

## Long-term vegetarians have low oxidative stress, body fat, and cholesterol levels

Mi Kyung Kim<sup>1</sup>, Sang Woon Cho<sup>1</sup> and Yoo Kyoung Park<sup>1,2§</sup>

<sup>1</sup>Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, 1732 Deogyong-daero, Giheung-gu, Yongin-si, Gyeonggi 446-701, Korea

<sup>2</sup>Research Institute of Medical Nutrition, Kyung Hee University, Seoul 130-701, Korea

### Abstract

Excessive oxidative stress and abnormal blood lipids may cause chronic diseases. This risk can be reduced by consuming an antioxidant- and fiber-rich vegetarian diet. We compared biomarkers of oxidative stress, antioxidant capacity, and lipid profiles of sex- and age-matched long-term vegetarians and omnivores in Korea. Forty-five vegetarians (23 men and 22 women; mean age, 49.5 ± 5.3 years), who had maintained a vegetarian diet for a minimum of 15 years, and 30 omnivores (15 men and 15 women; mean age, 48.9 ± 3.6 years) participated in this study. Their 1-day, 24-h recall, and 2-day dietary records were analyzed. Oxidative stress was measured by the levels of diacron reactive oxygen metabolites (d-ROM). Antioxidant status was determined by the biological antioxidant potential (BAP) and levels of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. We observed that vegetarians had a significantly lower body fat percentage (21.6 ± 6.4%) than that of omnivores (25.4 ± 4.6%;  $P < 0.004$ ). d-ROM levels were significantly lower in vegetarians than those in omnivores (331.82 ± 77.96 and 375.80 ± 67.26 Carratelli units;  $P < 0.011$ ). Additionally, total cholesterol levels in the vegetarians and omnivores were 173.73 ± 31.42 mg/dL and 193.17 ± 37.89 mg/dL, respectively ( $P < 0.018$ ). Low-density lipoprotein cholesterol was 101.36 ± 23.57 mg/dL and 120.60 ± 34.62 mg/dL ( $P < 0.005$ ) in the vegetarians and omnivores, respectively, indicating that vegetarians had significantly lower lipid levels. Thus, oxidative stress, body fat, and cholesterol levels were lower in long-term vegetarians than those in omnivores.

**Key Words:** Long-term vegetarian diet, oxidative stress, lipid profiles, d-ROM, Korean vegetarian

### Introduction

Oxidative stress is caused by excessive reactive oxygen species (ROS) that are produced during metabolic processes. Excess ROS act as free radicals and cause various diseases by inducing damage to proteins or DNA and destroying cell membranes [1]. Therefore, oxidative stress is a major cause of atherosclerosis, cardiac diseases, diabetic vascular diseases, cancers, and even early aging [2-4]. Antioxidant capacity is the protective ability of cells against oxidative stress and involves intrinsic factors such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), uric acid, and nitroxide, as well as extrinsic factors, such as vitamins A, C, and E and as phytochemicals. Extrinsic factors are mostly derived from food, particularly fruits and vegetables [5]. In contrast, excess consumption of red meat or processed food leads to increased oxidative stress [6,7].

The Western diet is one of the leading causes of the increased prevalence of metabolic syndrome and intractable diseases. For these reasons, there has been increased interest in vegetarian diets. A number of studies have been conducted on long-term vegetarian dietary habits, and several studies have reported that a long-term vegetarian diet reduces death rate [8,9], helps

regulate diabetes [10], and reduces the incidence of and mortality associated with vascular diseases [11-14]. Vegetarians usually consume more fruits and vegetables than omnivores while restricting their consumption of animal-sources of food; thus, they have lower intake of saturated fatty acids and increased intake of fiber and various kinds of antioxidants compared to those of omnivores [5]. This type of diet helps improve antioxidant status [15], lowers oxidative stress, and reduces blood lipid levels [14].

The vegetarian diet can be divided into several types depending on the kinds of food consumed: vegans avoid all animal food, lacto-vegetarians and ovo-vegetarians consume milk and dairy products or eggs; ovo-lacto-vegetarians consume milk, dairy products, and eggs, and semi-vegetarians consume small quantities of chicken and fish [16].

