. The mixture was lyophilized and then milled to a powder, which was used in the experiments.

Animals

We purchased Sprague-Dawley rats (4 weeks, male) from RaonBio (Yongin, Korea) and housed the rats at the Daejeon University (Daejeon, Korea). Animals were housed under controlled conditions of 22°C ± 2°C and 55 ± 15% humidity in a 12-h light/12-h dark cycle. The experimental animal facility and protocols were approved by the Institutional Animal Care and Use Committee of Daejeon University (DJUARB2017-035). All experimental procedures were performed in compliance with the NIH guideline for the care and use of laboratory animals and national animal welfare law in Korea.

Dyslipidemia model

The rats (n = 60) were divided into six groups (n = 10 per group). Dyslipidemia was induced in the five groups by the consumption of a HFD (D12336; Research Diets, New Brunswick, NJ, USA) and with 25% aqueous fructose (Danisco, Copenhagen, Denmark) solution for 14 weeks (from 4 to 20 weeks of age). The remaining group was fed a normal diet (D12337; Research Diets) and water without fructose.

Treatment

The rats were fed a normal diet and water without fructose as vehicle (Veh), HFD and 25% fructose solution as a negative control (NC), HFD and 25% fructose solution with oral administration of 260 mg/kg cholestyramine as a positive control (PC). Cholestyramine is used to treat dyslipidemia because it inhibits cholesterol absorption by sequestrating bile acids [15]. HFD and 25% fructose solution were orally administered with ID-BBR4401 10^7 cells (Low), 10^8 cells (Mid), and 10^9 cells (High) for 4 weeks (from 20 to 24 weeks of age) once per day.

Sample collection

The animals were anesthetized with isoflurane (Piramal Critical Care, PA, USA), and blood was obtained using a syringe and cardiac puncture into clean bottles without anticoagulant. The blood was allowed to stand at room temperature for 30 min and was then centrifuged (3000 rpm, 15 min) to separate the serum. Liver tissue and feces were added to 1 mL of PBS (Welgene, Gyeongsan, Korea) per 0.1 g of the weight. These samples were sonicated and

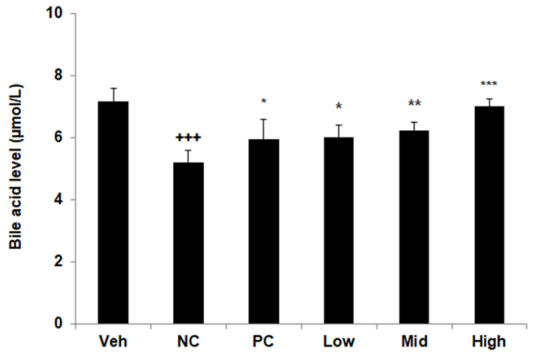

Figure 1. Effect of ID-BBR4401 on bile acid level in feces of HFD and 25% fructose solution-induced dyslipidemia animal model. The measured levels were expressed as the mean ± standard deviation (n = 10/group) (+++P < 0.001 compared to the Veh group, and *P < 0.05, **P < 0.01, ***P < 0.001 compared to the NC group).

centrifuged (3000 rpm, 15 min), after which the supernatants were separated. Separated serum and supernatants were prepared as 200-mL aliquots and stored at -80°C.

Biochemical assays

Total cholesterol, LDL-cholesterol, triglyceride, VLDL, and ApoB were determined in the serum or liver supernatants by using enzyme-linked immunosorbent assay kits from Mybiosource (San Diego, CA, USA). LDL-receptor, bile acyl-CoA synthetase, and cholesterol 7-α hydroxylase (CYP7α1) were determined in the liver supernatants and bile acid was checked in feces supernatants.

Data analysis

All data were expressed as the mean ± standard deviation of 10 animals and analyzed by one-way analysis of variance. The post hoc test was Tukey's honestly significant difference test which was performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). *P* values < 0.05 were considered as statistically significant.

Results

ID-BBR4401 supplementation increased bile acid content in feces

Rats fed the HFD showed a marked decrease in bile acid levels. Oral administration with cholestyramine (260 mg/kg) and ID-BBR4401 (10^7, 10^8, and 10^9 cells) increased bile acid levels in the feces. In all groups fed with ID-BBR4401, the level of bile acid was significantly augmented in a dose-dependent manner compared to in the NC group (**Figure 1**).

