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Clinical trials of kimchi intakes on the regulation of metabolic parameters and colon health in healthy Korean young adults

Hee-Young Kim[a], Kun-Young Park[b,c,*]

[a] Korean Medicine Research Center for Healthy Aging, Pusan National University, Yangsan, Gyeongsangnam-do 50612, Republic of Korea
[b] Department of Food Science and Biotechnology, Cha University, Gyeonggi-do 13488, Republic of Korea
[c] Chongqing Collaborative Innovation Center for Functional Food, Chongqing University of Education, Chongqing 400067, China

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ABSTRACT

Kimchi intakes significantly increased dietary fiber levels in volunteers. Intakes of standardized kimchi (SK) and functional kimchi (FK) tended to reduce body fat mass and percentage. Both kimchi groups showed reduced levels of LDL-C ($p < 0.05$), and increased levels of HDL-C ($p < 0.01$). However, FK intake was associated with reduction of TC, TG, and IL-6 levels, as well as an increase in adiponectin level ($p < 0.05$). In the fecal analysis, the SK and FK groups showed decreased pH, β-glucosidase, and β-glucuronidase levels ($p < 0.01$). Further, intakes of kimchi, especially FK, reduced the abundance of *Firmicutes*, but increased levels of *Bacteroidetes*. In addition, intakes of both types of kimchi increased the abundance of short chain fatty acid production related genera (*Faecalibacterium, Roseburia*, and *Phascolactobacterium*) and reduced *Clostridium* sp. and *Escherichia coli* group counts. Thus, kimchi intakes regulated metabolic parameters and colon health, and FK clearly increased health function in humans.

1. Introduction

Kimchi is a lactic acid bacteria fermented vegetable from Korea and is widely consumed worldwide. There are various types of kimchi, based on the type of main ingredient, including baechu cabbage (*Brassica pekinensis* Rupr.), radish, cucumber, leek, and so on, However, the most common type is known as baechu kimchi (made with baechu cabbage as the main ingredient). Kimchi is rich in dietary fiber, vitamin C, minerals, phytochemicals such as β-carotene, capsaicin, gingerol, chlorophylls and phenols, and lactic acid bacteria (LAB) (Park, 1995; Park & Ju, 2018, chap. 3). Kimchi undergoes spontaneous fermentation at low temperatures (around 5 °C), and many probiotic plant-derived LAB such as *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Weissella koreensis* are predominant (Jung, Kim, et al., 2013; Jung, Lee, et al., 2013). A previous study reported that plant-derived LAB can survive better in the context of gastric acid, bile, and pancreatic secretion than animal-derived LAB, suggesting plant-derived LAB, have greater health-promoting potential (Fumiko et al., 2010). Kimchi also exhibits various health functionalities such as anti-cancer (Park, 1995; Park, Jeong, Lee, & Daily, 2014), antioxidant (Lee et al., 2004), anti-obesity (Cui et al., 2015; Kim et al., 2011), and antidiabetic (Islam & Choi, 2009) activities. Cruciferous vegetables, the main ingredient of kimchi, have health beneficial functions such as improvement of immune system, prevention of cancer and constipation (Park, 1995). Garlic, ginger, red pepper powder, and several active compounds in kimchi (eg. β-sitosterol, thiocyanate, benzyl isothiocyanate, indole compounds), are related to anticancer, antioxidative, and antiobesity effects (Park, 1995; Park & Ju, 2018, chap. 3). The functionalities of kimchi can be enhanced by manipulation of the types and amounts of ingredients and fermentation conditions (temperature, container, etc.). The functioanl kimchi included various active compounds present in mustard leaves (glucosinolates and thioglucoside) (Kim, Kim, & Park, 2007), Chinese pepper(terpenylated coumarins, 4-quinolone alkaloid and integrifoliodiol) (Nguyen et al., 2016), mistletoe(lectin, viscotoxin, flavonoid, triterpene, polysaccharide, and alkaloid) (Ju, Do, Kwon, & Kim, 2009), and probiotics of *Lactobacillus plantarum* (Bong, Jeong, & Park, 2013). Further, adjustment of sub-ingredients can improve the antioxidant, antiobesity, antimutagenic, and anticancer effects of kimchi (Kim, Rhee, & Park, 2000; Park, Cho, & Rhee, 1998).