Unlike the number of studies on vegetarians in the West, only a few similar studies have been performed in Asian countries [17,18]. Even in Korea, many systematic studies have been conducted on various subjects from different perspectives [19-21], but few studies have reported on the effects of a long-term vegetarian diet.

Traditionally, Koreans have a lower meat intake than people in other Asian countries and the West [20,22], because the

§ Corresponding Author: Yoo Kyoung Park, Tel. 82-31-201-3779, Fax. 82-31-201-3819, Email. ypark@khu.ac.kr

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Korean diet is predominantly composed of rice as the staple food served with vegetable soup and several vegetable side dishes. However, because of modern industrialization, the dietary habits of Koreans have undergone rapid westernization.

The consumption of animal food was only 3% in 1969, but increased to 20.2% in 2009. The incidence of obesity, a major factor in metabolic syndrome, has steadily increased from 26% in 1998 to 31.3% in 2009 as reported by the Korea National Health and Nutrition Examination Survey-IV (KNHANES-IV). The common causes of death in people aged 65 years and older have been replaced by cancers, diabetes, and cardiovascular diseases [23], which are similar to those in Western countries.

Thus, Koreans are paying more attention to healthy lifestyle choices, and traditional Korean dietary habits [22] have returned to the spotlight with a focus on consumption of brown rice, fresh vegetables, and fresh fruits, including dishes such as namul (cooked vegetables eaten as a side dish), ssam (fresh vegetables similar to salad), and kimchi (Korean style salted cabbage with garlic and hot pepper).

Therefore, this study was designed to investigate ways to prevent chronic diseases via a vegetarian diet in Korea. The aim of our study was to evaluate the health status of people who have maintained a vegetarian diet for more than a decade. First, body composition and nutritional status were evaluated between long-term vegetarians and omnivores. Then, biomarkers of oxidative stress, antioxidant capacity, and lipid profiles were compared between long-term vegetarians and omnivores.

## Subjects and Methods

### *Subjects and methods*

This study was carried out in accordance with the Declaration of Helsinki and was conducted with approval of the Institutional Ethics Board of Kyung Hee University, Korea (KHU IRB 2010-011). Written informed consent was obtained from all study participants. All subjects in our study participated voluntarily. The vegetarian subjects were recruited through the Han-Nong society. The Han-Nong society is a religious group largely consisting of people who are Seventh Day Adventists and maintain a vegetarian diet. The Food Frequency Questionnaire (FFQ) was used to characterize the participants' dietary intake over the past year. We used the FFQ from the KNHANES-IV, and two more kimchi items were added to the survey to reflect the exact amount of kimchi intake, one of the most commonly eaten foods in the Korean diet. The vegetarian participants consumed plant foods and occasional dairy products (goat milk only) and eggs.

The participants were healthy (age range, 40-65 years and had maintained this diet for more than 15 years. The exclusion criteria included any condition that may affect oxidative stress levels and lipid profiles. The exclusion criteria included smoking, use of prescribed medications, and acute disease within 3 months

before the study or chronic illnesses within 10 years, and use of an antioxidant supplement (vitamins A, C, and E and phytochemicals) for at least 3 months prior to the study. Subjects who consumed one or more alcoholic beverages per week and those with a body mass index (BMI)  $\geq 30$  were also excluded. Age- and sex-matched omnivores were enrolled for the comparison group. In total, 45 vegetarians (10 vegans, 35 ovo-lacto-vegetarians; 23 men and 22 women) and 30 omnivores (15 men and 15 women) satisfied the inclusion criteria and consented to participate in the study. This survey was carried out in August 2010.

### *Anthropometry measurements*

The participants height and body weight were measured with an automatic height/weight measurement system (DS-102; Jenix<sup>®</sup>, Seoul, Korea). BMI ( $\text{kg}/\text{m}^2$ ) was calculated. Body fat was measured using a bioelectrical impedance analyzer (Jawon Medical, Seoul, Korea). Blood pressure was measured twice with an automatic blood pressure monitor (HEM-770A; Omron, Tokyo, Japan) after at least 10 min of rest.

