Original Article
Clin Nutr Res 2014;3:98-105
http://dx.doi.org/10.7762/cnr.2014.3.2.98
pISSN 2287-3732 · eISSN 2287-3740

CLINICAL NUTRITION RESEARCH

Immunomodulatory Effects of *Kimchi* in Chinese Healthy College Students: A Randomized Controlled Trial

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This study examined the potential immunomodulatory effects of *Kimchi*, a traditional fermented Korean vegetable, in healthy Chinese college students. The four-week clinical-trial (randomized, open-label, prospective, controlled) was followed by a one week wash-out period. Healthy Chinese college students (over 20 years of age with a body mass index of 18.5-23.0 kg/m^2) volunteered for this study. Forty-three students were randomly classified into two groups, *Kimchi* (n = 21, supplemented with 100 g of *Kimchi* per day) or non-*Kimchi* (n = 22, supplemented with 100 g of radish per day, control) groups. During the four-week intervention period, students were asked to maintain their usual diet and activity, and instructed not to take any medications, functional food products, or dietary supplements. Anthropometrics, nutritional intake, and blood immune parameters (lymphocyte subsets, cytokines, and immunoglobulins) were measured before and after the four weeks of intervention. Thirty-nine students (19 in the *Kimchi* group, 20 in the non-*Kimchi* group) finished the study. After the intervention, no significant changes were observed in lymphocyte subsets (T-cell, B-cell, NK cell), pro-inflammatory cytokines (IL-6, TNF-α), anti-inflammatory cytokines (IL-4 and IL-10), and immunoglobulins (Ig A, G, and M) between groups in either the *Kimchi* or non-*Kimchi*. These results suggest that the short-term consumption of *Kimchi* has no immunomodulatory effects in healthy Chinese college students.

Key Words: Immunomodulation, Fermentation, *Kimchi*, Randomized Controlled Trial, Chinese

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Received May 31, 2014
Revised June 30, 2014
Accepted July 7, 2014

Introduction

Kimchi, a traditional fermented vegetable, is an indispensable component of every meal in Korea [1]. There are about 187 kinds of *Kimchi* according to the ingredients and processing methods [2]. *Kimchi* is prepared by various ingredients, such as Chinese cabbage, garlic, onion, and red pepper in the presence of salt [3]. *Kimchi* is currently recognized worldwide as a nutritious and healthy food. Nutritionally, *Kimchi* is a low-calorie food (18 kcal/100 g) and an important source of vitamins, minerals, and fiber [4]. It is also a good source of phytochemicals (e.g., β-sitosterol, glucosinolates, isothiocyanate, indoles, allyl compounds) and probiotic strains (e.g., *Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc mesenteroides*) [5,6]. In regard to these nutrition properties, many functional properties of *Kimchi* have been reported including anti-oxidative activity [7,8], anti-mutagenic and anti-tumor activities [9], antiatherogenic activity [10], and weight-controlling activity [11].

Immune system is very complex and a delicate network associated with the balance between health and disease. Any biologically active substances or food, which can enhance, suppress or modulate the immune system, is called an immunomodulator. Typical examples such as vitamins (A, C, E, B6), minerals (Zn, Fe, Se), probiotics and other foods (tomato, garlic, mushroom, soybean etc.) were explored [12].

Kimchi has been reported to have potential effects on the immune system [13]. Previous studies also reported that *Kimchi* has beneficial effects on stimulating the growth of spleen cells, bone marrow cells, thymus cells, and B cell proliferation in the spleen lymphocytes of rats [14]. Also, it is often believed that taking *Kimch* strengthen the immunity, and has been reported to decrease the risk of acquiring avian influenza (AI), severe acute respiratory syndrome (SARS) and flu.

To our knowledge, no published study has examined the effect of *Kimchi* consumption on the blood immune parameters in a clinical trial setting. Thus the present study was designed to determine assessed immunomodulatory properties of *Kimchi* consumption in the Chinese healthy college student.

Meterials and Methods

Study design

This four-week clinical trial (randomized, open-labeled, prospective, and controlled) was carried out with healthy college students. The study was approved through the Institutional Review Board (IRB, KMC IRB1211-04) of Kyung Hee University Medical Center (Seoul, Korea).

