*Food Sci. Biotechnol. Vol. 15, No. 1, pp. 82 ~ 85 (2006)*

Food Science and Biotechnology



# Effects of the Fermented Milk Intake on Human Antioxidant Activity and Blood Alcohol Concentration

**Hae-Seon Nam*, Kyong-Ae Lee[1], Yong-Jin Lee[2], Yong-Bae Kim[3], Sung-Ho Kim[4], Sang-Han Lee[4], Sun-Hwa Lee, Yoon-Jin Lee[4], Ho-Young Kim, Young-Tae Ahn[5], Kwang-Sei Lim[5] and Chul-Sung Huh[5]**

*Department of Clinical Parasitology, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 330-090, Korea*
*[1]Department of Food Science and Nutrition, College of Natural Science, Soon, Asan, Chungnam 330-090, Korea*
*[2]Department of Industrial Medicine, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 330-090, Korea*
*[3]Department of Preventive Medicine, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 330-090, Korea*
*[4]Medical Science and Technology Center, College of Medicine, Soonchunhyang University, Cheonan, Chungnam 330-090, Korea*
*[5]Research & Development Center, Korea Yakult Co., Ltd., Kyunggi 449-901, Korea*

**Abstract** Randomized, double-blinded, placebo-controlled, cross-over clinical trial was performed to assess effects of fermented milk intake on antioxidant activities and blood alcohol levels of 26 healthy volunteers. All subjects received fermented milk (Kupffer's®, n=13) or placebo (n=13) twice daily for 2 weeks. After 3 weeks resting period, subjects under same test but in reverse role. In both tests, fermented milk intake significantly increased total antioxidant status ($p<0.05$) and decreased thiobarbituric acid reactive substance ($p<0.05$) levels compared to before fermented milk intake. Blood alcohol levels of fermented milk intake group were significantly lower than those of placebo group ($p<0.05$).

**Keywords:** blood alcohol level, clinical trial, fermented milk, TBA-reactive substance (TBARS), total antioxidant status (TAS)

## Introduction

Antioxidation involves the removal of free radicals before cell damages. The free radicals are produced as a part of normal cellular metabolism and as a part of host defense system; however, excess production of free radicals from endogenous or exogenous sources may induce uncontrolled tissue damage by reacting indiscriminately. In particular, excess lipid peroxidation reaction transforms the cell wall and destructs signal transduction system, eventually causing diseases or cancers. After alcohol consumption, the alcohol is absorbed rapidly into the bloodstream from the stomach and intestinal tract. The absorbed alcohol is metabolized mostly in the liver into acetaldehyde by alcohol dehydrogenase (ADH), then into acetate by aldehyde dehydrogenase (ALDH) (1). The acetaldehyde suppresses the synthesis of albumin and transferrin in the liver and enhances the production of free radicals, thereby causing oxidative liver cell damages. Some bacteria in the human gut also accomplish alcohol metabolisms. Although humans and other organisms possess antioxidant defense and repair systems, these systems are not effective enough to totally prevent the damages (2). Thus, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damages (3, 4).

The probiotic lactic acid bacteria have been suggested to be helpful to human health and as well as for treatment of some diseases (5, 6). Many researchers reported that some fermented milk and lactic acid bacteria can enhance antioxidative system (7-12) and act on alcohol or acetaldehyde metabolisms (13-15). In particular, some lactobacilli and bifidobacteria are known as scavengers of free radicals (7) and lipid peroxidation product malondialdehyde (MDA) (3). These bacteria are known to have ADH and ALDH activities and can reduce blood alcohol or acetaldehyde level (13-15) in animal experiments. Recent studies showed that fermented milk products such as fermented goat milk and Kefir containing probiotic lactic acid bacteria exert antioxidative effect in human (10) and rat (11, 12). In this study, we examined the effects of fermented milk intake on the serum antioxidant activity and blood alcohol level in human.