### *Dietary assessment*

Food consumption was estimated for 3 days from 1-day, 24 h recall and 2-day dietary records. Nutrient intake was calculated by CAN-Pro version 3.0, a computer-aided nutritional analysis program (Korea Institute of Nutrition, 2005). Twenty-four-hour recall was carried out with a one-on-one interview with a well-trained nutritionist. Using this method, all kinds and amounts of foods and beverages (including cooking methods, brands, and locations where the food was consumed) that the participant had consumed in the last 24 h were recorded. Portion sizes were estimated using food models and an atlas, which showed different portion sizes for individual food quantities. The participants were asked to describe the quantities that they had eaten in terms of fractions or multiples of the amounts of food shown in the food atlas.

### *Blood sampling and lipid profile measurements*

Blood samples (10 mL) were collected by venipuncture into an EDTA tube (Greiner Bio-one, Kremsmünster, Austria) and a Vacuette (Greiner Bio-one) after a 12-h overnight fast. All samples were immediately placed on ice, and plasma was separated by centrifugation at  $3,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Aliquots of plasma were placed in separate Eppendorf tubes and immediately frozen at  $-80^\circ\text{C}$  and then transferred to liquid nitrogen. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglyceride (TG) concentrations were measured using standard enzymatic colorimetric techniques. Plasma glucose concentrations were measured using the glucose oxidase method

with an automated glucose analyzer (Hitachi 7600-110; Hitachi, Tokyo, Japan). Analyses were performed by an accredited clinical chemical laboratory (Green Cross Reference Lab, Seoul, Korea).

#### *Oxidative stress and antioxidant status measurements*

Oxidative stress was assessed by measuring diacron reactive oxygen metabolites (d-ROM). Antioxidant status was determined with the biological antioxidant potential (BAP), CAT, SOD, and GPx. The d-ROM (Diacron s.r.l. Grosseto, Italy) and BAP test (Diacron s.r.l.) has been demonstrated as a very useful tool for measuring oxidative stress and antioxidant capacity. Both methods are highly reproducible and scientifically validated and have been widely used to investigate oxidative stress and antioxidant states [3,24-27]. The d-ROM test is used to measure ROM through both quantitative and qualitative methods. The d-ROM level represents the total level of peroxidized metabolites. This method is based on the ability of transition metals to catalyze the formation of free radicals in the presence of hydroperoxides, which are then trapped by an amine. The d-ROM test utilizes the *N, N*-diethyl-*para*-phenyldiamine (DEPPD). DEPPD reacts with free radicals to form a colored radical detectable at 505 nm as a result of a linear kinetic reaction. The results of the d-ROM test are expressed as Carratelli units (Carr U), with 1 Carr U equals 0.08 mg/100 mL H<sub>2</sub>O<sub>2</sub> [3,24-27]. The test was performed with an automated clinical chemistry analyzer (Architect ci8200, Abbott Diagnostics, Abbott Park, IL, USA). The BAP test was used to measure the antioxidant capacity in the body by measuring the ratio of ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>). Briefly, FeCl<sub>3</sub> was added to a cuvette containing thiocyanate reagent. Then, the serum sample was added to a cuvette containing the resulting colored solution, and the absorbance at 505 nm was recorded automatically. Levels are expressed as μmol/L. The BAP test was performed with an automated clinical chemistry analyzer [26,28].

Endogenous antioxidant enzymes were also measured in the serum (SOD, GPx, and CAT). SOD activity was measured using a commercial kit (OxiSelect™, Cell Biolabs, Inc., San Diego, CA, USA). GPx (BioVision Research Products, Mountain View, USA) and CAT (Catalase Fluorometric Detection kit, Enzo Life Sciences International, Inc., Zandhoven, Belgium) activities were also measured using commercial kits. All tests were performed according to the manufacturer's instructions.