Subjects

Recruitment was performed from July 2012 to September 2012, and the study was completed in November 2012. Forty-three healthy normal weight (BMI 18.5-23.0 kg/m^2) Chinese college students over 20 years-old were enrolled in the study. Subjects suffering from any kind of disease/disorder, have a history of medication in the past few months were excluded. Subjects possessing any underlying conditions which might affect immunity and smokers were also excluded. All subjects were assessed for eligibility and provided written informed consent for participation in the study.

Randomization and intervention

Subjects were randomly assigned, using a computer-generated randomization sequence, into two groups, a *Kimchi* (n = 21, 100 g/day) or a non-*Kimchi* (n = 22, control) group. All eligible subjects were instructed to discontinue *Kimchi* and dishes made with *Kimchi* (e.g., *Kimchi jjigae, Kimchi guk, Kimchi bokkeumbap, Kimchi bosaam*) during one week of washout period.

At the beginning of the study, subjects underwent anthropometric and dietary assessment. A 24-hour dietary record (using food models) by a registered dietitian was used to examine nutrient intake. Nutrient intake was analyzed using the Computer Aided Nutritional Analysis version 4.0 (CAN-pro 4.0, The Korean Nutrition Society, Seoul, Korea). During the four -week intervention period, the *Kimchi*-group consumed 100 g of *Kimchi*/day and the non-*Kimchi* group consumed 100 g of radish/day

Kimchi preparation

Kimchi, prepared with a standardized method by the Nonghyup *Kimchi* factory (Gyenggi, Korea) was provided every other day. The ingredients of *Kimchi* included 92.8 g cabbage preserved in salt, 1.4 g garlic, 2.1 g red pepper powder, 0.4 g ginger, 2.9 g onion, and 0.4 g salt.

Kimchi was fermented at room temperature then stored at 5°C to 10°C in a refrigerator until used. Fermentation was evaluated with a pH meter (Thermo Scientific, USA). The average pH of *Kimchi* was 4.2.

Blood analysis

Blood samples were drawn from the antecubital vein following an overnight fast (12-h) at the beginning and at the end of the study. Blood sample was obtained in ethylenediamine tetra-acetic acid-potassium (EDTA-K_2) anticoagulant tubes and serum-separate tubes (SST). SST was immediately centrifuged (3,000 x g, 4°C, for 10 min) and the supernatant used for analysis.

White blood cell subsets (neutrophils, eosinophils, basophils, monocytes, and lymphocytes) were analyzed using flow cytometry by the Sysmes X- 2,100 hematology analyzer (Sysmes, Kobe, Japan). Lymphocyte subset profiles were analyzed by flow cytometry (Beckman Coulter, USA). The absolute number and percentage of helper T cells (CD4+) and suppressor T cells (CD8+) were automatically calculated. Also, total T cells (CD3+), B cells (CD19+), and natural killer (NK) cells (CD16/56) were quantified using monoclonal antibodies against T cells, B cells, and NK cells, respectively. Antibody-bound cells were counted through flow cytometry using FACS can (Becton Dickinson, Franklin Lakes, NJ).

Serum total immunoglobulins (Ig A, G, and M) were measured using nephelometry by the Siemens Dimension Vista 500 automated analyzer according to the manufacturer's instructions (Siemens Healthcare Diagnostics Inc., Newark, DE, USA). Pro-inflammatory cytokines (IL-6, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) in serum were measured by the Luminex Multiplex Human High Sensitivity Cytokine Panel assay (Millipore, Billerica, USA). All assays were conducted according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using Statistical Package of Social Sciences (SPSS) version 20.0. The categorical variables of the two groups were compared with the Chi-square test, and the data presented as percentages or numbers. Continuous variables of two groups were compared with the independent or paired t-test, and data were presented as mean ± SD. The significance level was defined at $p < 0.05$.

Results

General characteristics

Among 43 healthy subjects two subjects from each group were withdrawn from the study due to personal reasons (moving a far distance away and taking medication). Thirty-nine students (20 in the *Kimchi* and 19 in the non-*Kimchi* groups) were able to finish the study. The average age of the subjects was 21.6 ± 2.1 years. The average height, weight, and BMI were 163.0 ± 6.4 cm, 54.9 ± 6.0 kg, and 20.6 ± 1.5 kg/m^2, respectively. All subjects were in the normal range and no differences were observed between the groups.