Consumption of ID-BBR4401 increased cholesterol-lowering proteins in the liver

Rats fed the HFD showed a marked decrease in cholesterol decomposition factor levels. Oral administration with cholestyramine (260 mg/kg) and ID-BBR4401 (10^7, 10^8, and 10^9 cells) increased LDL-receptor, bile acyl-CoA synthetase, and CYP7α1 levels in the liver. In the ID-BBR4401 High group, the levels of cholesterol-lowering factors were significantly augmented compared to in the NC group. Additionally, the ID-BBR4401 Mid group showed a significant increase in only CYP7α1 levels (**Figure 2A-C**).

ID-BBR4401 supplementation decreased lipid and ApoB content in the liver

Similar to the results in the serum, rats fed the HFD showed a marked increase in lipid metabolism factor levels. Oral administration with cholestyramine (260 mg/kg) and ID-BBR4401 (10^7, 10^8, and 10^9 cells) attenuated total cholesterol, LDL-cholesterol, triglyceride, VLDL, and ApoB levels in the liver. In all groups fed with ID-BBR4401, the total cholesterol and ApoB levels were significantly reduced in a dose-dependent manner compared to those in the NC group. Additionally, the ID-BBR4401 Mid group showed significantly decreased triglyceride and LDL-cholesterol levels. However, the VLDL level did not change in all groups fed with ID-BBR4401 (**Figure 3A-E**).

ID-BBR4401 supplementation decreased lipid and ApoB content in serum

The rats fed the HFD showed a marked increase in lipid metabolism factor levels, indicating that dyslipidemia had occurred. Oral administration with cholestyramine (260 mg/kg) and ID-BBR4401 (10^7, 10^8, and 10^9 cells) attenuated total cholesterol, LDL-cholesterol, triglyceride, VLDL, and ApoB levels in the serum. In all groups fed with ID-BBR4401, the levels of lipid metabolism factors were significantly reduced

