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Effects of Calcium Fortified Beverage Intake on Insulin Sensitivity and Antioxidant Metabolism in Healthy Elderly

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ABSTRACT

Calcium, one of the most important nutrients, determines the quality of life of the elderly. It has been reported that 7 out of 10 people over the age of 60 have insufficient calcium intake. The purpose of this study was to evaluate the effect of calcium fortified beverage (CFB) intake on insulin sensitivity and antioxidant metabolism in healthy elderly. A crossover clinical trial was performed and antioxidant status of healthy elderly (age above 65 years, n = 8) was analyzed. Subjects did not take CFB for 0–3 weeks. They then took it for 3–6 weeks. CFB supplementation decreased insulin levels (Δ3–6 weeks: 1.19 ± 0.65 μ IU/mL → Δ0–3 weeks: −0.58 ± 0.38 μ IU/mL). Increasing degree of fasting blood glucose level was suppressed by intake of CFB, although the suppression was not statistically significant. Except for insulin, there were no significant differences in results of biochemical analysis between 0–3 weeks and 3–6 weeks. Catalase activity was significantly increased by CFB supplementation (Δ3–6 weeks: 3.50 ± 5.30 K g/Hb) compared to the no CFB supplementation period (Δ0–3 weeks: −12.48 ± 4.37 K g/Hb). However, the activity of superoxide dismutase and glutathione-peroxidase were not significantly different between 0–3 weeks and 3–6 weeks. H_2O_2-induced DNA oxidative damage was also decreased significantly by CFB supplementation. Taken together, these results indicate that CFB has beneficial effect on insulin sensitivity and some antioxidant enzymes in healthy elderly.

Keywords: Calcium; Healthy aging; Insulin sensitivity; Antioxidant activity; Beverage

INTRODUCTION

With increasing number of the elderly due to the extension of life expectancy, it is important to extend a healthy life expectancy. Active seniors refer to the generation in their 50s and 60s who enjoy consumption and leisure even after retirement. They are defined as a new class of consumers who actively engage in economic activities [1]. They consider health and convenience when choosing food. They eat a high proportion of healthy snacks such as fruits and nuts. The market size of active seniors, attracting attention as bluesumers (blue ocean + consumer), has grown from KRW 9.33 trillion in 2012 to KRW 26 trillion in 2020 [2]. Accordingly, the demand for developing various products is increasing to secure a potential large-scale market for them.

Funding

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Conflict of Interest

The authors declare that they have no competing interests.

As a result of a nutrient intake survey of 2,876 elderly by the Korea Centers for Disease Control and Prevention in 2015, it was found that 1 out of 6 people had insufficient nutritional intake. Among those aged 65 years and over, only 25% of the elderly met the recommended daily caloric intake [3]. Among nutrients, deficiency rate of calcium intake (81.7%) was the highest, followed by that of vitamin B_2 (71.8%), fat (70.5%), vitamin C (66.3%), vitamin A (62.9%), and protein (30.1%). Reduced supplies of calcium are associated with a reduced bone mass and osteoporosis in elderly [4]. In recent years, various evidences were provided linking bone loss to reactive oxygen species (ROS) [5-7]. Therefore, it can be predicted that calcium supply to the elderly can enhance their antioxidant metabolism by the reduction of free radicals due to bone loss.

Calcium intake should be sufficient in daily life so that antioxidant system is not inhibited by calcium deficiency in the elderly. However, many elderly are unable to consume sufficient, let alone optimal, levels of certain nutrients (calcium, vitamin D, and vitamin B_{12}) solely by diet. A modified food guide pyramid for people over 70 years recommends the use of dietary supplements to bridge this gap [8]. Such levels of calcium can be obtained by drinking or eating the equivalent of 3 servings of calcium-rich dairy products (240 mL of milk, 56 g of hard cheese, 240 mL of yogurt, 240 mL of calcium fortified orange juice) per day. Foods for calcium intake are currently limited to milk and dairy products. However, many Asian elderly people are not milk drinkers due to lactose intolerance, perceived lactose intolerance, or thinking of milk as a child's food Also, the recommended daily intake of calcium could not be met by one food alone, but by a variety of foods. Therefore, if various products using food materials with high calcium content are developed, it will help the elderly to meet the daily calcium requirement.