Daily nutrition intake

The daily nutrient intake of both groups was compared with the Dietary Reference Intake for Koreans (KDRIs, 2010) (Figure 1). The average caloric intake was 80% of KDRIs and the intake of vitamins (B2, C) and minerals (Ca, K) were between 70 and 90% of KDRIs in both groups. There were no significant differences in the dietary intake of calories, proteins, vitamins, and minerals (except for Vitamin B6) between the groups during the study period.

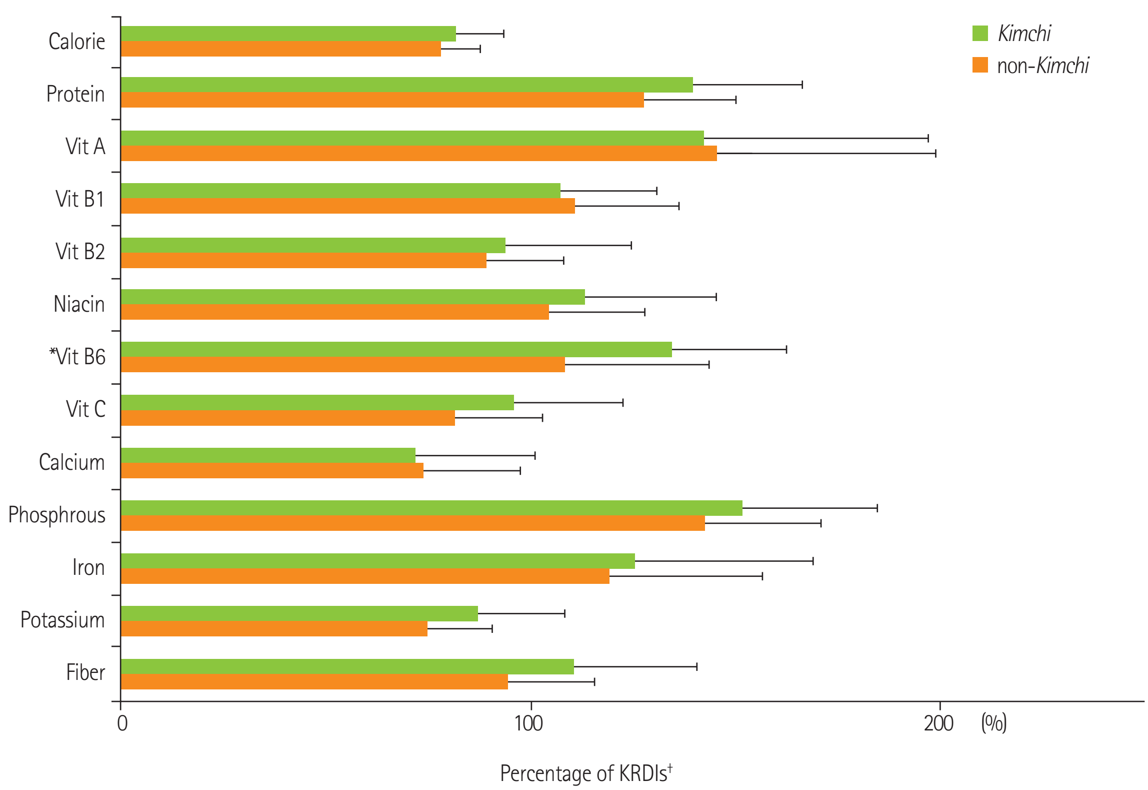

Figure 1. Comparison of nutrient intakes with KDRIs†. *Significant differences were shown between groups by independent t-test at $p < 0.05$. †KDRIs: dietary reference intake for Koreans, 2010.

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White blood cell subset and immunoglobulin

The blood levels of white blood cell subsets (neutrophils, lymphocytes, monocytes) and immunoglobulins (Ig A, G, M) are given in Table 1. The levels of WBC subsets were not different between two groups at baseline and after 4 weeks. All values were in the reference range, neutrophils (50-80%), eosinophils (0-5%), basophils (0-2%), monocytes (2-10%), and lymphocytes (25-50%) [15].

