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# Antihypertensive Effects of *Lactobacillus*-Fermented Milk Orally Administered to Spontaneously Hypertensive Rats

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**ABSTRACT:** Products fermented with lactic acid bacteria may show antihypertensive effects via substances such as angiotensin I-converting enzyme inhibitor (ACEI) and γ-aminobutyric acid (GABA). It was previously found that milk fermented with *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (101FM) or *Lactobacillus plantarum* NTU 102 (102FM) has ACEI and GABA activities. This study aimed to investigate the antihypertensive effects of 101FM and 102FM orally administered to spontaneously hypertensive rats (SHRs). Eight hours after a single oral administration or after 8 weeks of weekly (chronic) administration, 101FM and 102FM significantly decreased systolic and diastolic blood pressures in the SHRs. Microscopic examination of aortic tissue demonstrated that 101FM and 102FM reduced the disorganization of the media layer. These findings suggest that orally administered 101FM and 102FM have antihypertensive effects, possibly via ACEI and GABA activity, in SHRs. Therefore, 101FM and 102FM may be useful ingredients in physiologically functional foods to prevent hypertension.

**KEYWORDS:** hypertension, lactic acid bacteria, angiotensin I-converting enzyme inhibitor, γ-aminobutyric acid

## INTRODUCTION

Therapeutic control of high blood pressure has a fundamental role in cardiovascular disease prevention.[1] Lactic acid bacteria (LAB)-fermented products may prevent hypertension via substances such as γ-aminobutyric acid (GABA) and angiotensin I-converting enzyme inhibitors (ACEIs). In 2003, Fuglsang et al.[2] reported that administration of milk fermented with *Lactobacillus helveticus* CHCC637 and CHCC641 to rat results in the inhibition of angiotensin II conversion. Milk fermented with *L. helveticus* JCM1004 can produce two angiotensin-converting enzyme inhibitor tripeptides: Val-Pro-Pro and Ile-Pro-Pro.[3] The hypotensive properties of LAB-fermented products have been shown to include these tripeptides as the active ingredients.[4] Furthermore, LAB have been extensively studied for the production of GABA-rich foods and pharmaceuticals.[5,6]

We previously screened *Lactobacillus paracasei* subsp. *paracasei* NTU 101[7] and *Lactobacillus plantarum* NTU 102,[8] isolated from human feces and homemade Korean-style cabbage pickle, respectively. Their resistance to gastric juice and bile salt in the natural environment has been demonstrated, and their various bioactive properties have also been reported as follows: These LAB strains are effective in reducing cholesterol in the blood and liver.[9] Mice fed *L. paracasei* subsp. *paracasei* NTU 101 show up-regulation of the antigen-presenting ability of dendritic cells and expression of natural killer group-2 D molecules, triggering natural killer cell-mediated cytotoxicity; these mice also show significantly increased lymphocyte proliferation and antibody production.[10] Another study demonstrated that *L. paracasei* subsp. *paracasei* NTU 101 significantly enhances innate immunity and induces Peyer's patch-mediated gut mucosal immunity.[11] When the immunomodulatory activity of *L. paracasei* subsp. *paracasei* NTU 101 was investigated in enterohemorrhagic *Escherichia coli* O157:H7-infected BALB/c mice, the lactobacillus down-regulated the expression of proinflammatory cytokines and toll-like receptors on macrophages and chemokines induced by *E. coli* O157:H7 infection.[12] Soy-skim milk fermented with *L. paracasei* subsp. *paracasei* NTU 101 and supplemented with or without *Momordica charantia* is effective in preventing and slowing hyperlipidemia-induced oxidative stress and atherosclerosis.[13] *L. plantarum* NTU 102 induces superoxide dismutase (SOD) and phenol oxidase activities as an immune response in *Litopenaeus vannamei*.[14] Soy-skim milk fermented with *L. paracasei* subsp. *paracasei* NTU 101 or *L. plantarum* NTU 102 is useful for preventing acute gastric ulcers induced by pyloric ligation and acidified ethanol via prostaglandin $E_2$ and significantly enhances SOD activity.[15] Heat-killed cells and cytoplasmic fractions from these LAB strains also have inhibitory effects on cancer cell lines and antioxidant activities in vitro.[16]

In a preliminary study, we found that milk fermented with *L. paracasei* subsp. *paracasei* NTU 101 (101FM) or *L. plantarum* NTU 102 (102FM) has ACEI activity and GABA activity, and optimal production by submerged culture and response surface methodology (RSM) were practiced (data not shown). The aim of this study was to investigate the antihypertensive effects of 101FM or 102FM orally administered to spontaneously hypertensive rats (SHRs).

## MATERIALS AND METHODS

**Preparation of 101FM and 102FM.** One percent of *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 were inoculated into lactobacilli MRS broth and transferred monthly, respectively. To prepare the LAB-fermented milk products, skim milk powder

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(32.4% protein; 54.1% lactose; 0.8% fat) (Fonterra Ltd., Auckland, New Zealand) was reconstituted with demineralized water (w/v = 10%), sterilized (90 °C; 1 h), and inoculated with precultured *L. paracasei* subsp. *paracasei* NTU 101 or *L. plantarum* NTU 102 (w/w = 5%) for 3 or 6 days at 37 or 34 °C. *Lactobacillus*-fermented milk products were lyophilized and stored at 4 °C until further application. Metabolism of glutamic acid can result in the formation of GABA. *L. plantarum* NTU 102 has been demonstrated to produce GABA. To enhance the content of GABA, 1% of monosodium glutamate was added to the culture medium for *L. plantarum* NTU 102.