Sprouts of barley grass has young green leaves, and it starts from sprouts with young green leaves to seedling at 10 days after sprouting (barley sprout) to elongation stage (barley green) with nutritional peak before starting its reproductive cycle [9]. It contains 11 times more calcium than milk, 5 times more iron than spinach, 3 times more vitamin C than spinach, and 60 times more than apples [10]. Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries [11]. It is a nutritionally excellent food with high protein content, high contents of vitamins such as carotene, niacin, and folic acid, and high contents of minerals such as calcium, sodium, copper, manganese, and zinc [12].

Therefore, in this study, calcium fortified beverage (CFB) was prepared using calcium-rich sprouted barley grass and chickpea powder to increase calcium intake for healthy elderly. This effect was verified through insulin sensitivity and antioxidant metabolism analysis for the elderly.

MATERIALS AND METHODS

Subjects and study design

Subjects were recruited form the public advertisement. The inclusion criteria of subjects were that they should be able to live their daily lives with no assistance, be able to walk independently, and diseases such as hypertension, diabetes, hyperlipidemia, and sarcopenia should not affect their daily life at all. Exclusion criteria were people with food allergy symptoms, people who have difficulty without assistance, people with mobility difficulties,

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and people who have difficulties in daily living due to diseases such as high blood pressure (BP), diabetes, hyperlipidemia, and sarcopenia. In addition, patients with thyroid disease, cancer, liver failure and malnutrition, dialysis patients, patients currently receiving treatment for a serious disease, and patients admitted to nursing homes were excluded from the clinical trial. After the screening, 9 of Korean elderly (male 3, female 6) who signed the free and informed consent form were finally included in the analysis. Of the 9 participants eligible for the trial, one withdrew from the clinical trial for not being able to comply with the sample. The sample contained 12.90 g of sprouted barley grass powder and 17.10 g of chickpea powder per package, and a sample for 3 weeks was provided. The mixing ratios of these samples were determined using response surface analysis to maximize calcium conditions (not yet published). Subjects were instructed to take this powder mixed with 300 mL of water once daily. Thirty gram of powder contains 77.7 mg of calcium (% daily value 11%). Each subject was given a checklist and instructed to check after eating CFB. In addition, it was determined whether the sample was ingested through the checklist submitted on the end day of the test. The study was approved by the Institutional Review Board of the Kyungnam University (1040460-A-2020-033). The protocol was designed as a crossover clinical trial. Subjects participated in the clinical trial for a total of 6 weeks and did not consume samples for 1–3 weeks and took samples for 4–6 weeks.

Anthropometric parameters

Anthropometrics parameters were measured in subjects wearing light clothes and putting off shoes. Participants were examined for height, weight, total body fat percentage using an automatic body composition analyzer (HBF-306; Omron Inc., Matsusaka, Japan). Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2). Systolic and diastolic BP were obtained by automatic BP monitor (T5-M; Omron Inc.) in the arm in the seated position after 20 minutes of rest.

Calcium intake survey

The calcium content of daily food intake was obtained through the 24-hour recall method with a modification of the Korea National Health and Nutrition Examination Survey form through face-to-face interview. It was calculated using the Computer Aided Nutritional analysis program (CAN-Pro 5.0; The Korean Nutrition Society, Seoul, Korea).

Blood collection and biochemical analysis

Blood samples were collected after 12 hours fasting by vein puncture at 0, 3, and 6 weeks. Collection was performed by a duly trained nursing professional. The collected sample was placed on a heparinized (anti-coagulation solution) tube. Blood was centrifuged at 3,000 rpm and 4ºC for 30 minutes to obtain the plasma and at 3,000 rpm for 10 minutes to obtain the serum. After separation of plasma, red blood cells were suspended in 10 volumes of isotonic phosphate buffer pH 7.4 and centrifuged at 3,000 rpm for 10 minutes. This was repeated 3 times to obtain the erythrocyte. Analysis of fasting glucose, triglyceride, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), insulin was performed in EONE laboratories (Incheon, Korea).