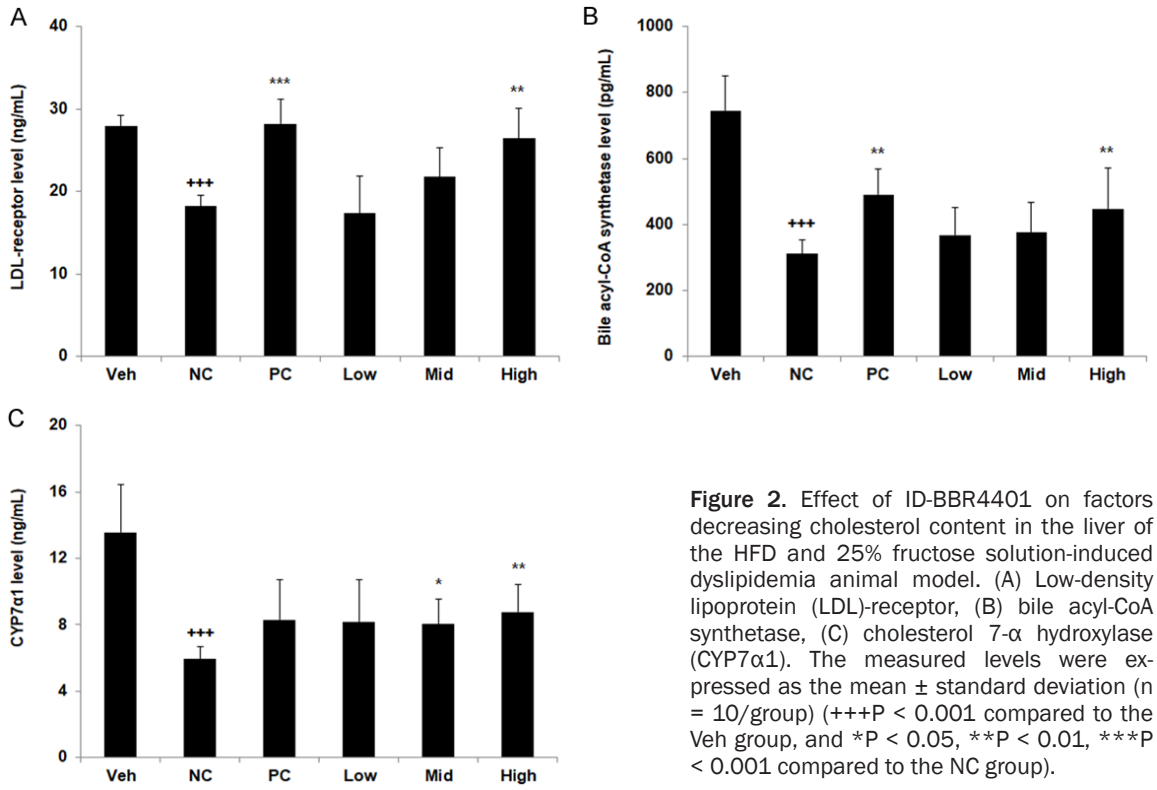

Figure 2. Effect of ID-BBR4401 on factors decreasing cholesterol content in the liver of the HFD and 25% fructose solution-induced dyslipidemia animal model. (A) Low-density lipoprotein (LDL)-receptor, (B) bile acyl-CoA synthetase, (C) cholesterol 7-α hydroxylase (CYP7α1). The measured levels were expressed as the mean ± standard deviation (n = 10/group) (+++P < 0.001 compared to the Veh group, and *P < 0.05, **P < 0.01, ***P < 0.001 compared to the NC group).

in a dose-dependent manner compared to those in the NC group (**Figure 4A-E**).

Discussion

We found that ID-BBR4401 showed similar efficacy to cholestyramine in animal models of dyslipidemia induced by the HFD and 25% aqueous fructose solution. In this study, ID-BBR4401 increased bile acid levels in the feces and up-regulated LDL-receptor, bile acyl-CoA synthetase, and CYP7α1 levels in the liver. These results indicate that ID-BBR400 increases the production of bile acids and metabolism of cholesterol in the body.

ID-BBR4401 significantly attenuated total cholesterol, LDL-cholesterol, triglyceride, VLDL, and ApoB levels in the liver. In addition, the serum level of lipid metabolism factors except for VLDL were reduced compared to those in the NC group. Thus, ID-BBR4401 exhibited significantly improved dyslipidemia and may be effective for treating dyslipidemia.

Bile acid is synthesized by the breakdown of cholesterol in the liver, which is a major pathway for cholesterol metabolism in the liver [16, 17]. Bile acid biosynthesis is regulated by cholesterol 7 alpha-hydroxylase (CYP7α1) in the normal pathway, and overexpression of CYP7α1 promotes the production and secretion of bile acids in the liver and decreases cholesterol levels in the body [18-20]. The LDL receptor binds to LDL, the major cholesterol-transporting lipoprotein in the blood, and transports it through endocytosis into the cell, which then causes degradation [21, 22]. It also increases the expression of LDL receptors and, as a result, reduces LDL cholesterol levels in the blood [23]. The use of non-statin treatment is increasing because of the side effects of statins, among which cholestyramine (Questran) is a bile acid sequestrant that inhibits cholesterol absorption [24].

Cholestyramine promotes the excretion of bile acids, and the liver increases cholesterol degradation to increase the synthesis of bile acids to maintain homeostasis of excreted bile acids [25]. This results in a decrease in the blood cholesterol level.

Other previous studies showed that lipid metabolism was improved when using a combination of various microorganisms and typically