**Determination of the GABA Concentration.** The supernatants of 101FM and 102FM were filtered, derivatized with *o*-phthalaldehyde (OPA), and analyzed in triplicate by reverse phase high-performance liquid chromatography (RP-HPLC) as previously described.[17,18] The chromatographic eluent pump (PU2089 plus, Jssco Co., Tokyo, Japan), injector (7725i, Rheodyne Co., Robert Park, CA), $C_{18}$ column (25 cm × 4.6 mm inner diameter, 5 $\mu$m, Discovery, Supelco, Inc., Bellefonte, PA), fluorescence detector FL-1 (Rainin Co., Wobum, MA), and 1−16 mg/L GABA (Sigma Chemical Co., St. Louis, MO) were used for calibration.

**Determination of ACEI Activity.** ACEI activity was measured in triplicate by using a previously described assay method[18−20] with some modifications. Forty-five microliter supernatants of 101FM and 102FM were incubated with 45 $\mu$L of 0.1 M borate buffer (pH 8.3) containing 0.3 M NaCl and 0.033 unit/mL ACE (Sigma Chemical Co.) at 37 °C for 10 min. Then, 45 $\mu$L of 3 mM hippuric acid−histidine−leucine (HHL, Sigma Chemical Co.) was added, and the reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped with 150 $\mu$L of 1 N HCl. The reaction solution was then filtered with a 0.45 $\mu$m pore size filter and analyzed by HPLC using a Discovery $C_{18}$ column (25 cm × 4.6 mm). The mobile phase consisting of 50% methanol and 50% water was eluted as a flow rate of 0.6 mL/min. The HHL and hippuric acid were detected by ultraviolet detector (UV2075 plus, Jasco) set at 228 nm. For calibration, inhibitory percentage (*I*%) was calculated as follows: 100% × (Hc − Hs)/(Hc − Hb), where Hs is optical density in the presence of both ACE and 101FM or 102FM, Hc is optical density without the FMs, and Hb is optical density without ACE. Next, ACEI activity (mU/mL) = ACE total activity (mU/mL) × *I*%/sample ($\mu$L), I% between 15 and 85%.

**Animal Experiment and Experimental Schedule.** Seventy-two male SHRs were housed in individual plastic cages and subjected to a 12 h light−dark cycle with 60% relative humidity at 25 ± 2 °C. The animals were given free access to regular rodent chow and water for 1 week to adapt to the new environment. They were weighed and randomly assigned to nine groups of eight animals each before the animal experiment. The experimental protocol was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taiwan University.

Experimental feeding doses of 101FM and 102FM were calculated on the basis of ACE activity and GABA concentrations. Effective doses were estimated on the basis of reference dosages acknowledged for Calpis (2380 U/kg BW/day) as well as GABA (1.36 mg/kg BW/day). For the 101FM intervention trial, experimental animals were assigned to a control group (0 mU ACE), Calpis group (2380 mU ACE), 101FM-0.5× group (1190 mU ACE), 101FM-1× group (2380 mU ACE), or 101FM-2× group (4760 mU ACE); for the 102FM intervention trial, animals were assigned to a control group (0 mg GABA/kg BW/day), GABA group (1.36 mg GABA/kg BW/day), 102FM-0.5× group (0.68 mg GABA/kg BW/day), 102FM-1× group (1.36 mg GABA/kg BW/day), or 102FM-5× group (6.80 mg GABA/kg BW/day). Body weights were recorded once a week until 8 weeks.

At 24 h before sacrifice, all food was removed. The animals were anesthetized and sacrificed by $CO_2$ inhalation. Their whole blood, plasma, and serum samples were collected, prepared, and then stored at −80 °C. Their aortas were excised, rinsed frequently with 0.8% NaCl solution to eliminate any blood, placed in buffered formalin (10%), and fixed for microscopic examination by H&E staining method.

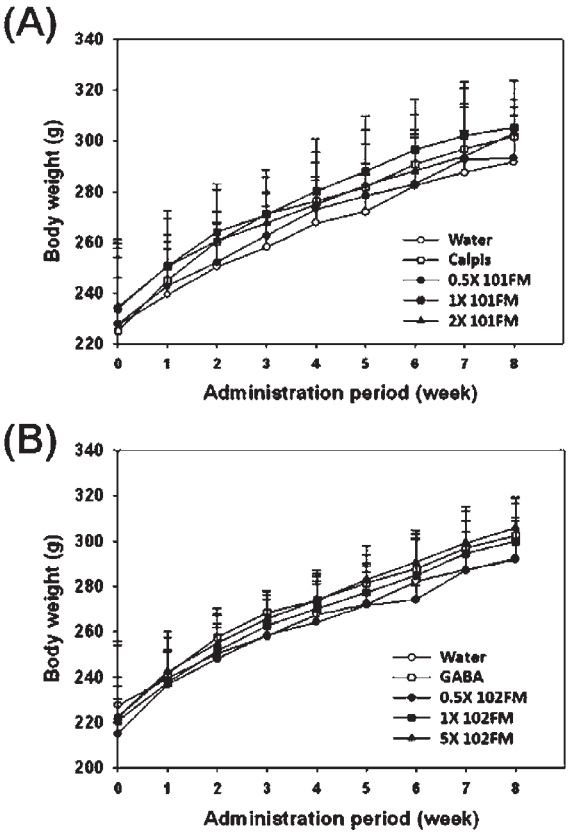


**Figure 1.** Body weight of SHR chronically administered 101FM (A) and 102FM (B). Calpis and GABA were used as positive controls in this experiment. ∗, $p < 0.05$, $p$ estimated by one-way ANOVA and versus the control group ($n = 8$).