The levels of Ig A and Ig G in the *Kimchi* group at baseline (194.6 ± 66.9 and 1207.1 ± 193.1 mg/dL) and after 4 weeks (194.1 ± 74.2 and 1173.5 ± 172.9 mg/dL) were significantly lower than the non-*Kimchi* group (baseline, 270.5 ± 74.5 and 1321.1 ± 105.2 mg/dL; after 4 weeks, 269.3 ± 75.5 and 1302.3 ± 132.9 mg/dL) ($p < 0.05$). These parameters were not significantly changed during the study period. Levels of Ig M were not different between two groups as well as between two periods.

Lymphocyte subsets

The levels (%) of lymphocyte subsets (T, B and NK cells) at baseline and after four weeks are given in Figure 2. Total T-cells and B-cells were not different between groups and was not significantly changed during the four weeks of intervention. Helper T cells at baseline and after four weeks in the *Kimchi* group (40.4 ± 6.5% and 40.8 ± 5.5%, respectively) were significantly higher than those of the non-*Kimchi* group (36.3 ± 5.5% and 35.8 ± 6.5%, respectively). NK cells at baseline and after four weeks in the *Kimchi* group (11.7 ± 2.8% and 10.6 ±

Table 1. Blood levels of WBC subsets and immunoglobulins of the subjects

		Kimchi (n = 19)	non-*Kimchi* (n = 20)	p value
Neutrophil, %	Baseline	57.8 ± 8.4	57.7 ± 6.3	0.952
	After 4 weeks	59.2 ± 8.2	58.5 ± 7.3	0.777
	Change	1.4 ± 7.3	0.8 ± 6.9	0.807
Eosinophils, %	Baseline	1.5 ± 0.8	1.3 ± 0.6	0.323
	After 4 weeks	1.4 ± 0.8	1.4 ± 0.6	0.854
	Change	-0.1 ± 0.5	0.0 ± 0.5	0.264
Basophils, %	Baseline	0.4 ± 0.2	0.3 ± 0.2	0.673
	After 4 weeks	0.4 ± 0.2	0.4 ± 0.2	0.519
	Change	0.0 ± 0.2	0.0 ± 0.2	0.767
Monocyte, %	Baseline	6.5 ± 1.2	6.3 ± 1.4	0.643
	After 4 weeks	6.9 ± 2.3	6.6 ± 1.6	0.686
	Change	0.4 ± 2.0	0.3 ± 1.6	0.895
Lymphocyte, %	Baseline	33.9 ± 8.0	34.2 ± 5.9	0.898
	After 4 weeks	32.3 ± 7.1	33.2 ± 7.1	0.685
	Change	-1.6 ± 6.4	-1.0 ± 6.7	0.763
Ig A, mg/dL	Baseline	194.6 ± 66.9[†]	270.5 ± 74.5	0.002
	After 4 weeks	194.1 ± 74.2[†]	269.3 ± 75.5	0.004
	Change	-0.5 ± 11.4	-1.2 ± 14.3	0.882
Ig G, mg/dL	Baseline	1207.1 ± 193.1[†]	1321.1 ± 105.2	0.037
	After 4 weeks	1173.5 ± 172.9[†]	1302.3 ± 132.9	0.018
	Change	-33.6 ± 68.6	-18.8 ± 55.9	0.482
Ig M, mg/dL	Baseline	128.8 ± 56.5	154.9 ± 46.8	0.137
	After 4 weeks	126.8 ± 53.5	153.1 ± 47.0	0.121
	Change	-2.0 ± 8.2	-1.8 ± 12.1	0.959

Values are expressed as means ± SD.
[†]Values are significantly different between two groups by independent t-test at $p < 0.05$.