*Corresponding author: Tel: 82-41-570-2460; Fax: 82-41-577-2464
E-mail: namhs@sch.ac.kr
Received September 23, 2005; accepted December 26, 2005

## Materials and Methods

**Study design** Randomized, double-blinded, placebo-controlled cross-over clinical trial was conducted over a 7-week period to evaluate the effects of fermented milk intake on antioxidant activities and blood alcohol levels in 26 selected healthy volunteers. All subjects were randomized into two groups. One group (Group A, n=13) received the placebo for 2 weeks in the first test and, after three weeks resting period, followed by the fermented milk consumption for further 2 weeks in the second test. The other group (Group B, n=13) received the fermented milk in the first test and placebo in the second test. All subjects ingested two bottles (150 mL/bottle) of samples per day (morning and evening) during the two test periods. For each test, four blood samples were collected for analysis of total antioxidant status (TAS) and thiobarbituric acid reactive substance (TBARS) at immediately before starting and the last day of the test. At the last day of each test, all subjects drank same amount of alcohol (50 mL,

40% vol), and additional eight blood samples were collected through heparinized angio-catheter just before the drinking to after 4 hr for analysis of the blood alcohol levels (0, 20, 40, 60, 90, 120, 180, and 240 min).

**Samples** All samples for the clinical trial were produced by Korea Yakult Co. Fermented milk used was Kupffer's®, which contains probiotics (HY7401, HY8001, CSG, and CS332), known as lactic acid bacteria having alcohol dehydrogenises activity (14, 15) and restrictive functions against hazardous materials in the gut (9, 16). Kupffer's also contains betaine and multi-vitamin complex (vitamin $B_1$, $B_2$, $B_3$, $B_6$, $B_9$, $B_{12}$, C, and E), among others. Placebo was manufactured as follows: all the effective components of Kupffer's® were extracted. For the purpose of maintaining similar taste, mixed juice concentrate (Northwest Naturals, WA, USA) and high fructose corn syrup (Samyang Jenex, Co., Korea) were mixed.

**Analysis**

**TAS (total antioxidant status)** Total Antioxidant Status assay kit (Randox Lab. Ltd., UK) was used. Plasma (20 μL) was mixed with 1 mL chromogen solution and incubated for 1 min at room temperature. The absorbance ($A_1$) was measured at 600 nm. The substrate solution (200 μL) was then added (3 min incubation), and the absorbance ($A_2$) was measured at the same wavelength. TAS (mmol/L) was determined using the following equation.

$A_2$-$A_1$ = ΔA of sample/standard/blank

$$\text{Total Antioxidant Status ; Factor} = \frac{\text{conc. of standard}}{(\Delta A \text{ blank} - \Delta A \text{ standard})}$$

mmol/L = Factor × (ΔA Blank - ΔA Sample)

**TBARS (thiobarbituric acid reactive substance)** Lipid peroxidation assay kit (Calbiochem, CA, USA) was used with 1,1,3,3-Tetramethoxypropane (TMOP) as the standard. Plasma (200 μL) was added to 650 μL N-methyl-2-phenylindole in acetonitrile solution. After 3-4 sec vortex-mixing, 150 μL methanesulfonic acid solution was added, and the mixture was incubated for 60 min at 45°C. The absorbance was measured at 586 nm and malondialdehyde (MDA) level was calculated using the standard curve.

**Blood alcohol concentration** Cobas Integra system (Integra 400, Roche, Germany) was used to determine the blood alcohol concentrations. After adding 2 μL serum to 120 μL buffer reagent (300 mmol/L 1,3-diamino-2-hydroxy-propane, pH 9.0), 40 μL enzyme reagent (50 mmol/L sodium citrate, 36 mmol/L NAD, and ≥2000 μkat/L ADH) was then mixed. Total volume was adjusted to 245 μL with distilled water, and the absorbance was measured at 378/409 nm, and the blood alcohol concentration (%) was calculated.

**Statistics** The data were collated using Microsoft Excel, and analyzed with the statistical package SPSS Version 12.0. The results were expressed as means and standard deviations. Mean comparisons between groups were analyzed through ANOVA and Student's t-test for independent samples. Two paired samples *t*-tests were performed to compare the means of TAS and TBARS levels of before and after sample intake. The mean changes in blood alcohol concentration were compared both within each group and between groups using repeated measures ANOVA, followed by Bonferroni method for posthoc multiple comparisons.

## Results and Discussion

**General features of subjects** There were no significant differences between the two groups on age, height, weight, and BMI ($p>0.05$) (Table 1).