Metabolic syndrome related diseases such as cardiovascular disease (CVD), type 2 diabetes, dyslipidemia, and hypertension are important health problems worldwide (Choi et al., 2013; Kim et al., 2011). Metabolic syndrome, especially obesity, is highly related with colorectal cancer, and thus metabolic parameters such as serum lipid level are important to prevent both metabolic and colonic diseases (Pais, Silaghi, Silaghi, Rusu, & Dumitrascu, 2009). Most metabolic diseases are

* Corresponding author at: Department of Food Science and Biotechnology, Cha University, Gyeonggi-do 13488, Republic of Korea.
E-mail address: kunypark@cha.ac.kr (K.-Y. Park).

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incurable, and thus prevention is very important. Obesity is the most well-known metabolic disorder (Kim et al., 2011). To prevent occurrence of metabolic diseases, body weight and life style management with a focus on exercise and correct dietary habits is recommended. Specifically, intake of whole grains (Esmaillzadeh, Mirmiran, & Azizi, 2005) and dietary fiber (Papathanasopoulos & Michael, 2010) is an effective way to reduce risk of metabolic disease.

Currently, intestinal disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and colorectal cancer (CRC) have become very common. Most intestinal disorders are related to the environment, inflammatory factors, dietary habits, and human gut microbiota (Ling, Korpela, Mykkänen, Salminen, & Hänninen, 1994). To survey the composition of the human gut microbiota, 16S ribosomal RNA (rRNA) analysis and pyrosequencing methods are commonly used, and fecal samples can be used instead of direction intestinal examination (Tremaroli & Bäckhed, 2012). The human gut microbiota is a complex community of 100 trillion microbial cells consisting of more than 1000 species (Tremaroli & Bäckhed, 2012). The composition of the commensal bacterial microbiota is associated with not only IBD and CRC but also other metabolic diseases (Holmes, Li, Athanasiou, Ashrafian, & Nicholson, 2011). Further, the bacterial enzymes β-glucuronidase, nitroreductase, and β-glycosidase are related with toxicants and carcinogens. Therefore, modification of the colonic microflora and bacterial enzymes by foods is an important topic of study (Ling et al., 1994).

To prevent or improve metabolic and intestinal diseases, consumption of dietary fiber and probiotics is recommended (Albenberg, Lewis, & Wu, 2012; Chmielewska & Szajewska, 2010; Heizer, Southern, & Mcgovern, 2009; Papathanasopoulos & Michael, 2010). Therefore, we hypothesize that kimchi, which contains abundant amounts of dietary fiber and LAB, can improve metabolic parameters and intestinal indicators. For this, we examined the various functionalities of kimchi, including its antiobesity and anticancer effects. We further developed functional kimchi with anti-colorectal cancer activity by adding phytochemical-containing sub-ingredients both *in vitro* and *in vivo* (Kim, Song, Chang, Kang, & Park, 2014; Park & Ju, 2018, chap. 3). We conducted a clinical study to confirm the beneficial effects of newly developed functional kimchi on colon health and other health metabolic parameters.

2. Materials and methods

2.1. Study design and participants

To recruit subjects, we posted an announcement on the Pusan National University homepage, and 28 healthy young adults (age range 18–36 years) were recruited. All subjects finished the study, and the study protocol was approved by the Institutional Review Board (IRB) of Pusan National University (Busan, South Korea) for ethical procedures (Approval number: PNU IRB/2014_24_HR). Informed consent was obtained from all cases and controls enrolled in the study. Consent for fecal sample collection was obtained from subjects on a voluntary basis.

The subjects were randomly assigned into the SK (intake of standardized kimchi) or FK (intake of functional kimchi) group. SK (n = 14) and FK (n = 14) groups each consisted of nine men and five women (total n = 28), and no participants dropped out of the study. Mean age of the SK group was 22.6 ± 2.2 years while that of the FK group was 24.1 ± 4.8 years. Heights of the SK and FK groups were 166.3 ± 8.5 cm and 170.0 ± 6.9 cm, respectively. Body weights of the SK and FK groups were 65.6 ± 10.5 kg, and 70.3 ± 14.7 kg, respectively.