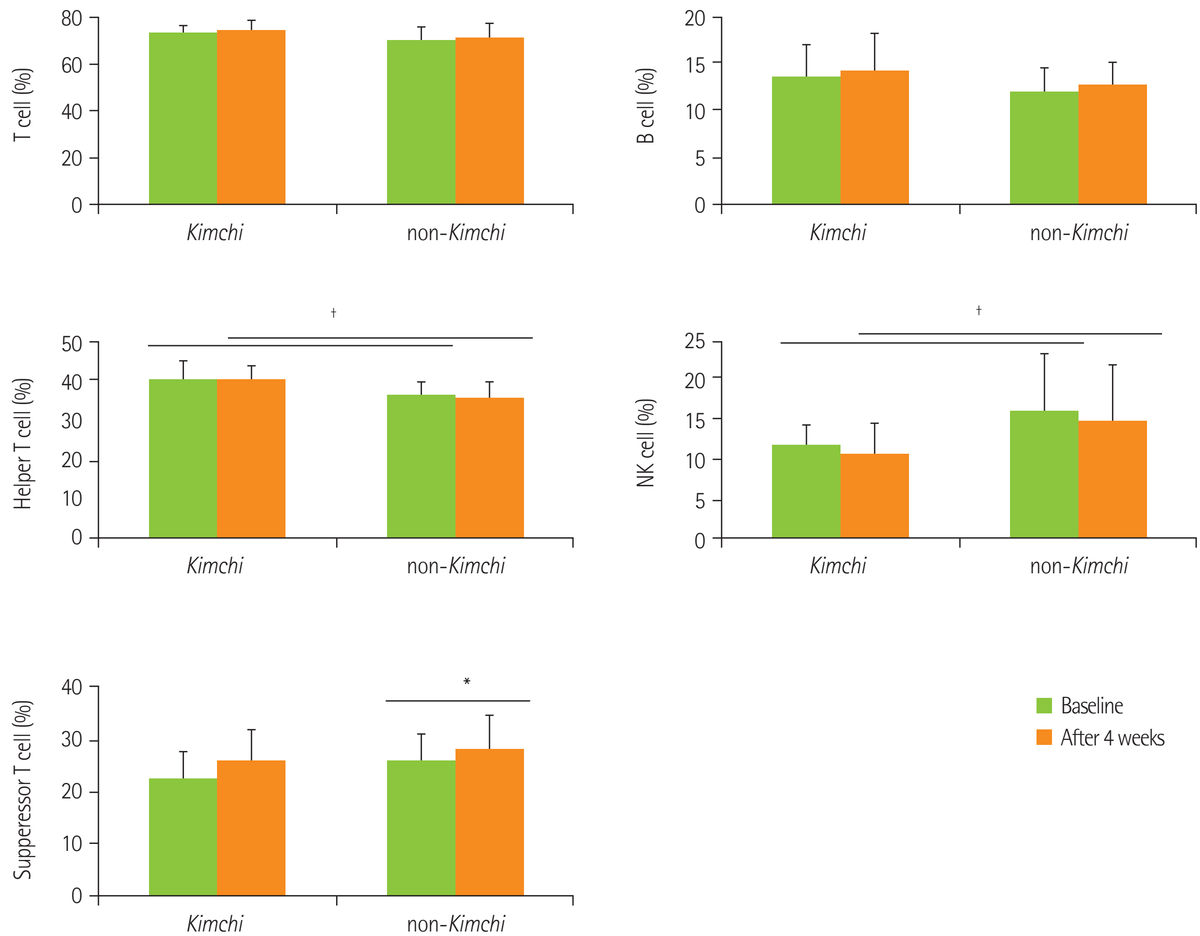

Figure 2. Lymphocyte subsets (T, B, and NK cell) at baseline and after 4 weeks. *Values are significantly different from the baseline values by paired t-test at $p < 0.05$. †Significant differences were shown between the groups by independent t-test at $p < 0.05$.

3.9%, respectively) were significantly lower than those in the non-*Kimchi* group (15.9 ± 7.0% and 14.7 ± 6.8%, respectively) ($p < 0.05$). These values were not significantly changed after four weeks of intervention in both experimental groups.

Suppressor T cells were not different between two groups at baseline. However, after four weeks, the level of suppressor T-cells were significantly increased in the non-*Kimchi* group (from 26.4 ± 5.5% to 28.8 ± 6.7%) but not in the *Kimchi* group (from 23.2 ± 5.2% to 26.5 ± 5.9%).

Pro- and anti-inflammatory cytokines

Blood levels of pro-inflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokines (IL-4 and IL-10) are shown in Figure 3. The levels of TNF-α and IL-6 were not different between the groups; however values were significantly decreased in the *Kimchi* group (TNF-α; from 5.5 ± 1.7 to 4.7 ± 1.6 pg/mL, IL-6; from 0.5 ± 0.2 to 0.4 ± 0.2 pg/mL) and in the non-*Kimchi* group (TNF-α; from 5.3 ± 1.6 to 4.5 ± 1.6 pg/mL, IL-6; from 0.5 ± 0.2 to 0.4 ± 0.1 pg/mL) ($p < 0.05$).