#### *Statistical analysis*

Data were analyzed using the Statistical Package for the Social Sciences for Windows version 19 (SPSS Inc., Chicago, IL, USA). The homogeneity of variances was assessed with Levine's test and normally distributed data were analyzed with the Kolmogorov-Smirnov test. Continuous variables are expressed as mean ± SD. Differences in demographic characteristics, anthropometry, nutrient intake, and biochemical measurements between vegetarians and

omnivores were determined using Student's *t*-test. When the data were skewed rather than normally distributed, differences were determined by the Mann-Whitney rank test. Pearson's correlation coefficients were calculated to assess the relationship between antioxidant status, oxidative stress, and nutrient intake parameters. Results were considered statistically significant at *P* < 0.05.

## Results

Seventy-five subjects finished the study, including 45 vegetarians and 30 omnivores. The general characteristics of the vegetarian and omnivore subjects are shown in Table 1. The mean age of the vegetarian and omnivore groups was 49.4 and 48.9 years, respectively. The mean duration of vegetarianism was 24.6 years (range, 17-35 years). The mean age, height, and weight were similar between the groups. The median BMIs of the vegetarian and omnivore groups were 22.1 and 23.4, respectively, and fell within the normal range (Korea Health Statistics 2009: KNHANES IV). However, body fat was significantly lower in vegetarians (21.6%) than that in omnivores (25.4%). Systolic and diastolic blood pressures tended to be slightly higher in vegetarians than those in omnivores, but the difference was not significant.

Energy intake, macro- and micronutrients, and antioxidant vitamin intake in vegetarians and omnivores are shown in Table 2. No difference was observed between the energy intake of the two diet groups. Vegetarians had a significantly higher intake of carbohydrates than that of omnivores (302.8 g and 258.6 g, respectively). However, fat intake was significantly higher in omnivores (50.6 g) than that in vegetarians (31.1 g). Total protein intake tended to be higher in the vegetarian group, but the difference was not statistically significant. The percentage of energy vegetarians obtained from carbohydrate, protein, and fat was 66.1%, 18.6%, and 15.3%, respectively. In omnivores, carbohydrate, protein, and fat consumption was 58.3%, 16.6%, and 25.1% respectively. Vegetarians had significantly higher mean intakes of dietary fiber, vitamins A and C, and β-carotene antioxidant vitamins than those of omnivores. Vegetarians also had higher intakes of folate, total iron, and potassium, and significantly lower mean intakes of fat, retinol, cholesterol, fatty

**Table 1.** General characteristics of the vegetarian and omnivore subjects

Variables	Vegetarians (n = 45)	Omnivores (n = 30)	<i>P</i>
Age (yrs)	49.4 ± 5.2	48.9 ± 3.6	0.567
Duration of vegetarianism (yrs)	24.6 (17-35)	-	0.000
Height (cm)	161.3 ± 8.9	163.6 ± 7.6	0.254
Weight (kg)	57.8 ± 10.9	62.9 ± 10.7	0.530
BMI (kg/m <sup>2</sup> )	22.1 ± 2.8	23.4 ± 2.7	0.720
Body fat (%)	21.6 ± 6.4	25.4 ± 4.6	0.004
Systolic blood pressure (mmHg)	120.2 ± 12.9	115.8 ± 13.9	0.070
Diastolic blood pressure (mmHg)	74.3 ± 8.4	73.6 ± 8.6	0.534

Values are means ± SD, as assessed by Student's *t*-test and chi-square test. BMI, body mass index