**Blood Pressure Measurements.** At 0, 4, 8, and 24 h after a single oral administration of the fermented product solutions, individual rats were gently placed in a constant-temperature holder at 37 ± 1 °C for a few minutes. Then, their systolic and diastolic blood pressures (SBP and DBP, respectively) were measured by tail cuff plethysmography with a photoelectric system (Visitech BP-2000, Napa Place, NC) controlled with a personal computer. The mean value from at least five consecutive readings was used for the calculations. To assess the hypotensive effects of the samples individually, the blood pressures and heart rate were measured 0 and 4 h after a single administration. During the chronic administration, the SBP and DBP were measured before the weekly administration of samples.

**Statistical Analysis.** The data are expressed as the mean ± standard deviation (SD). The statistical significance of the behavioral and biochemical effects was determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS (SPSS, Inc., Chicago, IL), followed by ANOVA with Duncan's test. Differences with $p < 0.05$ were considered to be statistically significant.

## RESULTS

**Changes in BW and Daily Intake.** The average BWs of the SHRs were not significantly different during the experiment (Figure 1). The daily intake of the SHRs increased normally, without a significant difference among the groups during the experiment (data not shown).

**Table 1. Active Ingredients of GABA Content and ACEI Activity in LAB-Fermented Milks**

| | active ingredients from LAB-fermented milk | |
|---|---|---|
| | GABA content (mg/L) | ACEI activity (mU/mL) |
| 101FM[a] | | 85 |
| 102FM[b] | 970 | 93 |

[a] *L. paracasei* subsp. *paracasei* NTU 101 was cultured in 10% skim milk at 37 °C for 3 days. [b] *L. plantarum* NTU 102 was cultured in 10% skim milk with 1% MSG at 37 °C for 6 days.

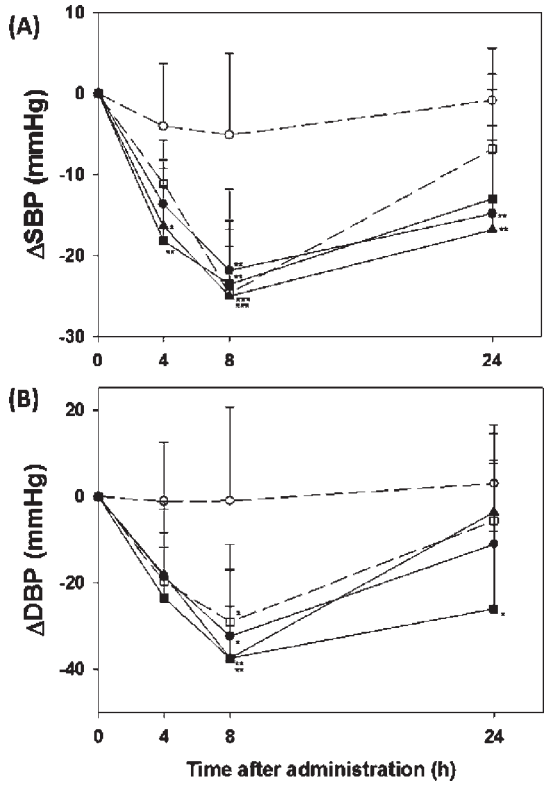


**Figure 2.** Effect of a single oral administration of 101FM product on (A) SBP and (B) DBP in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (control group, ○). The other SHRs were administered a 1-fold dose of Calpis (15 mL/kg/day including 2380 mU of ACEI activity) (Calpis group, □), a 0.5-fold dose of 101FM (14 mL/kg BW/day including 1190 mU of ACEI activity) (101FM-0.5× group, ●), a 1-fold dose of 101FM (28 mL/kg BW/day including 2380 mU of ACEI activity) (101FM-1× group, ■), and a 2-fold dose of 101FM (56 mL/kg BW/day including 4760 mU of ACEI activity) (101FM-2× group, ▲). *, $p < 0.05$, $p$ estimated by one-way ANOVA and versus the control group ($n = 8$).

**GABA Content and ACEI Activity of 101FM and 102FM.** Table 1 shows that 101FM and 102FM had 85 and 93 mU/mL of ACEI activity, respectively. Furthermore, 102FM produced GABA (970 mg/L), which was not detected in 101FM (non-GABA-producing strain).