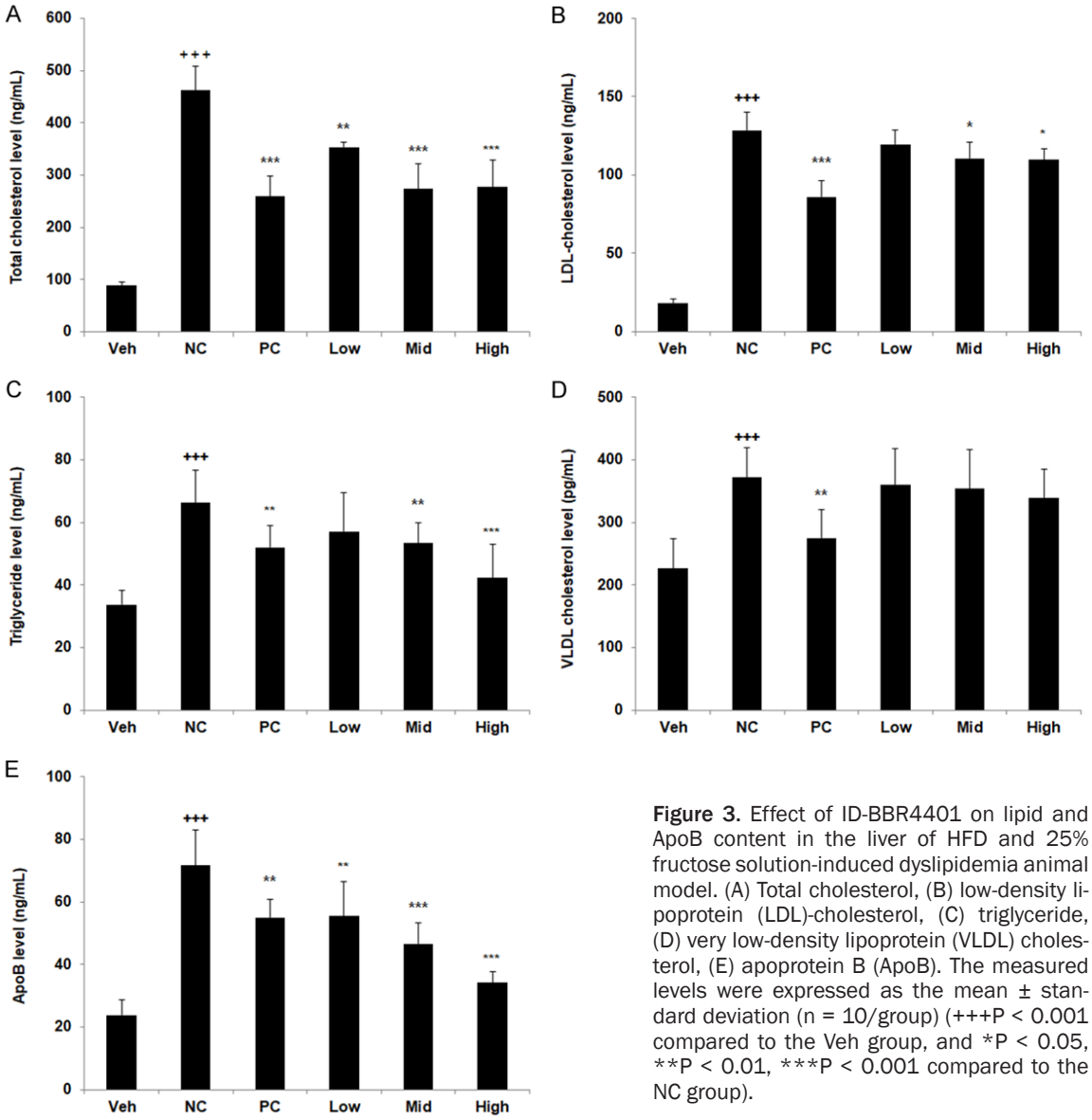

Figure 3. Effect of ID-BBR4401 on lipid and ApoB content in the liver of HFD and 25% fructose solution-induced dyslipidemia animal model. (A) Total cholesterol, (B) low-density lipoprotein (LDL)-cholesterol, (C) triglyceride, (D) very low-density lipoprotein (VLDL) cholesterol, (E) apoprotein B (ApoB). The measured levels were expressed as the mean ± standard deviation (n = 10/group) (+++P < 0.001 compared to the Veh group, and *P < 0.05, **P < 0.01, ***P < 0.001 compared to the NC group).

not with a single microorganism [26-31]. Consistent with these studies, experiments evaluating *Bifidobacterium bifidum* TMC3115 alone resulted in decreased serum total cholesterol and LDL-cholesterol levels [11], and *B. breve* B-3 resulted in decreased serum total cholesterol and triglyceride levels [14]. Additionally, *B. breve* B-3 slightly decreased triglyceride levels and improved HDL cholesterol in healthy pre-obese adults [13].

In our study, we evaluated the detailed effects of *B. breve* ID-BBR4401 on lipid and bile acid metabolism. ID-BBR4401 reduced the levels of total cholesterol by 35.8%, LDL-cholesterol by 32.7%, triglyceride by 58.3%, VLDL-cholesterol by 33.6%, and ApoB by 55.2% in the serum. Thus, *B. breve* ID-BBR4401 was more effective for improving lipid metabolism compared to *B. breve* CBG-C15 or *B. bifidum* TMC3115. ID-BBR4401 also increased the levels of bile acid by 25.6%, LDL-receptor by 31.0%, bile acyl-CoA synthetase by 30.5%, and CYP7α1 by 32.4%. Studies are needed to further evaluate the reduction of cholesterol in the body through bile acid metabolism. These studies may lead to the use of ID-BBR4401 for treating dyslipidemia. ID-BBR4401 improved dyslipidemia symptoms in an HFD and high-fructose induced dyslipidemia model. The