**Table 2.** Daily nutrient intake of the vegetarian and omnivore subjects

Variables	Vegetarians (n = 45)	Omnivores (n = 30)	P
Energy (kcal)	1,832 ± 407	1,790 ± 420	0.651
Carbohydrate, in kcal (%)	66.1	58.3	0.000
Total protein, in kcal (%)	18.6	16.6	0.779
Total fat, in kcal (%)	15.3	25.1	0.000
Carbohydrate (g)	302.8 ± 70.1	258.6 ± 55.0	0.005
Total protein (g)	85.1 ± 29.9	74.6 ± 22.2	0.087
Plant protein (g)	81.5 ± 29.5	39.0 ± 9.0	0.000
Animal protein (g)	3 ± 4.26	35.6 ± 19.7	0.000
Total fat (g)	31.1 ± 13.1	50.6 ± 17.3	0.000
Plant fat (g)	27.8 ± 12.1	25.3 ± 9.3	0.346
Animal fat (g)	3.4 ± 4.3	25.3 ± 13.8	0.000
Fiber (g)	39.9 ± 11.4	23.7 ± 5.2	0.000
Total calcium (mg)	551.2 ± 226.0	532.8 ± 161.9	0.701
Plant calcium (mg)	481.6 ± 210.8	323.3 ± 93.1	0.000
Animal calcium (mg)	69.6 ± 117.0	209.5 ± 107.9	0.000
Total iron (mg)	19.1 ± 4.9	14.8 ± 5.1	0.000
Plant iron (mg)	18.8 ± 4.8	11.5 ± 4.5	0.000
Animal iron (mg)	0.3 ± 3.3	3.3 ± 1.6	0.000
Sodium (mg)	3,077.8 ± 1,741.1	4,436.6 ± 1,676.0	0.001
Potassium (mg)	4,243.0 ± 1,198.8	3,177.8 ± 865.8	0.000
Vitamin A (µg RE)	1,311.9 ± 657.7	873.6 ± 447.9	0.002
Retinol (µg)	38.7 ± 47.5	100.3 ± 72.6	0.000
β-Carotene (µg)	7.2 ± 3.8	4.3 ± 2.6	0.000
Vitamin B <sub>1</sub> (mg)	1.7 ± 0.5	2.4 ± 4.1	0.355
Vitamin B <sub>2</sub> (mg)	1.1 ± 0.4	1.2 ± 0.9	0.587
Niacin (mg)	18.2 ± 5.0	19.7 ± 7.5	0.330
Vitamin C (mg)	170.6 ± 78.9	109.9 ± 71.4	0.001
Folate (µg)	385.6 ± 116.5	244.5 ± 66.9	0.000
Vitamin E (mg)	15.7 ± 5.3	14.1 ± 4.9	0.212
Cholesterol (mg)	115.0 ± 148.3	262.5 ± 138.0	0.000
Fatty Acid (g)	11.9 ± 7.1	23.0 ± 12.5	0.000
SFA (g)	2.3 ± 1.6	7.3 ± 4.8	0.000
NSFA (g)	5.8 ± 3.8	8.5 ± 4.9	0.000

Values are means ± SD, as assessed by Student's *t*-test, SFA, saturated fatty acids; NSFA, non-saturated fatty acid

acid, and sodium than those of omnivores. Additionally, vegetarians and omnivores consumed similar amounts of total calcium, although vegetarians obtained their calcium mostly from plant sources. The results of the hematological and biochemical measurements, including lipid profiles, are shown in Table 3. All mean hematological values were within the reference range. There was a significant difference in TC between vegetarians (173.73 ± 31.42 mg/dL) and omnivores (193.17 ± 37.89 mg/dL). LDL cholesterol was also significantly different between vegetarians (101.36 mg/dL) and omnivores (120.60 mg/dL). In contrast, HDL cholesterol and TG did not significantly differ between the groups.

The level of oxidative stress (d-ROM), total antioxidant status (BAP), and antioxidant enzymes in vegetarians and omnivores are shown in Table 4. The levels of ROM were significantly lower in the vegetarian group than that in omnivores ( $P < 0.011$ ).