Erythrocytic antioxidant enzyme activities

For measuring superoxide dismutase (SOD) activity, 500 μL of erythrocytic hemolysate was dissolved in ethanol and chloroform. After centrifugation at 10,000 × g for 2 minutes, supernatant stood at 25°C for 10 minutes. The reaction was initiated by the addition of 20

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μL of pyrogallol and measured at 320 nm for 180 seconds using a spectrophotometer. SOD activity was defined as the amount of the SOD which inhibits the reaction by 50%.

Catalase (CAT) activity was measured as follows. Erythrocytic hemolysate 100 μL preparation was dissolved in 50 mM phosphate buffer 50 mL (pH 7), and 2 mL of the mixture was added to a cuvette. The reaction was initiated by the addition of 1 mL H_2O_2 30 nM at 20°C. The H_2O_2 decomposition rate was measured at 240 nm for 30 seconds using a spectrophotometer.

Erythrocytic hemolysates were prepared by the dilution of erythrocytes to 1:20 (glutathione peroxidase [GSH-Px], SOD) and 1:500 (CAT) with distilled H_2O. For measuring GSH-Px activity, erythrocytic hemolysate 10 μL preparation was added to 100 μL of 1M Tris-HCl 5 mM ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0), 20 mL of glutathione 0.1 M, 100 μL of glutathione reductase 10 U/mL, and 100 μL of NADPH 2 mM, and filled with H_2O to a final volume of 1 mL. After 10 minutes of incubation at 37°C, the reaction was initiated by the addition of 10 mL of t-butyl hydroperoxide, and the absorbance was measured at 340 nm. The reaction was run for 90 seconds, and the loss of NADPH was monitored by the change in A 340 nm/min.

Lipid peroxidation

The plasma thiobarbituric acid reactive substances (TBARS) content was analyzed using the method of Puntel et al. [13]. For the analysis of plasma TBARS content, 200 μL of plasma and 2 mL of the reaction solution (15% (w/v) trichloroacetic acid, 0.38% (w/v) thiobarbituric acid [TBA], 0.25 N hydrochloric acid) were mixed, and boiled at 100°C for 20 minutes. After centrifugation of the reaction solution, it was read at 540 nm against the reagent blank and compared with standards prepared from 1,1,3,3-tetraethoxypropane.

The baseline LDL conjugated diene (CD) levels were determined according to the methods outlined by Ahotupa et al. [14], with slight modifications. Plasma (100 μL) was added to 700 μL of heparin citrate buffer (0.064 M trisodium citrate, 50,000 IU/L heparin, pH 5.05), and this suspension was incubated for 10 minutes at room temperature. The insoluble lipoproteins were then sedimented by centrifugation at 1,000 × g for 10 minutes. The pellet was resuspended in 100 μL of 0.1 M Na-phosphate buffer containing 0.9% NaCl (pH 7.4). Lipids were extracted from 100 μL of the LDL suspension with chloroform-methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed at 234 nm using a spectrophotometer (Shimadzu, Tokyo, Japan). EDTA was added to the samples to prevent oxidation during sample preparation.

Comet assay

The alkaline comet assay was conducted according to Singh et al. [15] with minor modification. The isolated lymphocytes were suspended in phosphate-buffered saline with 100 μM H_2O_2 for 5 minutes on ice to induce oxidative stress. Frosted slides were prepared with a basal layer of 0.5% normal melting agarose, and lymphocytes mixed with 75 μL of 0.7% low melting agarose (LMA) were added to the slides. The slides were covered again with coverslips and kept in the refrigerator for 10 minutes. Then coverslips were removed and a top layer of 75 μL 0.7% LMA was added and the slides were again kept cold for 10 minutes. After removal of the coverslips, the slides were immersed in a jar containing cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauroylsarcosine, pH 10; 1% Triton X-100 and 10% dimethyl sulfoxide were added freshly) and stored in a refrigerator for 1 hour. After lysis, the slides were placed in a horizontal electrophoresis tank (Threeshine Co.,