**Effect of a Single Oral Administration of LAB-Fermented Milk.** Figure 2 displays changes of average resting SBP and DSP (0 h) of SHR upon single administration of 101FM. One-way ANOVA showed independent effects of time in the 101FM groups ($p < 0.05$). Compared with the control group, the 0.5-, 1-, and 2-fold doses of 101FM and Calpis groups showed decreased SBP by 13 mmHg , 18 mmHg ($p < 0.01$), 16 mmHg ($p < 0.05$), and 11 mmHg, respectively, 4 h after administration; furthermore, 8 h after administration, they showed decreased SBP by 22 mmHg ($p < 0.01$), 24 mmHg ($p < 0.01$), 25 mmHg ($p < 0.001$), and 25 mmHg ($p < 0.001$), respectively. There was a significant difference in SBP between the control and 101FM groups by 8 h after dosing. Compared with the control group, the 0.5-, 1-, and 2-fold dose 101FM and Calpis groups also showed decreased DBP by 19 mmHg , 25 mmHg ($p < 0.01$), 18 mmHg ($p < 0.05$), and 20 mmHg, respectively, 4 h after administration; furthermore, 8 h after administration, they showed decreased DBP by 32 mmHg ($p < 0.05$), 38 mmHg ($p < 0.01$), 37 mmHg ($p < 0.01$), and 29 mmHg ($p < 0.05$), respectively. Neither the SBP nor the DBP returned to the baseline value by 24 h after a single oral administration of 101FM.

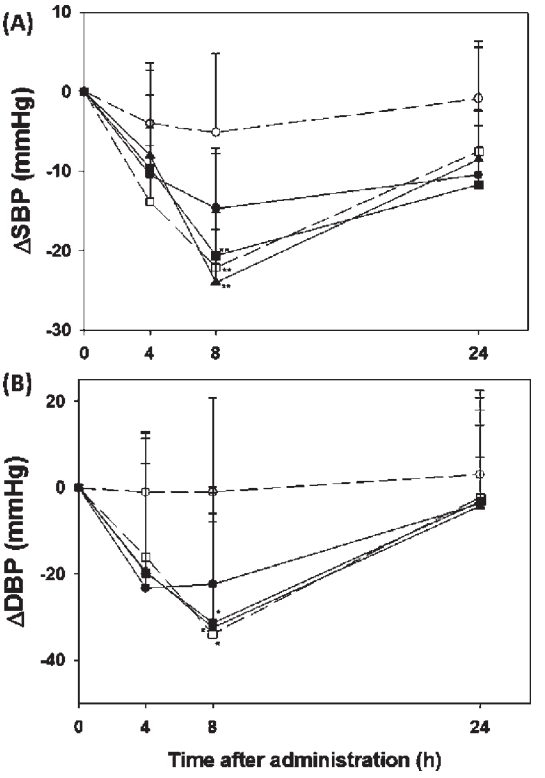


**Figure 3.** Effect of a single oral administration of 102FM product on (A) SBP and (B) DBP in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (control group, ○). The other SHRs were administered a 1-fold dose of GABA (1.36 mg/kg BW/day) (GABA group, □), a 0.5-fold dose of 102FM (0.7 mL/kg BW/day including 0.68 mg of GABA) (102FM-0.5× group, ●), a 1-fold dose of 102FM (1.4 mL/kg BW/day including 1.36 mg of GABA) (102FM-1× group, ■), and a 5-fold dose of 102FM (7 mL/kg BW/day including 6.8 mg of GABA) (102FM-5× group; ▲). *, $p < 0.05$, $p$ estimated by one-way ANOVA and versus the control group ($n = 8$).

Figure 3 shows changes of average resting SBP and DSP (0 h) of SHR upon a single administration of 102FM. One-way ANOVA again showed independent effects of time ($p < 0.05$). Compared with the control group, 4 and 24 h after administration, all dose groups of 102FM and GABA demonstrated no significant effects on the SBP (Figure 2A) and DBP (Figure 2B). However, 8 h after administration, the GABA, 1-fold dose 102FM,

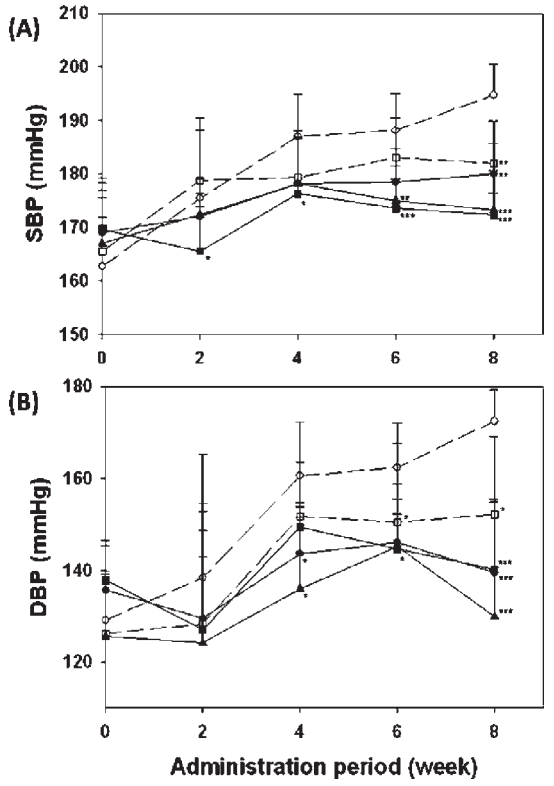


**Figure 4.** Effect of chronic administration of 101FM product on (A) SBP and (B) DBP in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (control group, ○). The other SHRs were administered a 1-fold dose of Calpis (15 mL/kg/day including 2380 mU of ACEI activity) (Calpis group, □), a 0.5-fold dose of 101FM (14 mL/kg BW/day including 1190 mU of ACEI activity) (101FM-0.5× group, ●), a 1-fold dose of 101FM (28 mL/kg BW/day including 2380 mU of ACEI activity) (101FM-1× group, ■), and a 2-fold dose of 101FM (56 mL/kg BW/day including 4760 mU of ACEI activity) (101FM-2× group, ▲). ∗, $p < 0.05$, $p$ estimated by one-way ANOVA and versus the control group ($n = 8$).