**Table 3.** Lipid profiles and other hematologic assays of the vegetarians and omnivores

Variables	Reference value	Vegetarians (n = 45)	Omnivores (n = 30)	P
TC (mg/dL)	Below 220	173.73 ± 31.42	193.17 ± 37.89	0.018
LDL Cholesterol (mg/dL)	0~130	101.36 ± 23.57	120.60 ± 34.62	0.005
HDL Cholesterol (mg/dL)	30~75	47.64 ± 9.45	50.30 ± 10.12	0.250
TG (mg/dL)	Below 200	132.36 ± 79.96	111.07 ± 53.44	0.205
Fasting glucose (mg/dL)	70~110	95.8 ± 7.0	101.3 ± 7.0	0.002
Hemoglobin (g/dL)	12.7~17.1	13.6 ± 1.5	13.8 ± 1.9	0.612
RBC (× 10 <sup>6</sup> /µL)	4.04~5.82	4.5 ± 0.4	4.6 ± 0.4	0.219
Hematocrit (%)	38~53	41.2 ± 3.8	41.5 ± 4.7	0.740
White blood cells (× 10 <sup>3</sup> /µL)	4~10.0	4.4 ± 1.2	5.1 ± 1.1	0.017
Platelet (× 10 <sup>3</sup> /µL)	145~375	223.7 ± 63.9	227.7 ± 38.2	0.761
AST (units/L)	0~37	24.11 ± 6.8	23.83 ± 5.8	0.855
ALT (units/L)	0~40	21.07 ± 13.8	22.70 ± 10.9	0.588
BUN (mg/dL)	4.5~23.5	14.4 ± 3.6	15.15 ± 4.2	0.411
Creatinine (mg/dL)	0.7~1.3	0.95 ± 0.1	0.99 ± 0.14	0.138

Values are means ± SD, as assessed by the Student's *t*-test and Mann-Whitney rank test.

TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; TG, triglycerides; RBC, red blood cell

**Table 4.** Oxidative stress and antioxidant status of vegetarians and omnivores

Variables	Vegetarians (n = 45)	Omnivores (n = 30)	P
d-ROM (Carr U)	331.82 ± 77.96	375.80 ± 67.26	0.011
BAP (µmol/L)	2,072.07 ± 166.95	2,122.80 ± 171.76	0.210
SOD activity (%)	14.37 ± 4.10	14.72 ± 6.45	0.802
GPx (mU/mL)	1.96 ± 1.08	2.22 ± 0.69	0.337
CAT (U/mL)	126.85 ± 49.68	119.73 ± 42.39	0.577

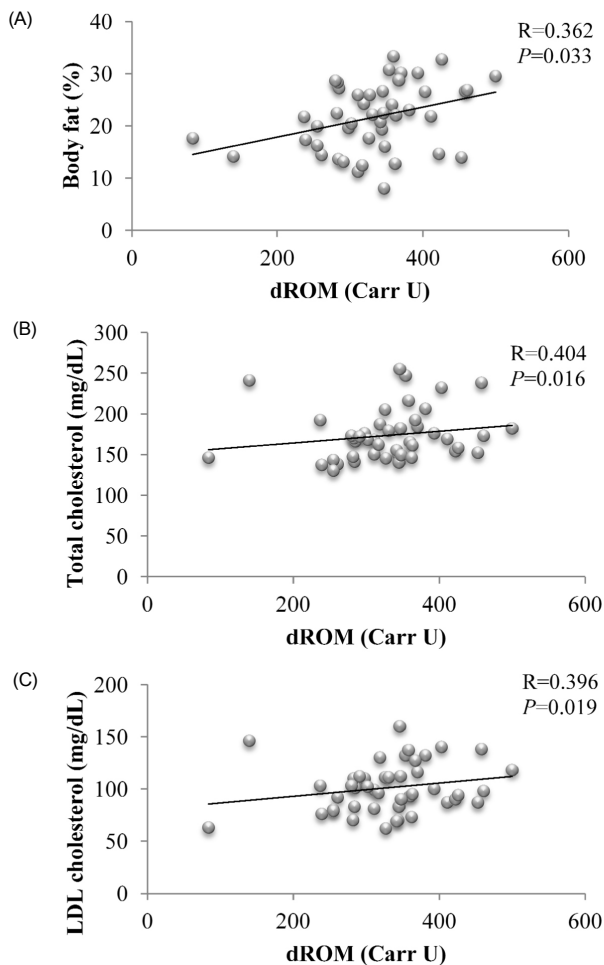
Values are means ± SD, as assessed by Student's *t*-test.

d-ROM, diacron reactive oxygen metabolites; 1 Carr U = 0.08 mg H<sub>2</sub>O<sub>2</sub>/dL; BAP, biological antioxidant potential; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase