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Ltd., Daejeon, Korea). The slides were covered with fresh alkaline buffer (300 mM NaOH, 10 mM Na_2EDTA, pH 13.0) at 4°C for 40 minutes. To electrophorese the DNA, an electric current of 25 V/300 ± 3 mA was applied for 20 minutes at 4°C. The slides were washed 3 times with neutralizing buffer (0.4 M Tris, pH 7.5) for 5 minutes at 4°C and then treated with ethanol for another 5 minutes. All of these steps were conducted under dark conditions to prevent additional DNA damage. The 50 μL of ethidium bromide (20 μL/mL) was added to each slide followed by analysis using a fluorescence microscope (LEICA DMLB, Bensheim, Germany). Images of 100 randomly selected cells (50 cells from each of 2 replicate slides) were analyzed from each subject. Measurements were made by image analysis (Komet 5.0; Kinetic Imaging, Liverpool, UK) to determine the percentage of DNA in the tail.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics (ver. 23.0; IBM, Chicago, IL, USA). Biochemical data was presented as means and standard error. The data was evaluated by one-way analysis of variance and the differences between the means were assessed using Duncan's multiple-range test. The all parameters between Δ0–3 weeks and Δ3–6 weeks were considered significant at $p < 0.05$ by Student's t-test.

RESULTS

Baseline characteristics and biochemical parameters

Changes of baseline characteristics and biochemical parameters of study participants are presented in **Table 1**. No side effects were reported in any subjects. The average BMI of subjects was 24.0 ± 0.9 kg/m^2, which was considered in the overweight range. Their systolic BP was 128.1 ± 4.3 mmHg, which was within the border range. Their diastolic BP was 79.8 ± 2.1 mmHg, which was within the normal range. The average fasting blood glucose level of subjects was 125.6 ± 4.8 mg/dL, indicating that they had impaired fasting glucose level. Their average daily intake of calcium was 343.2 ± 66.9 mg, which was insufficient according to the suggested dietary reference intake of calcium for Koreans 2020 (male: 700 mg, female:

Table 1. Changes in anthropometric and biochemical parameters with/without CFB intake

Variables	0 wk	3 wk	6 wk	Δ3–0 wk	Δ6–3 wk	p value
Age (years)		63.8 ± 3.2				
Height (cm)		164.5 ± 11.2		-	-	-
Weight (kg)	64.1 ± 3.6	64.5 ± 3.6	64.6 ± 3.7	0.3 ± 0.1	0.1 ± 0.4	0.560
BMI (kg/m^2)	24.0 ± 0.9	23.8 ± 0.5	23.7 ± 0.5	−0.2 ± 0.4	−0.1 ± 0.2	0.735
Systolic BP (mmHg)	128.1 ± 4.3	132.9 ± 4.2	129.8 ± 4.8	4.8 ± 4.9	−3.1 ± 2.6	0.175
Diastolic BP (mmHg)	79.8 ± 2.1	77.6 ± 2.8	78.6 ± 3.2	−2.1 ± 1.4	1.0 ± 2.1	0.246
Total body fat (%)	30.4 ± 2.6	29.9 ± 2.8	30.1 ± 2.7	−0.5 ± 1.1	0.2 ± 0.4	0.567
Calcium intake (mg)	343.2 ± 66.9	459.4 ± 89.9	537.0 ± 110.3	116.16 ± 64.90	77.64 ± 91.50	0.736
Fasting glucose (mg/dL)	125.6 ± 4.8	133.1 ± 5.0	138.0 ± 5.9	7.5 ± 3.1	4.9 ± 5.2	0.672
Triglyceride (mg/dL)	99.4 ± 13.3	97.4 ± 15.2	116.0 ± 19.1	−2.0 ± 13.5	18.6 ± 9.9	0.239
HDL-cholesterol (mg/dL)	66.4 ± 4.6	65.6 ± 5.4	61.2 ± 4.4	−0.8 ±1.4	−4.4 ± 1.8	0.150
LDL-cholesterol (mg/dL)	122.2 ± 15.3	114.6 ± 9.5	111.4 ± 9.5	−7.4 ± 9.5	−2.6 ± 0.9	0.626
Total cholesterol (mg/dL)	213.9 ± 21.2	201.3 ± 13.0	194.3 ± 13.9	−12.6 ± 11.8	−7.0 ± 2.6	0.650
AST (U/L)	44.9 ± 10.9	29.0 ± 3.0	29.4 ± 2.3	−15.9 ± 9.2	0.4 ± 1.1	0.122
ALT (U/L)	27.6 ± 6.8	22.9 ± 3.6	19.3 ± 1.8	−4.8 ± 4.9	−3.6 ± 2.1	0.835
γ-GTP (U/L)	18.6 ± 2.4	18.3 ± 2.2	16.9 ± 1.8	−0.4 ± 1.2	−1.4 ± 0.8	0.512
Insulin (μ IU/mL)	2.7 ± 0.7	3.9 ± 0.4	3.3 ± 0.3	1.2 ± 0.6	−0.6 ± 0.4*	0.034
Grip strength (kg)	34.9 ± 3.3	34.9 ± 3.5	35.0 ± 3.7	−0.1 ± 1.1	0.1 ± 0.8	0.887