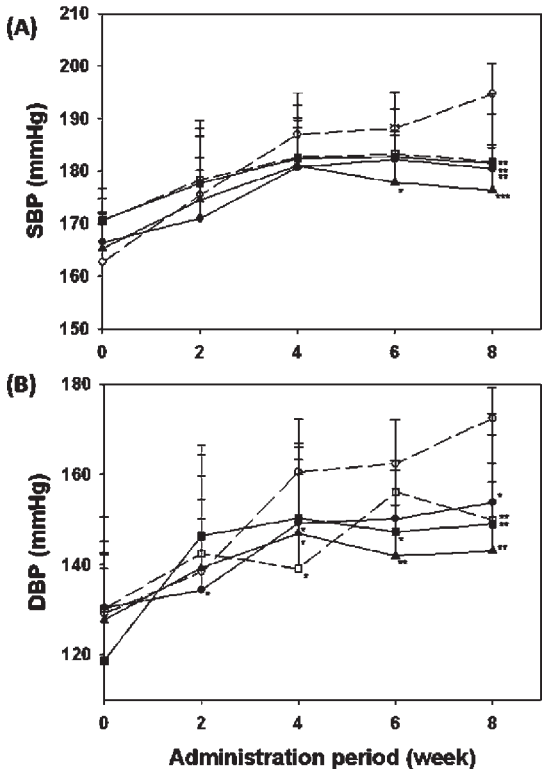


**Figure 5.** Effect of chronic administration of 102FM product on (A) SBP and (B) DBP in SHR. One group of the SHRs was fed a normal diet without the administration of test materials (control group; ○). The other SHRs were administered a 1-fold dose of GABA (1.36 mg/kg BW/day) (GABA group; □), a 0.5-fold dose of 102FM (0.7 mL/kg BW/day including 0.68 mg of GABA) (102FM-0.5× group, ●), a 1-fold dose of 102FM (1.4 mL/kg BW/day including 1.36 mg of GABA) (102FM-1× group, ■), and a 5-fold dose of 102FM (7 mL/kg BW/day including 6.8 mg of GABA) (102FM-5× group, ▲). ∗, $p < 0.05$, $p$ estimated by one-way ANOVA and versus the control group ($n = 8$).

and 5-fold dose 102FM groups had significantly decreased SBP (22, 21, and 24 mmHg, respectively; $p < 0.05$) and DBP (34, 31, and 33 mmHg, respectively; $p < 0.05$). The 0.5-fold dose of 102FM had no effect on the blood pressures upon a single oral administration.

**Effect of Chronic Administration of LAB-Fermented Milk.** A significantly slower increase in SBP was observed 4 and 6 weeks after the start (at 8 weeks of age) of chronic feeding with 101FM and 102FM, respectively, than with the control, and this difference was maintained throughout the experiment. After 8 weeks of feeding, the SBP (Figure 4A) in the control (water), 0.5-fold dose 101FM, 1-fold dose 101FM, 2-fold dose 101FM, and Calpis groups was 195 ± 6, 182 ± 8 mmHg ($p < 0.01$), 172 ± 8 mmHg ($p < 0.005$), 173 ± 8 mmHg ($p < 0.005$), and 182 ± 6 mmHg ($p < 0.01$), respectively; moreover, the DBP (Figure 4B) was 173 ± 4mmHg , 140 ± 7 mmHg ($p < 0.005$), 140 ± 5 mmHg ($p < 0.005$), 130 ± 6 mmHg ($p < 0.005$), and 152 ± 7 mmHg ($p < 0.05$), respectively.

After 8 weeks of feeding, the SBP (Figure 5A) in the control (water), GABA, 0.5-fold dose 102FM, 1-fold dose 102FM, and 5-fold dose 102FM groups was 195 ± 6mmHg , 183 ± 8 mmHg ($p < 0.01$), 180 ± 8 mmHg ($p < 0.01$), 182 ± 8 mmHg ($p < 0.01$), and 176 ± 6 mmHg ($p < 0.005$), respectively, and the DBP (Figure 5B) was 173 ± 5 mmHg , 156 ± 6 mmHg ($p < 0.05$), 154 ± 8 mmHg ($p < 0.01$), 149 ± 4 mmHg ($p < 0.01$), and 143 ± 6 mmHg ($p < 0.01$), respectively.

**Effect of LAB-Fermented Products on Vascular Histology.** Microscopic examination of the aortic sections (Figure 6) showed that the elastin fibers in the 101FM- and 102FM-treated SHRs were significantly straighter than those in the controls (water). The arrows indicate the location of difference.