**Antioxidative effects of fermented milk** In Group A at the first test (placebo intake), no significant changes were observed in TAS and TBARS levels. On the other hand, in the second test (fermented milk intake), TAS significantly ($p<0.05$) increased from 1.05±0.14 to 1.19±0.13 and TBARS significantly ($p<0.05$) decreased from 25.2±4.5 to

**Table 1. General features of 26 healthy subjects**

| | No | Sex (M/F) | Age | Height (cm) | Weight (kg) | BMI (kg/m$^2$) |
|---|---|---|---|---|---|---|
| Group A | 13 | 8/5 | 26.8±5.1 | 165.7±8.4 | 58.9± 9.3 | 21.4±2.4 |
| Group B | 13 | 5/8 | 30.0±5.6 | 167.9±8.2 | 62.3±13.3 | 21.9±3.6 |
| p-value† | | | .588 | .754 | .175 | .211 |
| Total | 26 | 13/13 | 28.4±5.5 | 166.8±8.2 | 60.6±11.4 | 21.7±3.0 |

†: Comparison by Student's *t*-test for independent samples.
Group A: received placebo in the first test and after 3 weeks resting periods then fermented milk (Kupffer's®) in the second test. Group B: received fermented milk (Kupffer's®) in the first test and after 3 weeks resting periods then placebo in the second test.

**Table 2. Changes of TAS values (mmol/L) of the cross-over two tests**

| | First test | | Second test | |
|---|---|---|---|---|
| | 0 week | 2 weeks | 0 week | 2 weeks |
| Group A | .99±0.10 | .96±0.12 | 1.05±0.14* | 1.19±0.13* |
| Group B | 1.01±0.18† | 1.13±0.17† | 1.08±0.17 | 1.07±0.12 |

*,†: $p<0.05$. Comparison by Student's *t*-test for paired samples.
Group A: received placebo in the first test and after 3 weeks resting periods then fermented milk (Kupffer's®) in the second test. Group B: received fermented milk (Kupffer's®) in the first test and after 3 weeks resting periods then placebo in the second test.

**Table 3. Changes of TBARS values (μM) of the cross-over two tests**

| | First test | | Second test | |
|---|---|---|---|---|
| | 0 week | 2 weeks | 0 week | 2 weeks |
| Group A | 23.7±4.7 | 24.9±4.7 | 25.2±4.5* | 21.7±4.5* |
| Group B | 22.2±5.6† | 17.1±6.0† | 22.2±6.3 | 22.2±6.6 |

*, †: $p<0.05$. Comparison by Student's *t*-test for paired samples.
Group A: received placebo in the first test and after 3 weeks resting periods then fermented milk (Kupffer's®) in the second test. Group B: received fermented milk (Kupffer's®) in the first test and after 3 weeks resting periods then placebo in the second test.

21.7±4.5 (Table 2 and 3). In Group B, TAS significantly ($p$ <0.05) increased from 1.01±0.18 to 1.13±0.17 and TBARS significantly ($p$<0.05) decreased from 22.2±5.6 to 17.1±6.0 in the first test (fermented milk intake), whereas in the second test (placebo intake), no significant changes in TAS and TBARS levels were observed (Table 2 and 3). Above results showed that fermented milk intake increased the TAS level and decreased the TBARS level in humans. Recently, several researchers have reported similar results. Han *et al.* (9) reported *L. brevis* HY7401, *L. acidophilus* CSG, and *B. longum* HY8001 in Kupffer's® decreased lipid peroxidation in the mouse liver treated with $CCl_4$, and Ahn *et al.* (12) reported that fermented milk intake (Kupffer's®) improved hepatic antioxidative system in alcohol-treated rats due to antioxidative activities of lactic acid bacteria and ingredients in Kupffer's®. The antioxidative activity of lactic acid bacteria is known to be due to their metal ion-chelating ability, scavenging reactive oxygen species, and reducing activity (8). Many kinds of antioxidants such as melanoidins, glycated proteins, and glucose-glycine reaction products, which have radical-scavenging activity and chelate metal ion, are produced during the manufacturing of fermented milk (17-19). Thus, we thought that various antioxidants, such as probiotics and vitamins, in Kupffer's® exhibited the antioxidative activities in humans.