CFB, calcium fortified beverage; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.
*Δ3–0 weeks vs. Δ6–3 weeks, p value < 0.05.

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800 mg). Their triglyceride and HDL-cholesterol levels were 99.4 ± 13.3 mg/dL and 66.4 ± 4.6 mg/dL, respectively, which were within their normal ranges. Their LDL-cholesterol level was 122.2 ± 15.3, which was close to the normal range (optimal < 100, near optimal 100–129, borderline high 130–159). Their total cholesterol level was at a borderline level of 213.9 ± 21.2 mg/dL (desirable < 200 mg/dL, borderline high 200–239 mg/dL, high ≥ 240 mg/dL). Their AST level was 44.9 ± 10.9 U/L, which was higher than the normal level (≤ 40 U/L). Their ALT level was 27.6 ± 6.8 U/L, which was within the normal range (≤ 40 U/L). Their γ-GTP level was 18.6 ± 2.4 U/L, which was also within the normal range (9–64 U/L). Their average insulin level and grip strength were 2.7 ± 0.7 mg/dl and 34.9 ± 3.3 kg, respectively. All participants were apparently healthy. However, these parameters (except insulin) did not show significant differences between the period of no CFB intake and the period of CFB intake. Insulin secretion was increased during the period without CFB intake (2.7 ± 0.7 → 3.9 ± 0.4 μ IU/mL), but decreased during CFB intake (3.9 ± 0.4 → 3.3 ± 0.3 μ IU/mL). These differences were significant (Δ3–0 weeks: 1.2 ± 0.6 vs. Δ6–3 weeks: −0.6 ± 0.4, p = 0.034). The increase in fasting blood sugar was somewhat moderate in the period of CFB intake compared to that in the period without CFB intake. The difference between the 2 period was not statistically significant. Daily calcium intake was also increased after CFB intake. However, it showed no significant difference between the 2 periods.

Effect of CFB intake on erythrocyte antioxidant enzyme activity

Changes of erythrocyte antioxidant enzyme activity are shown in **Table 2**. SOD was increased after CFB intake, although such increase was not statistically significant. GSH-Px was decreased after CFB intake, although such decrease was not statistically significant. However, CAT was found to be significantly increased after CFB intake (Δ3–0 weeks: −12.5 ± 4.4 vs. Δ6–3 weeks: 3.5 ± 5.3, p = 0.036).

Effect of CFB intake on lipid peroxidation

Effects of CFB on lipid peroxidation of subjects are shown in **Table 3**. Both malondialdehyde (MDA) and CD were decreased after CFB intake. However, these values showed no significant differences after CFB intake.

Effect of CFB intake on H_2O_2 induced DNA damage

Figure 1 shows effects of CFB on markers of DNA damage as evaluated by the comet assay. % DNA in tail in cells induced by exposure to H_2O_2 was significantly decreased by CFB intake.