## DISCUSSION

Angiotensin is part of the renin−angiotensin system, stimulating the release of aldosterone, increasing sodium and water reabsorption, and leading to vasoconstriction and increased blood pressure. Its inactive precursor, angiotensinogen, is converted by renin to inactive angiotensin I and by ACE to angiotensin II. In the milk fermentation process, the proteolytic system of LAB plays the key role because it enables these bacteria to grow in milk, thereby ensuring successful fermentation. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids.[21] Milk is widely considered to be the substrate for LAB to produce ACEI peptides. A number of ACEI peptides have been shown to be effective in lowering the blood pressure of SHRs.[4,22]

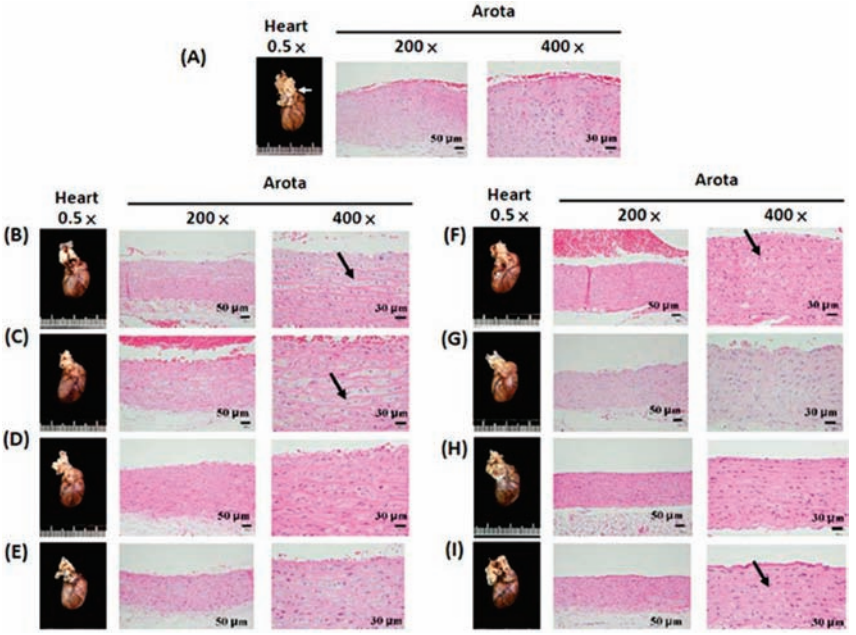


**Figure 6.** Heart appearance and microscopic examination (200× and 400×) of aorta biopsy on experimental SHRs: (A) control group; (B) Calpis group; (C) GABA group; (D) 101FM-0.5× group; (E) 101FM-1× group; (F) 101FM-2× group; (G) 102FM-0.5× group; (H) 102FM-1× group; (I) 102FM-5× group. (The arrow points out the location of difference.)

We found that 101FM and 102FM exhibit ACEI activities of 85 and 93 mU/mL, respectively. In the single oral administration, 101FM and 102FM had significant hypotensive effects on the SBP and DBP of the SHRs. Although the blood pressure returned to the baseline value 24 h after 102FM administration, this phenomenon was not observed 24 h after 101FM administration and should be studied in the future.

GABA is a major inhibitory neurotransmitter in mammalian brains and has several well-known physiological functions, including neurotransmission, induction of hypotensive effects, diuretic effects, treatment of epilepsy, and tranquilizer effects.[23] It has been reported to decrease blood pressure in experimental animals and humans after oral and systemic administration. GABA can be produced by microorganisms including bacteria, fungi, and yeast. Glutamate decarboxylase, the key enzyme for the bioconversion of GABA, has been found in bacteria. Recently, many studies have focused on the GABA-producing LAB such as *L. brevis*,[24] *L. lactis*,[25] *L. paracasei*,[25] *L. delbrueckii* subsp. *bulgaricus*,[25] *L. buchneri*,[26] and *L. plantarum*.[25] Besides discussing the optimal culture condition, Huang et al. indicated that immobilized *L. brevis* cells are stable and efficient for biosynthesis of GABA.[27] Furthermore, a single oral dose of GABA or LAB-fermented product (*L. casei* Shirota and *Lactococcus lactis* YIT 2027) significantly decreases the blood pressure of SHRs from 4 to 8 h after administration.[28] *L. plantarum* NTU 102 has been demonstrated as a novel GABA-producing LAB (970 mg/L of 102FM) that could also produce 93 mU/mL of ACEI activity. For GABA production, *L. plantarum* NTU 102 has the largest amount of GABA production at 6 days (data not shown). In the food industry, the fermentation time is usually <24 h. In the future, we may change the fermentation process to enhance the inoculate volume (w/w = 5−10%).

After 8 weeks of chronic oral administration, both 101FM (0.5-, 1-, and 2-fold doses) and 102FM (0.5-, 1-, and 5-fold doses) performed a statistically significant antihypertensive effect on SBP and DBP. Chronic kidney disease is directly associated with cardiovascular complications. Heart remodeling, including fibrosis, hypertrophy, and decreased vascularization, is frequently observed in renal diseases.[29] The kidneys play a central role in the long-term regulation of blood pressure,[30] and in tissues, the renin−angiotensin system also regulates long-term changes. Thus, the hypotensive effects by the chronic administration in our study may also have inhibited ACE activity. We used animals that were not fed a high-salt diet and water because we attempted to determine the relationship between the LAB-fermented products and genetic hypertension. Importantly, we found that the LAB-fermented products prevented age-induced genetic hypertension.