The vegetarian subjects had ROM levels of 331.82 ± 77.96 Carr U, whereas omnivores had ROM levels of 375.80 ± 67.26 Carr U. However, BAP levels did not differ significantly between the two groups. BAP measurements in vegetarians and omnivores were 2,072.07 ± 166.75 µmol/L and 2,122.80 ± 171.76 µmol/L, respectively. No significant differences were observed in the activities of endogenous antioxidant enzymes such as GPx, SOD, or CAT. Fig. 1 shows the Pearson's correlation coefficient for the biochemical variables and oxidative stress in vegetarians. Body fat, TC, and LDL cholesterol were significantly and positively associated with ROM level.

## Discussion

Many health conscious people in Korea want to eat more vegetables and reduce their intake of saturated fatty acids and also wish to know the ultimate benefits and advantages of a vegetarian diet for preventing disease [29]. They also want to



**Fig. 1.** Pearson's correlation coefficient between body fat and lipid profiles with diacron reactive oxygen metabolite (d-ROM) level in vegetarians

understand the nutritional imbalances that are likely to appear in those who have maintained a vegetarian diet for a prolonged period of time. Several studies have been conducted about different health views of the vegetarian diet in Korea [19-21, 30-31] in populations such as Buddhists or postmenopausal women. However, very few studies have been conducted on the effects of a long-term vegetarian diet in a healthy general population. This is the first study that has investigated the long-term overall health effect of eating a vegetarian diet on oxidative stress.

We compared oxidative stress, antioxidant capacity, lipid profiles, and body composition between long-term vegetarians and omnivores. The long-term vegetarians were similar to the omnivores in weight, height, BMI, and blood pressure but had a particularly lower body fat percentage. Thus, unlike omnivores, long-term vegetarians consume more fiber and less saturated fatty acids, because vegetarian diets have low energy density, resulting in less fat accumulation [32]. A previous study with a large cohort showed that, in comparison to meat eaters who consume a relatively small amount of fiber, vegetarians (particularly vegans)

have significantly lower BMIs [33]. Body fat has been recently chosen as a more useful measure of obesity than BMI for Koreans, and, hence, our results can be regarded as more encouraging.

Approximately 1,832 kcal per day were consumed by the vegetarians participating in this study, which was comprised of carbohydrate, protein, and fat intakes of 66.1%, 18.6%, and 15.3%, respectively, compared to the control group's intake of 58.3%, 16.6%, and 25.1%, respectively, and 1,790 kcal per day. Some similarities were observed between the groups for total calories and the main energy source (carbohydrate), but the long-term vegetarians had a significantly lower fat intake than that of omnivores. In particular, the consumption of saturated fatty acids, known as one of the main causes of metabolic syndrome, averaged 2.3 g in the vegetarian group, accounting for only 1% of the average calorie intake. This intake of saturated fatty acids is even lower than that in the traditional Okinawa diet, known as a key diet to longevity, in which the intake of saturated fatty acids is 2% [34]. The vegetarians in our study consumed a small quantity of goat's milk instead of cow's milk and consumed eggs, whereas the Okinawa menu includes fish and lean meat. Both studies included ample amounts of fresh vegetables and fruits. In addition, the percentage intake of protein was 14.6%, higher than the usual intake of protein, as reported in the 2009 KNHANES IV. This could be because those who participated in this study maintained a nutritionally well-balanced diet with various kinds of legume-based foods as a main source of vegetables. Both the long-term vegetarians and omnivores exhibited blood biochemical parameter levels within normal reference ranges. Although anemia may be caused by a long-term vegetarian diet and has been a main concern of previous studies [35], hemoglobin levels, hematocrit, and red blood cell (RBC) counts were also in the normal range, and no differences were found between the groups. In contrast, fasting glucose, TC, and LDL were significantly lower in the vegetarian group than those in the omnivore group, which is consistent with previous studies. In particular, oxidized LDL is associated with cancer, arthritis, cataracts, and other chronic diseases in addition to atherosclerosis disorders [36]. High d-ROM level is correlated with highly oxidized LDL [24].