Table 2. Changes in erythrocyte antioxidant enzymes with/without CFB intake

Variables	0 wk	3 wk	6 wk	Δ3–0 wk	Δ6–3 wk	p value
SOD (IU g/Hb)	1,746.4 ± 167.7	1,749.0 ± 132.7	2,050.0 ± 109.3	2.6 ± 221.1	301.0 ± 212.3	0.347
GSH-Px (U g/Hb)	19.0 ± 3.5	19.1 ± 2.3	16.7 ± 1.5	0.1 ± 2.2	−2.4 ± 1.3	0.352
CAT (K g/Hb)	80.9 ± 4.0	68.4 ± 4.2	71.9 ± 6.8	−12.5 ± 4.4	3.5 ± 5.3*	0.036

CFB, calcium fortified beverage; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.
*Δ3–0 wk vs. Δ6–3 wk, p value < 0.05.

Table 3. Changes in lipid peroxidation with/without CFB intake

Variables	0 wk	3 wk	6 wk	Δ3–0 wk	Δ6–3 wk	p value
MDA (nM)	2.5 ± 0.3	5.9 ± 2.7	3.5 ± 0.9	3.4 ± 3.9	−2.4 ± 3.0	0.265
CD (μM)	6.7 ± 0.5	6.7 ± 0.4	6.4 ± 0.4	0.08 ± 0.1	−0.4 ± 0.4	0.340

CFB, calcium fortified beverage; MDA, malondialdehyde; CD, conjugated diene.

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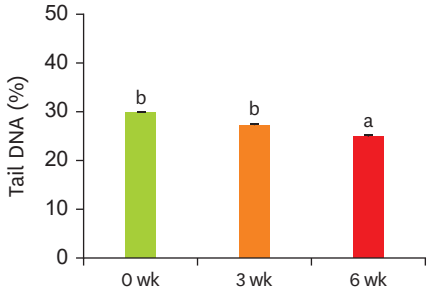

Figure 1. Changes in H_2O_2-induced DNA damage with/without CFB intake. Means with different superscript letters are significantly different at $p < 0.05$ by a Duncan's multiple range tests.
CFB, calcium fortified beverage.

DISCUSSION

Most older people, except for some in the city, have an unbalanced diet, which is usually low in calories, protein, vitamins, and minerals [16]. In particular, the lack of calcium intake was found to be serious. The average daily calcium intake was 305.9–347.1 mg for some elderly women living in Seoul [17] and 393.5–439.3 mg for the elderly living in Gyeongsan [18]. The average daily calcium intake was only 321.3–377 mg for long-lived elderly in Ganghwa. According to these studies, the calcium intake of the elderly was found to be less than 50% of the Dietary Reference Intake for Koreans 2020. In addition, the average daily calcium intake of the elderly who participated in this study was 343.2–459.4 mg, which was similar to those of previous studies. Reduction of calcium content in bone increases ROS production [19], which can lead to an imbalance in the antioxidant system, leading to bone loss [20,21].

Most studies on calcium intake in the elderly analyzed effects of calcium supplementation on fractures and osteoporosis [22-24]. Those studies confirmed that calcium supplementation had an ameliorating effect on osteoporosis and fractures. However, there have been few studies on the effect of calcium intake in the form of food on antioxidant metabolism in healthy elderly subjects. Therefore, in this study, as part of diversification of calcium sources, CFB was prepared using sprouted barley grass and chickpea powder with high calcium content. Sprouted barley grass consists of significant quantities of Zn, Fe, Ca, Mg, K, β-carotene, folic acid, chlorophyll, vitamin C, vitamin B_{12} and pantothenic acid [25]. It is not only utilized as a common, green-colored drink [26], but also used to inhibit chronic diseases, particularly diabetes and circulatory disorders. It also had cholesterol reducing effect, anti-obesity, anti-cancer, anti-arthritis, anti-inflammation, and antioxidant activities [25]. Chickpea is a relatively inexpensive source of different vitamins, minerals [27-29], and several bioactive compounds (phytates, phenolic compounds, oligosaccharides, enzyme inhibitors, and so on) that could potentially lower the risk of chronic diseases. Due to its potential nutritional value, chickpea is gaining consumer acceptance as a functional food.