The levels of anti-inflammatory cytokines, IL-4 and IL-10, in *Kimchi* and non-*Kimchi* groups were not significantly different. However, in the *Kimchi* group, the levels of IL-4 was significantly increased (from 0.7 ± 0.7 to 3.9 ± 5.7 pg/mL) however, IL-10 was not significantly changed (from 0.6 ± 0.4 to 0.5 ± 0.4 pg/mL).

Discussion

The present study was conducted to evaluate the potential immunomodulatory effects of *Kimchi* on selected immunological measure in healthy Chinese college students.

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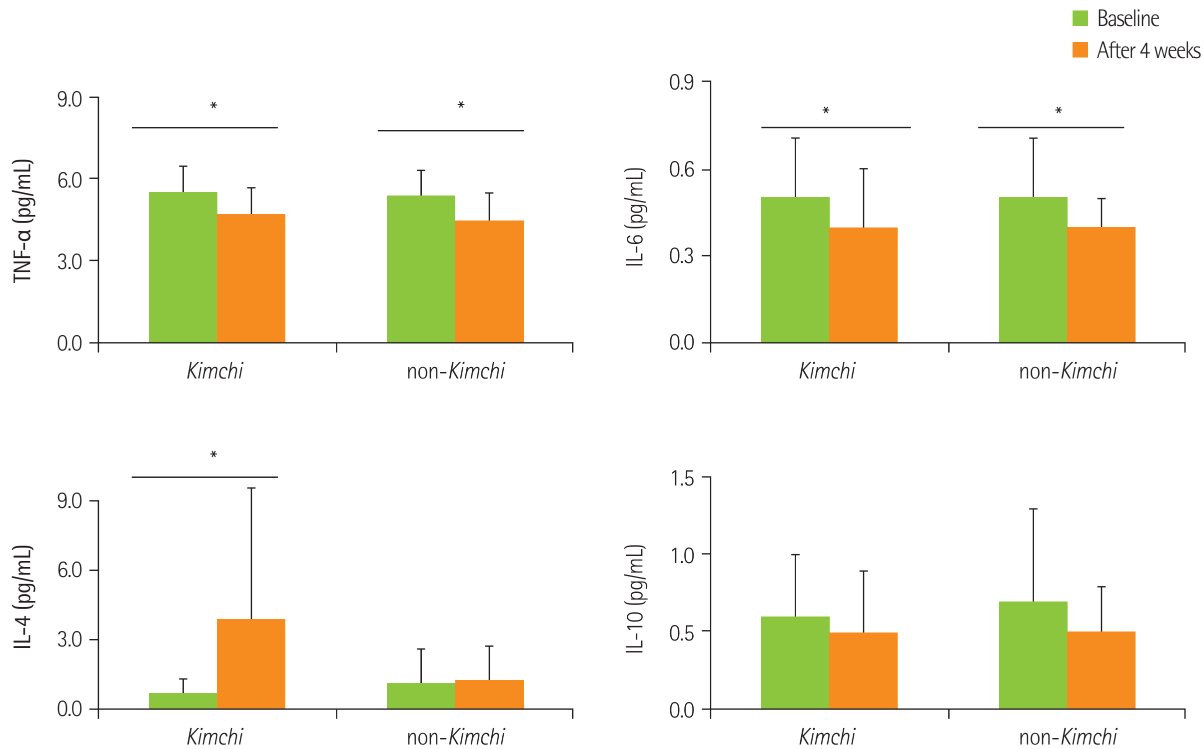

Figure 3. Blood levels of cytokines (TNF-α, IL-6, IL-4 and IL-10) at baseline and after 4-weeks. No differences were shown between groups by independent t-test at $p < 0.05$. *Values are significantly different from the baseline values by paired t-test at $p < 0.05$.

Our findings indicated that there is no clear effect of *Kimch* intake on immune parameters, which is somewhat surprising. Because previous reports have suggested that various *Kimchi* ingredients (cabbage, garlic, onion, red pepper, and ginger) or probiotic strains improve immune markers both in vivo and in vitro studies, we hypothesized that fermented *Kimchi* exerts immunomodulatory effects.