The d-ROM level, reflecting oxidative stress, was significantly lower in vegetarians ( $331.82 \pm 77.96$  Carr U) than that in omnivores ( $375 \pm 67.26$  Carr U). Normally, numerous factors are associated with a high level of oxidative stress, such as increasing age [37], hard labor, smoking, drinking, and dietary habits that include consumption of meat or meat products and low intakes of vegetables and fruits [3]. The level of d-ROM in Mongolians ( $429.7 \pm 95.2$  Carr U), who have a very low consumption of vegetables, is significantly higher level than that of the same-aged Japanese group ( $335.3 \pm 59.8$  Carr U) [3] whose dietary habits are very similar to Koreans. In our study, we confirmed that vegetarians had lower levels of oxidative stress than those of age-matched omnivores.

The body's defense mechanism is the antioxidant capacity, and total antioxidant capacity is reflected by the BAP level. Interestingly, no significant difference was observed in the BAP level between vegetarians ( $2,072.07 \pm 166.95 \mu\text{mol/L}$ ) and omnivores ( $2,122.80 \pm 171.77 \mu\text{mol/L}$ ). Previous data from healthy individuals indicate that a healthy BAP level is approximately  $2,200 \mu\text{mol/L}$  [29]. The BAP level in subjects in our study was relatively high, suggesting that even the omnivores participating in this study led a healthy life.

We also found no significant differences in the concentrations of antioxidant enzymes, such as SOD, GPx, and CAT between the groups. In general, high oxidative stress reduces total antioxidant capacity. This tendency is observed in patients with cancer; patients with progressive cancer show a particular tendency toward increasing ROM levels and decreasing SOD activity. Unfortunately, this tendency does not appear in every situation, and as ROS increases, antioxidant molecules are stimulated. As oxidative stress increases, SOD may increase, while the BAP level is positively correlated with ROM level in Mongolian subjects [26]. Palauan people have a higher ROM level than that of the Japanese but a similar BAP level compared with that of Japanese [27]. Other studies have reported that the reason for the lack of a difference in the antioxidant capacity between the two diet groups might partly be due to the body's tendency to maintain homeostasis [3,27,38]. Another reason was speculated to be the "healthy volunteer effect" [38]. The omnivore subjects in this study were relatively health conscious and did not consume alcohol, smoke, or use medication.

Body fat, TC, and LDL cholesterol levels were weakly positively correlated with d-ROM in vegetarians, suggesting that even in the vegetarian group (which consumes a high amount of antioxidants) excess lipid levels and higher body fat is highly correlated with increased oxidative stress. In a previous study, ROM levels were significantly correlated with body fat ratio in Palauan people, although they consume large amount of fruits and vegetables [27]. This indicates that fruit and vegetable intake does not suppress excessive oxidative stress caused by many factors such as abdominal obesity, smoking, and excess sunlight exposure. Additionally, their high ROM levels significantly correlated with body fat ratio.

In terms of dietary intake, the long-term vegetarians consumed about twice more antioxidant vitamins such as vitamins A and C in their diets than those of omnivores. Although the intake of other antioxidants was not measured in the long-term vegetarian diet, it could easily be estimated that the vegetarian diet is associated with lower oxidative stress. Fruits and vegetables contain various phytochemicals such as polyphenols and flavonoids, which are powerful antioxidants.

The limitation in this study was a failure to include a homogenous vegetarian diet group. In this study, vegans and ovo-lacto-vegetarians were both included but were not separately analyzed. Future studies should recruit subjects based on their vegetarian type. Another limitation is that the control group did

not exhibit any serious health risks compared with the study group, mainly because the control omnivores were relatively highly health conscious, which may explain their high level of total antioxidant capacity.

In our study, people who maintained a vegetarian diet for a long period of time (more than 20 years) not only had lower body fat and blood cholesterol levels but also a lower degree of oxidative stress. Additionally, long-term vegetarians did not show any serious or potential nutritional problems including anemia. The results of this study will be useful for those who consider a vegetarian diet to prevent disease and promote health.

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