Other substances in *Lactobacillus* might contribute to the antihypertensive effects observed in this study. Endothelium-dependent relaxation to acetylcholine and other agonists is impaired in animal models of hypertension. Endothelial dysfunction is associated with decreased production of nitric oxide and/or increased production of endothelial-contracting factors, which prevent the development of oxidative stress, such as reactive oxygen species.[31] Current research on the antioxidant ability of LAB has shown that some LAB strains cannot reduce the risk of reactive oxygen species accumulation through food ingestion but can degrade the superoxide anion and hydrogen peroxide.[32] In our study, 101FM and 102FM had antioxidant activity in vitro (data not shown), which may be another way to decrease blood pressure.

Hypertension may also be induced by structural changes in the resistance-vasculature wall.[33] Histopathologically, several modes of remodeling can be distinguished: hypertrophy, hypotrophy, or eutrophy of the vascular wall.[33] The elastin fiber is an important determinant of arterial dispensability.[34] Several models of genetic hypertension show abnormalities of large arteries in terms of their elastin content or structure.[35] As shown in Figure 6, 101FM and 102FM prevented the rearrangement of the vascular wall;

therefore, their hypotensive effects may also depend on decreased peripheral vascular resistance.

In conclusion, our results show that both 101FM (including 85 mU/mL of ACEI activity) and 102FM (including 93 mU/mL of ACEI activity and 970 mg/L of GABA) have antihypertensive effects in SHRs and can reduce the disorganization of the aortic media layer. We consider that these antihypertensive effects depend on GABA and ACEI activity. Therefore, 101FM and 102FM may be useful ingredients in physiologically functional foods for preventing hypertension.

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## ABBREVIATIONS USED

ACEI, angiotensin I-converting enzyme inhibitor; GABA, γ-aminobutyric acid; LAB, lactic acid bacteria; SOD, superoxide dismutase; RSM, response surface methodology; SHRs, spontaneously hypertensive rats; OPA, *o*-phthalaldehyde; RP-HPLC, reverse phase high-performance liquid chromatography; SBP, systolic blood pressure; DBP, diastolic blood pressure.

## REFERENCES

(1) Psaty, B. M.; Lumley, T.; Furberg, C. D.; Schellenbaum, G.; Pahor, M.; Alderman, M. H.; Weiss, N. S. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. *JAMA, J. Am. Med. Assoc.* **2003**, *289*, 2534–2544.

(2) Fuglsang, A.; Rattray, F. P.; Nilsson, D.; Nyborg, N. C. Lactic acid bacteria: inhibition of angiotensin converting enzyme *in vitro* and *in vivo*. *Antonie Van Leeuwenhoek* **2003**, *83*, 27−34.

(3) Pan, D. D.; Luo, Y. K.; Tanokura, M. Antihypertensive peptides from skimmed milk hydrolysate digested by cell-free extract of *Lactobacillus helveticus* JCM1004. *Food Chem.* **2005**, *91*, 123–129.

(4) Nakamura, Y.; Yamamoto, N.; Sakai, K.; Okubo, K.; Yamazaki, S.; Takano, T. Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *J. Dairy Sci.* **1995**, *8*, 777–783.

(5) Komatsuzaki, N.; Shima, J.; Kawamoto, S.; Momose, H.; Kimura, T. Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol.* **2005**, *22*, 497–504.

(6) Xu, J. J.; Jiang, B.; Xu, S. Y. Screening of lactic acid bacteria for biosynthesis of γ-amino butyric acid. *Chinese J. Food Sci. Technol.* **2002**, *10*, 7–10.

(7) Lin, F. M.; Chiu, C. H.; Pan, T. M. Fermentation of a milk-soymilk and *Lycium chinense* Miller mixture using a new isolate of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Bifidobacterium longum*. *J. Ind. Microbiol. Biotechnol.* **2004**, *31*, 559–564.

(8) Pan, T. M.; Chiu, C. H.; Guu, Y. K. Characterization of *Lactobacillus* isolates from pickled vegetables for use as dietary or pickle adjuncts. *Foods Food Ingred. J. Jpn.* **2002**, *206*, 45–51.

(9) Chiu, C. H.; Lu, T. Y.; Tseng, Y. Y.; Pan, T. M. The effects of *Lactobacillus*-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 238–245.

(10) Tsai, Y. T.; Cheng, P. C.; Fan, C. K.; Pan, T. M. Time-dependent persistence of enhanced immune response by a potential probiotic strain *Lactobacillus paracasei* subsp. *paracasei* NTU 101. *Int. J. Food Microbiol.* **2008**, *128*, 219–225.

(11) Tsai, Y. T.; Cheng, P. C.; Liao, J. W.; Pan, T. M. Effect of the administration of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 on Peyer's patch-mediated mucosal immunity. *Int. Immunopharmacol.* **2010**, *10*, 791–798.