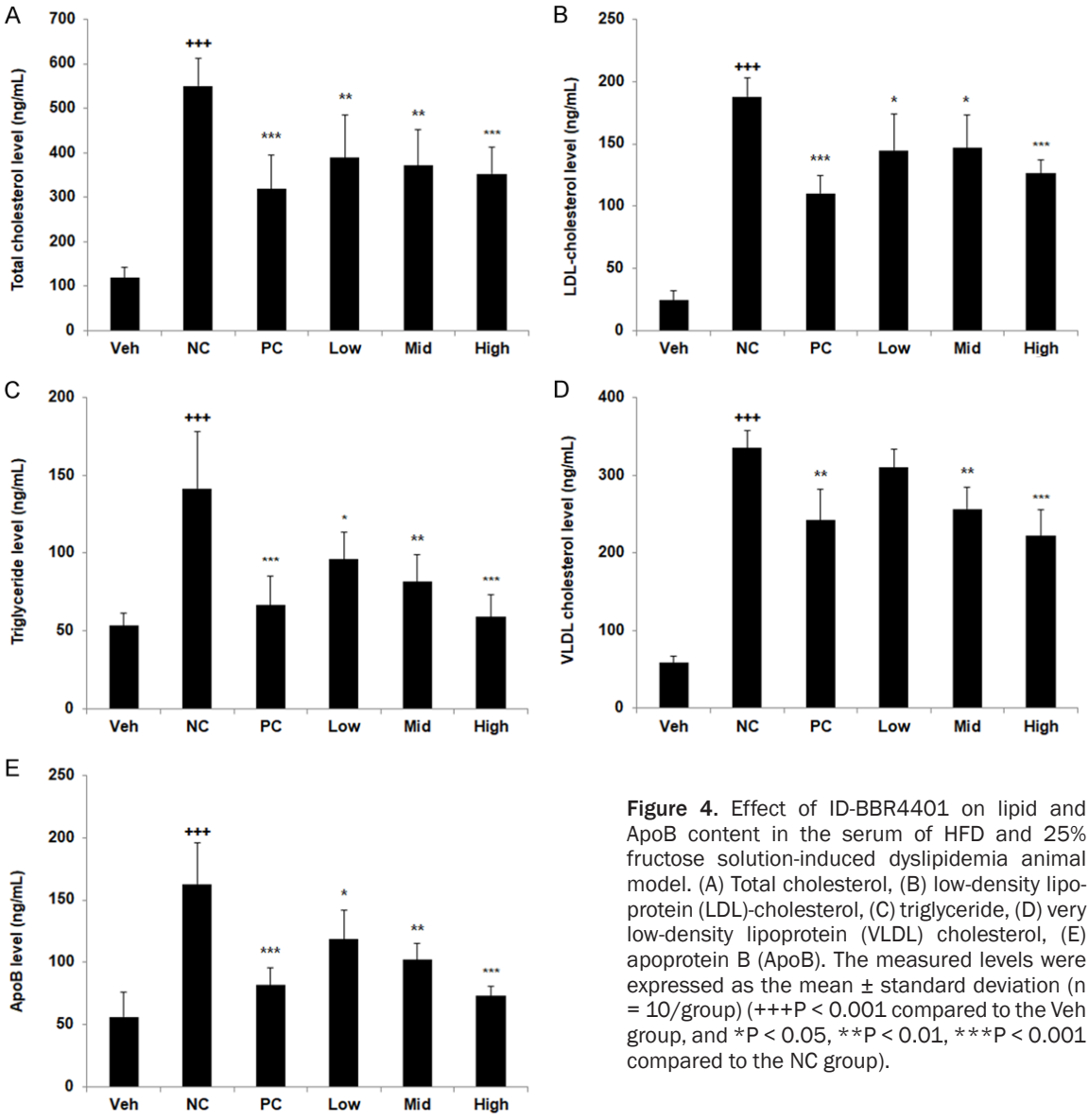

Figure 4. Effect of ID-BBR4401 on lipid and ApoB content in the serum of HFD and 25% fructose solution-induced dyslipidemia animal model. (A) Total cholesterol, (B) low-density lipoprotein (LDL)-cholesterol, (C) triglyceride, (D) very low-density lipoprotein (VLDL) cholesterol, (E) apoprotein B (ApoB). The measured levels were expressed as the mean ± standard deviation (n = 10/group) (+++P < 0.001 compared to the Veh group, and *P < 0.05, **P < 0.01, ***P < 0.001 compared to the NC group).

ID-BBR4401-treated group showed a dose-dependent increase in the cholesterol to bile acid conversion by promoting cholesterol metabolism in the body and reduced lipid metabolism factors in the blood. Therefore, if the efficacy of ID-BBR4401 in improving dyslipidemia symptoms is demonstrated in clinical trials, it may be possible to develop health functional foods containing this organism.

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Disclosure of conflict of interest

None.

Abbreviations

HFD, high-fat diet; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; ApoB, Apolipoprotein B; CoA, coenzyme A; CYP7α1, cholesterol 7-α hydroxylase.

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