**Changes of blood alcohol concentration** In the first test, blood alcohol concentration of Group B (fermented milk intake) was lower than that of Group A (placebo intake). In particular, there was a significant difference between Group A (0.010938±0.00719%) and Group B (0.005123± 0.00471%) 40 min after alcohol consumption ($p$<0.05) (Fig. 1). On the other hand, in the second test, results opposite to those of the first test were found. The blood alcohol levels of Group A (Fermented milk intake) were lower than those of Group B (placebo intake), with a significant difference between Group A (0.006885± 0.00490%) and Group B (0.010962±0.00047%) ($p$<0.05) on 40 min data (Fig. 2). These results suggest that fermented milk intake partly affect the human alcohol

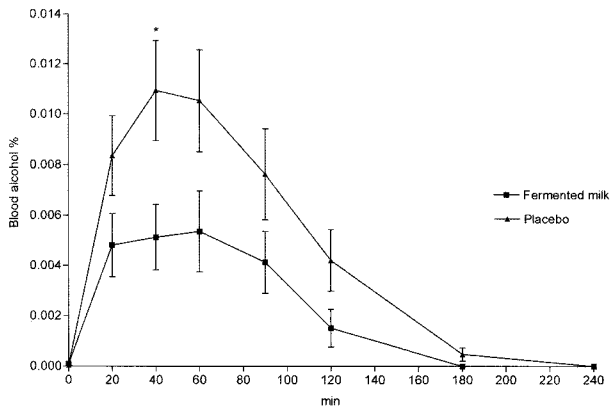


**Fig. 1. Blood alcohol concentrations of two groups (first test) followed by alcohol consumption (50 mL, 40% vol).** Each bar represents standard error of the mean. ■ : fermented milk, ▲ : placebo, *: $p$<0.05.

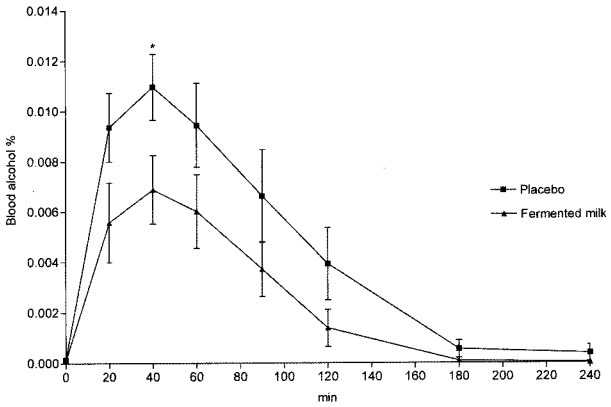


**Fig. 2. Blood alcohol concentration of two groups (second test for cross-over) followed by alcohol consumption (50 mL, 40% vol).** Each bar represents standard error of the mean. ■ : placebo, ▲ : fermented milk, * : $p$<0.05.

metabolism. In general, the alcohol is uptaken from gut and metabolized in the liver. There are some reports that several kinds of intestinal microbes have ADH and ALDH activities (20-23). Furthermore, a possibility of intestinal alcohol metabolism by some lactobacilli and bifidobacteria having ADH and ALDH activities has recently been introduced (13-15). The Kupffer's® has many kinds of functional lactic acid bacteria, among which *L. brevis* HY7401 (14) and *L. fermentum* CS332 (15) are known to possess ADH and ALDH activities. Ahn *et al.* (14) reported that, in experi-ments of potent metabolism of ethanol and acetaldehyde *in vitro* and *in vivo* by *Lactobacillus* strains, *L. brevis* HY7401 exhibited highest ADH and ALDH activities, and its cell intake significantly decreased the serum ethanol level in rats fed ethanol (4 g/kg BW) compared to control groups. In addition, Yang *et al.* (15) reported that breakdown of ethanol and the conversion of acetaldehyde into acetate were observed in mice intestines *by L. fermentum* CS332 after ethanol intake. Based on these data, we suggest that Kupffer's® intake has a beneficial impact on decreasing the alcohol level after alcohol consumption in humans.