All volunteers consumed 210 g/day (70 g for each meal) of kimchi for 28 days in addition to eating regular meals, except for probiotics. None of the volunteers had consumed any other medicine or probiotics prior to or during the experimental period.

For baseline information on nutritional and exercise levels, 3-day dietary records and 7-day activity records of the subjects were taken. In addition, food intake and exercise records of the last 3-day and 7-day periods were collected. Nutrient intakes were analyzed using the diet analysis program Can-pro (The Korean Nutrition Society, Seoul, Korea).

2.2. Kimchi preparation

SK and FK were prepared according to previously developed recipes (Kim et al., 2014). SK was prepared using the following ingredients: 13.0 g of sliced radish, 3.5 g of red pepper powder, 2.0 g of cut green onion, 1.4 g of chopped garlic, 0.6 g of chopped ginger, 2.2 g of anchovy juice, and 1.0 g of sugar, in 100 g of brined baechu cabbage. FK was prepared using the following ingredients: 11.0 g of sliced radish, 2.5 g of red pepper powder, 2.0 g of cut green onion, 2.8 g of chopped garlic, 0.6 g of chopped ginger, 1.0 g of sugar, 7.5 g of cut mustard leaf, 0.1 g of Chinese pepper, 2.8 g of chopped pear, 5.0 g each of *Lentinus edodes* and sea tangle juice, 0.05% mistletoe extract powder, and 10^6 CFU/g of *Lactobacillus plantarum* PNU in 100 g of brined organic baechu cabbage.

Organic baechu cabbage purchased from Joeunharu Co. (Seoul, South Korea) was used to make FK. Baechu cabbage (for preparing SK) and other sub-ingredients such as red pepper powder, radish, garlic, ginger, green onion, dried *Lentinus edodes,* sea tangle, and anchovy juice were purchased at a local market in Busan, South Korea. In addition, Korean mistletoe extract powder was purchased from Mistle Biotech Co. (Pohang, South Korea). The starter *Lactobacillus plantarum* PNU was isolated from well-fermented kimchi (pH 4.3), and this strain has probiotic and anti-colorectal cancer effects (Bong et al., 2013; Lee, Bong, Lee, Kim, & Park, 2016). Both types of kimchi were fermented at 5 °C. When the kimchi samples reached pH 4.3, each kimchi was individually packed (70 g), and participants consumed three packs per day (210 g/day) for 28 days.

2.3. Anthropometric measurements and blood analysis

Anthropometric measurements and blood collection were conducted on the initial and final days of the experimental period. Height was measured using an anthropometer, and body weight, body mass index (BMI), body fat mass and fat percentage, and skeletal muscle mass were measured using InBody 770 (InBody Co., Ltd, Seoul, South Korea). Blood was collected by a nurse from GC Labs (Yongin, South Korea), and serum triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), AST, ALT, ALP, and insulin were analyzed using serum assay kits (Roche, Germany) and Modular analytics (PE, Roche, Germany). Leptin, adiponectin, interleukin (IL)-6, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, and high-sensitivity C-reactive protein (hs-CRP) were automatically analyzed using immunoassay kits (R&D systems Inc., MN, USA) and γ-counter (COBRA Quantum E5010, Packard, CT, USA) or Microplate Reader (VersaMax, Molecular device, CA, USA) by GC Labs.

2.4. Fecal pH and enzyme activity

One gram of fecal sample (SK: n = 4; FK: n = 4) was homogenized four times with distilled water (w/v) and centrifuged (13,000 rpm) for 30 min. The pH of the supernatant liquid was measured using a pH meter (M220, Corning, MA, USA) (Lee, Choi, & Ji, 1996).

Enzyme activities were measured according to a slightly modified method (Lee et al., 1996). All fecal samples were diluted with 0.1 M sodium phosphate buffer (pH 6.0).

β-glucosidase: 40 μL of 10 μM para-nitrophenyl-β-D-glucoside was treated to 20 μL of sample and reacted at 5 min at 45 °C. To stop the reaction, 800 μL of 0.5 M Na_2CO_3 was added, followed by centrifugation (4000 × *g*) and absorbance measurement (400 nm) using the sample supernatant.

β-glucuronidase: 25 μL of 10 μM phenolphthalein-β-D-glucuronic