Chinese cabbage, a main ingredient of *Kimchi*, is rich in minerals, vitamin C, dietary fibers, and especially phytochemicals [16]. Also, cabbage contains several organic sulfur compounds (OSCs), such as isothiocyanates and dithiolethiones. In a previous study [17], these OSCs were shown to exert diverse biological effects including the inhibition of tumor cell proliferation, antimicrobial effects, and free radical scavenging. Another ingredient, garlic, contains various sulfur-contained compounds; S-allyl-l-cysteine, S-allyl-l-cysteine sulfoxides and alliin [18]. It suppress the production of inflammatory cytokines, such as TNF-a, IL-1, IL-6, and interferon-ɣ [19]. Red pepper contains high levels (25-80 mg%) of capsaicin. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is involved in physiological functions related to immune response [20].

Probiotics are living micro-organisms that have a health benefit for their host. Orally ingested probiotic bacteria are able to modulate the immune system; however, differences exist in the immunomodulatory effects of different probiotic strains [21]. Especially, lactic acid bacteria (LAB) produced during the fermentation process from *Kimchi : Leu. mesenteroides, Leu. citreum, Leu. gasicomitatum, Leu. kimchii, Leu. inhae, Weissella Koreensis, Weissella cibaria, Lac. plantarum, Lac. sakei, Lac. delbrueckii, Lac. buchneri, Lac. brevis, Lac. fermentum, Ped. acidilactici and Ped. Pentosaceus* [22]. According to the Lee et al. [23], suppressor T cells and NK cells are increased with *L. casei and Bifidobacterium longumi* treatment. However, in present study, T-helper cells and suppressor T cells were not affected by the consumption of *Kimchi*. T cells play central roles in the immune system, in which their major function assisting B cells in the production of antibodies. Serum Ig levels are routinely measured in clinical practice to examine immune balance. Typical ranges are suggested (Ig A; 1.4-0.4 mg/mL, Ig G; 8-16 mg/mL, and Ig M; 0.5-2.0 ng/mL). Low levels of Ig were observed in humoral immunodeficiency, while high levels of Ig were observed in chronic inflammatory diseases. Until now, many studies showed that *Kimchi* inhibited Ig E levels in the NC/nga mice atopic animal model [24,25]. Also, *lactobacillus*

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plantarum isolated from *Kimchi* increased the production of Ig A in normal or S180-bearing BALB/C tumor-induced mouse [26,14]. On the other hand, 4 weeks of *Kimchi* supplementation does not changes of Ig A, G, M.

Cytokines, protein mediators produced by immune cells, are involved in immune regulation. The levels of pro-inflammatory cytokines are increased in chronic inflammatory diseases while the levels of anti-inflammatory cytokines are decreased. Kim et al. [11] showed that the consumption of fermented *Kimchi* (300 g/day for 4 weeks) had no effects on the levels of TNF-α and IL-6 in overweight and obese patients (22 subjects, mean age of 38.6 $\pm$ 8.5 years). In our study, the levels of TNF-α and IL-6 were significantly decreased in the *Kimchi* and non-*Kimchi* groups. It is unclear why the levels of pro-inflammatory cytokines in the non-*Kimchi* group were decreased.

In previous of clinical trials, anti-obesity, anti-hypertension, anti-hypercholesterol effects of *Kimchi* have been investigated however, their immunomodulatory effects are not reported. The strength of this study is that the effects of the *Kimchi* supplementation on blood immune parameters are examined. These study results are useful information in further research of the patients with dysregulated immune responses. On the other hand, small sample size and short duration may limit the power in detecting differences between groups. Further studies examining a various subjects with unbalanced immune system, larger samples for longer periods are necessary to determine the immune-modulation of fermented *Kimchi*. In addition, the decreased levels of TNF-α and IL-6 in placebo group may be due to the use of radish. Because nutritional intakes of the *Kimchi* and non-*Kimchi* groups during the 4 weeks of intervention were similar in this study, more research is need to interpret changes observed in the control group.

Conflict of Interests

No conflict interests were declared by any of the authors.

Acknowledgement

This work was supported by the World Institute of *Kimchi*, An Annex of Korea Food Research Institute, Republic of Korea (Project Number: KE1201-3).

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