## References

1. Jung BS. Metabolic effects of alcohol. Korean J. Soc. Food Sci. Nut. 4: 207-211 (1991)
2. Simic MG. Mechanisms of inhibition of free-radical processed in mutagenesis and carcinogensis. Mutat. Res. 202: 377-386 (1988)
3. Lin MY, Yen CL. Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. J. Agric. Food Chem. 47: 3661-3664 (1999)
4. Ito M, Ohishi K, Yoshida, Y, Yoki W, Sawada H. Antioxidative effects of lactic acid bacteria on the colonic mucosa of iron-overloaded mice. J. Agric. Food. Chem. 51: 4456-4460 (2003)
5. Jayaprakasha HM, Yoon YC, Paik HD. Probiotic functional dairy foods and health claims: an overview. Food Sci. Biotechnol. 14: 523-528 (2005)
6. Kim SJ. Potential probiotic properties of lactic acid bacterial isolated from Kimchi. Food Sci. Biotechnol. 14: 547-550 (2005)
7. Lin MY, Yen CL. Reactive oxygen species and lipid peroxidation product-scavenging ability of yogurt organisms. J. Dairy Sci. 82: 1629-1634 (1999)
8. Lin MY, Yen CL. Antioxidative ability of lactic acid bacteria. J.

Agric. Food Chem. 47: 1460-1466 (1999).
9. Han SY, Huh CS, Ahn YT, Lim KS, Baek YJ, Kim DH. Hepato-protective effect of lactic acid bacteria. J. Microbiol. Biotechnol. 15: 887-890 (2005)
10. Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M. Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br. J. Nutr. 90: 449-456 (2003)
11. Güven A, Güven A, Gülmez M. The effect of Kefir on the activities of GSH-Px, GST, CAT, GSH and LPO levels in carbon tetrachloride-induced mice tissues. J. Vet. Med. 50: 412-416 (2003)
12. Ahn YT, Bae JS, Kim YH, Lim KS, Huh CS. Effect of fermented milk intake on hepatic antioxidative systems in alcohol treated rats. Korean J. Food Sci. Technol. 37: 631-635 (2005)
13. Nosova T, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M. Acetaldehyde production and metabolism by human indigenous and probiotic *Lactobacillus* and *Bifidobacterium* strains. Alcohol Alcohlism 35: 561-568 (2000)
14. Ahn YT, Kim YH, Bae JS, Lim KS, Huh CS, Yang WY, Kim HY, Baek YJ. Effect of *Lactobacillus brevis* HY7401 intake on the serum ethanol concentration in rats. Korean J. Food Sci. Technol. 36: 604-608 (2004)
15. Yang WY, Ahn YT, Lim KS, Huh CS, Baek YJ, Kim HS. Ethanol metabolism by probiotic lactic acid bacteria *in vivo* (abstract no W288). In: 2004 Joint Annual Meeting. July 25-29, America's Center, St. Louis, Missouri, USA. American Dairy Science Association, American Society of Animal Science, and Poultry Science Association, USA (2004)
16. Han SY, Huh CS, Ahn YT, Lim KS, Baek YJ, Kim DH. Hepato-protective effect of lactic acid bacteria, inhibitors of β-glucuronidase production against intestinal microflora. Arch. Pharm. Res. 28: 325-329 (2005)
17. Okamoto G, Hayase F, Kato H. Scavenging of active oxygens species by glycated protein. Biosci. Biotech. Biochem. 56: 928-931 (1992)
18. Terasawa N, Murta M, Homma S. Separation of model melanoidin into components with copper chelating Sepharose 6B column chromatography and comparison of chelating activity. Agric. Biol. Chem. 55: 1507-1514 (1991)
19. Yoshimura Y, Iijima T, Watanabe T, Nakazawa H. Antioxidative effect of Maillard reaction products using glucose-glycine model system. J. Agric. Food Chem. 45: 4106-4109 (1997)
20. Nosova T, Jokelainen K, Kaihovaara P, Jousimies-Somer H, Siitonen A, Heine R, Salaspuro M. Aldehyde dehydrogenase activity and acetate production by aerobic bacteria representing the normal flora of human large intestine. Alcohol Alcohlism 31: 555-564 (1996)
21. Salaspuro M. Microbial metabolism of ethanol and acetaldehyde and clinical consequences. Addict. Biol. 2: 35-46 (1997)
22. Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M. Ciprofloxacin decreases the rate of ethanol elimination in humans. Gut 44: 347-352 (1999)
23. Nosova T, Jokelainen K, Kaihovaara P, Vakevainen S, Rautio M, Jousimies-Somer H, Salaspuro M. Ciprofloxacin administration decreases enhanced ethanol elimination in ethanol-fed rats. Alcohol Alcohlism. 34: 48-54 (1999)