Daily calcium intake increased as much as calcium content in CFB at 6 weeks of taking CFB compared to that at 0 and 3 weeks without CFB. However, there was no significant difference between these values. When CFB was not taken for weeks 0–3 and when it was taken for weeks 4–6, differences in values of all anthropometric and biochemical parameters except insulin by periods were not significant. Although the subjects in this study consisted of healthy elderly, their average blood glucose was 125.6 mg/dL at 0 weeks, which was found to be impaired fasting glucose. However, after CFB ingestion, insulin concentration decreased

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significantly and fasting blood sugar showed a tendency to decrease, so it could be said that it is effective in improving insulin sensitivity. Calcitriol might affect insulin sensitivity by stimulating insulin receptor expression and insulin reactivity during glucose transport in cultured human promonocytic cells. Calcitriol can also stimulate intracellular free calcium [30] and increase insulin secretion, which has a beneficial effect on both insulin secretion and sensitivity, thereby improving glucose metabolism [31].

Activities of SOD and GSH-Px as components of antioxidant defense mechanisms were unaffected by CFB intake. SOD, a first-line enzymatic antioxidant, plays an important role in protecting cells from oxidative damage by catalyzing the transformation of superoxide anion to H_2O_2 and molecular oxygen. H_2O_2 is reduced to water by CAT and GSH-Px [32]. In the present study, levels of CAT were significantly increased at 4–6 weeks than those at 0–3 weeks. Osteoporotic women were found to have significantly depressed activities of CAT, GPx and SOD, compared to those found in healthy control women [33-35]. Furthermore, concentrations of the antioxidant enzymes SOD and CAT have been positively correlated with BMD which demonstrates a link between antioxidant status and BMD in postmenopausal women [35].

TBARS assay using TBA as a reagent is an indirect measure of ROS. TBARS are formed as by-products of lipid oxidative damage (i.e., as degradation products of fats). TBA can react with MDA, one of the several low-molecular weight end products formed from the decomposition of some primary and secondary lipid peroxidation products [36]. CD is the first substance produced by lipid peroxidation. It indicates the degree of oxidation of LDL [37]. CFB showed a tendency to decrease MDA, a product of lipid peroxidation in plasma. It also showed a tendency to inhibit LDL oxidation. However, such tendency did not reach statistical significance. Decrease in antioxidant enzyme activity due to osteoporosis increases serum MDA levels, and intake of antioxidant nutrients, including calcium, may have a positive effect on this mechanism [38]. However, since the subjects of this study did not have osteoporosis disease, the production of lipid peroxide was not excessive, and CFB intake only showed a tendency to suppress it.

Comet assay is a relatively fast, simple, and sensitive technique for the analysis of DNA damage in all cell types. It has been applied for screening chemicals, biomonitoring, and intervention studies [39]. Hydrogen peroxide challenge assay was used in this study to detect DNA damage in leukocytes in response to CFB consumption. CFB consumption decreased DNA damage levels in elderly's leukocytes upon H_2O_2 treatment compared to no CFB intake. Decreased oxidative DNA damage has also been reported after the use of calcium supplements in normal human colorectal mucosa [40]. The secretion of high insulin by dietary calcium deficiency is a leading cause of oxidative stress [41,42]. Therefore, adequate calcium supplementation is thought to inhibit DNA damage by reducing oxidative stress through amelioration of insulin-potentiating effects and some antioxidant enzymes.

However, this study has some limitations, including its small sample size, short intervention period, experimental design, and duration. Based on limitations of existing literature, future studies should aim to improve several aspects. A sufficiently large sample should be used to obtain more precise estimates. More rigorous experimental designs such as RCT should be adopted to clearly demonstrate causal effects. Finally, the experiment and follow-up periods need to be sufficiently long enough to assess the evolution and long-term effect of the intervention.

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In conclusion, in a group of healthy and free-living elderly, a short-term CFB intervention was well accepted. Consumption of CFB increased antioxidant status as shown in the improvement of insulin sensitivity, CAT activity, and H_2O_2-induced DNA damage. These results suggest that CFB is a promising agent for the management of diabetes and antioxidant metabolism. However, future studies with large sample sizes and more rigorous experimental design considering long-term effects are needed.

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