(12) Tsai, Y. T.; Cheng, P. C.; Pan, T. M. Immunomodulating activity of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 in enterohemorrhagic *Escherichia coli* O157:H7-infected mice. *J. Agric. Food Chem.* **2010**, *58*, 11265–11272.

(13) Tsai, T. Y.; Chu, L. H.; Lee, C. L.; Pan, T. M. Atherosclerosis-preventing activity of lactic acid bacteria-fermented milk-soymilk supplemented with *Momordica charantia*. *J. Agric. Food Chem.* **2009**, *57*, 2065–2071.

(14) Chiu, C. H.; Guu, Y. K.; Liu, C. H.; Pan, T. M.; Cheng, W. Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum*. *Fish Shellfish Immunol.* **2007**, *23*, 364–377.

(15) Liu, C. F.; Hu, C. L.; Chiang, S. S.; Tseng, K. C.; Yu, R. C.; Pan, T. M. Beneficial preventive effects of gastric mucosal lesion for soy-skim milk fermented by lactic acid bacteria. *J. Agric. Food Chem.* **2009**, *57*, 4433–4438.

(16) Liu, C. F.; Pan, T. M. *In vitro* effects of lactic acid bacteria on cancer cell viability and antioxidant activity. *J. Food Drug Anal.* **2010**, *18*, 77–86.

(17) Herbert, P.; Barros, P.; Ratola, N.; Alives, A. HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives. *J. Food Sci.* **2000**, *65*, 1130–1133.

(18) Wu, C. L.; Lee, C. L.; Pan, T. M. Red mold dioscorea has a greater antihypertensive effect than traditional red mold rice in spontaneously hypertensive rats. *J. Agric. Food Chem.* **2009**, *57*, 5035–5041.

(19) Cushman, D. W.; Cheung, H. S. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem. Pharmacol.* **1971**, *20*, 1637–1648.

(20) Boyd, E. *The Growth of the Surface Area of Human Body*; University of Minnesota Press: Minneapolis, MN, 1935.

(21) Savijoki, K.; Ingmer, H.; Varmanen, P. Proteolytic systems of lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 394–406.

(22) Masuda, O.; Nakamura, Y.; Takano, T. Antihypertensive peptides are present in aorta after oral administration of sour milk containing these peptides to spontaneously hypertensive rats. *J. Nutr.* **1996**, *126*, 3063–3068.

(23) Wong, C. G.; Bottiglieri, T.; Snead, O. C. GABA, γ-hydroxybutyric acid, and neurological disease. *Ann. Neurol.* **2003**, *6*, S3–S12.

(24) Huang, J.; Mei, L.; Xia, J. Application of artificial neural network coupling particle swarm optimization algorithm to biocatalytic production of GABA. *Biotechnol. Bioeng.* **2007**, *96*, 924–931.

(25) Siragusa, S.; De Angelis, M.; Di Cagno, R.; Rizzello, C. G.; Coda, R.; Gobbetti, M. Synthesis of γ-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl. Environ. Microbiol.* **2007**, *73*, 7283–7290.

(26) Cho, Y. R.; Chang, J. Y.; Chang, H. C. Production of γ-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. *J. Microbiol. Biotechnol.* **2007**, *17*, 104–109.

(27) Huang, J.; Le-He, M.; Wu, H.; Lin, D. Q. Biosynthesis of γ-aminobutyric acid (GABA) using immobilized whole cells of *Lactobacillus brevis*. *World J. Microbiol. Biotechnol.* **2007**, *23*, 865–871.

(28) Hayakawa, K.; Kimura, M.; Kasaha, K.; Matsumoto, K.; Sansawa, H.; Yamori, Y. Effect of a γ-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Br. J. Nutr.* **2004**, *92*, 411–417.

(29) Di Marco, G. S.; Reuter, S.; Kentrup, D.; Ting, L.; Ting, L.; Grabner, A.; Jacobi, A. M.; Pavenstädt, H.; Baba, H. A.; Tiemann, K.; Brand, M. Cardioprotective effect of calcineurin inhibition in an animal model of renal disease. *Eur. Heart J.* **2010**online.

(30) Wyss, J. M.; Carlson, S. H. The role of the central nervous system in hypertension. *Curr. Hypertens. Rep.* **1999**, *1*, 246–253.

(31) Chen, T. I.; Lai, C. J.; Hsieh, C. J.; Tsai, K. L.; Yang, K. T. Differences in left ventricular cardiomyocyte loss induced by chronic intermittent hypoxia between spontaneously hypertensive and Wistar-Kyoto rats. *Sleep Breath.* **2010**online.

(32) Lin, M. Y.; Yen, C. L. Antioxidative ability of lactic acid bacteria. *J. Agric. Food Chem.* **1999**, *47*, 1460–1466.

(33) Mulvany, M. J. Small artery remodeling in hypertension. *Curr. Hypertens. Rep.* **2000**, *4*, 49–55.

(34) Dobrin, P. B. Mechanical properties of arteries. *Physiol. Rev.* **1978**, *58*, 397–460.

(35) Boumaza, S.; Arribas, S. M.; Osborne-Pellegrin, M.; McGrath, J. C.; Laurent, S.; Lacolley, P.; Challande, P. Fenestrations of the carotid internal elastic lamina and structural adaptation in stroke-prone spontaneously hypertensive rats. *Hypertension* **2001**, *37*, 1101–1107.