

Effects of Paprika (*Capsicum annuum* L.) on Serum Lipid Profile and α -Tocopherol Concentration in Rats Fed a High-Cholesterol Diet

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파프리카 급여가 고콜레스테롤 식이 흰쥐의 혈청 지질과 α -Tocopherol 농도 변화에 미치는 영향

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Abstract

본 연구는 추출물 형태에 따른 파프리카 첨가 식이가 고콜레스테롤 식이 흰쥐의 혈청 지질과 α -tocopherol 농도에 미치는 영향을 알아보기 위해 Sprague-Dawley 수컷 25마리를 대조군(NC), 고콜레스테롤군(HC), 고콜레스테롤+동결 건조 파프리카 분말 섭취군(HC-FDP), 고콜레스테롤+파프리카 물 추출물 섭취군(HC-WEP), 고콜레스테롤+파프리카 에탄올 추출물 섭취군(HC-EEP)으로 나누어 실험 식이를 6주간 공급하였다. 6주 후 HC군과 파프리카 급여군에서 체중 증가는 각각 85.2%와 65.5~68.8%로 HC군에서 체중이 유의적으로 증가하였다. 그리고 내장지방 무게는 HC군보다 파프리카 급여군에서 감소하였으며, 파프리카 급여군의 중성지질, 총 콜레스테롤, LDL-콜레스테롤과 atherogenic index 역시 HC군보다 유의적으로 감소하였다($p<0.05$). 그리고 파프리카 급여군들 중에서는 HC-EEP 군이 혈청지질 농도 감소 효과가 가장 큰 것으로 나타났다. 혈청 α -tocopherol 농도는 HC-EEP에서 $30.5\pm 0.6 \mu\text{mol}/\ell$ 로 가장 높았고, 그 다음으로는 HC-WEP($30.0\pm 0.8 \mu\text{mol}/\ell$)>HC-FDP($29.1\pm 0.5 \mu\text{mol}/\ell$)>HC($27.7\pm 0.3 \mu\text{mol}/\ell$)이었다. 이는 고콜레스테롤 식이에 의한 산화적 스트레스를 감소시키기 위하여 혈청 α -tocopherol이 소모되는 것을 파프리카 급여로 절약하는 효과를 나타낸 것으로 사료된다. 따라서 본 연구를 통해 파프리카는 혈중 지질 농도 저하 효과를 보였고, α -tocopherol 절약 작용을 통해 체내 항산화제로서 활용 가능성을 확인할 수 있었다.

키워드: 적파프리카, 고콜레스테롤혈증, 혈청지질, α -토코페롤, 카로티노이드

INTRODUCTION

The intake of foods abundant in carotenoids is reported to correlate with decreasing death rates from cardiovascular disease (Peto et al. 1981; Gaziano & Hennekens 1993). Diseases such as arteriosclerosis, myocardial infarction, and stroke are known to be affected by diet. Their risk factors are high blood cholesterol, hypertension, smoking, diabetes, and obesity (Glowinska

et al. 2002). Therefore, many studies have been conducted to isolate hypocholesterolemic factors in foods and to determine their physiological functions scientifically.

Paprika (*Capsicum annuum* L.) is an important vegetable used for daily consumption (Materska & Perucka 2005). It is rich in capsanthin, capsorubin, and capsanthin 3,6-epoxide, which are categorized as xanthophylls (Peto et al. 1981). Capsanthin and capsorubin are known to scavenge singlet oxide and to inhibit

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the production of free radicals such as superoxide anion (O_2^-) and nitric oxide induced by lipid peroxidation (Murakami et al. 2000). In addition, various xanthophylls including capsanthin and β -carotene have been shown to inhibit lipid oxidation in animal and culture cell studies (Seppande & Csallany 2002). In particular, capsanthin is more effective in preventing the photooxidation of linoleic acid and destruction of polyunsaturated fatty acids than β -carotene, lutein, and lycopene, showing better antioxidant effects against free radicals and singlet oxygen exposed to plasma lipoprotein (Ojima et al. 1993).

Carotenoids are transported in the plasma with lipoproteins, predominantly low density lipoproteins (Krinsky et al. 1958). It is possible that they function, in part, to protect LDL from oxidation due to their ability to work as antioxidants (Sies et al. 1992). Such protection would be expected to decrease atherosclerosis and disease processes hypothesized to involve LDL oxidation (Berliner & Heinecke 1996.).

Therefore, this study was performed to investigate the effects of paprika on serum lipid concentrations and α -tocopherol levels in rats fed a high cholesterol diet.

MATERIALS AND METHODS

1. Preparation of Red Paprika

To prepare the freeze-dried paprika powder, red paprika was divided into four parts, cut by 3 mm in width with a food processor (Rondo 2500, TEFAL, Burgundy, France), freeze-dried (Bondiro, Ilshin Lab Co., Ltd, Korea) and grinded with a grinder (SQ-107, Ilzinjunggong Co., Korea). And then the grinded paprika passing a 20 mesh sieve was used as freeze-dried paprika powder. For water extract paprika (WEP), it was added distilled water which was 10 times of its weight (w/v) at a room temperature overnight and centrifuged at $1,000\times g$. It was filtered with a percolator (Whatman No. 4, Whatman Intl Ltd, England). The phase were separated and the extraction was repeated successively 2 times. After adding distilled water with five times of the weight to the sediment, it was extracted using stirrer for three hours and filtered it. Collected supernatant was used as its water extracts by freeze-drying. Filtered remainder was used for lipid soluble extracts. Lipid soluble extracts were prepared by following the same procedures of the water extracts using ethanol instead of distilled water. Yield rates of the materials were 10%, 8.6% and 4.3% in freeze dried paprika powder, WEP, and EEP, respectively.

2. Animals and Diets

Five week old male Sprague-Dawley (Charles River Laboratory Inc, MA, USA) rats were allowed free access to water and fed for the first week with a commercially prepared pellet diet for adjustment. The rats were then randomly divided into 5 groups of 5 rats and fed an tocopherol-stripped AIN-93G based control diet, supplementation with 1% cholesterol (HC), 1% cholesterol with 7.5% freeze dried red paprika (FDP), 1% cholesterol with 6.4% WEP, or 1% cholesterol with 3.2% EEP for 6 weeks. The supplemented amounts of paprika were high but safe. Jang et al. (1992) reported that *Capsicum annum* was fed at levels of 0.5, 1, 2.5, 5, 7.5, and 10% by weight to B6C3F1 mice. This was similar species paprika used in our research and supplemented as powder. The amounts of paprika extracts were equal to 7.5 g/100g FDP power. Table 1 shows diet composition for each group. The temperature and relative humidity of the breeding room were maintained at $22\pm 2^\circ C$ and $55\pm 5\%$, respectively, with 12 hr light and dark cycle. Animals in this study had a free access to water and diet, and their weight was measured once a week. Food intake was measured by placing a stainless steel dish containing a weighed amount of food into each cage and reweighing it 24 h later. Food efficiency ratio (weight gain/feed intake) describing the entire test period were calculated.

3. Collection of Blood Sample and Organ

Blood collection was conducted in 0, 3 and 6 weeks. Fasting blood were collected in ophthalmic venous plexus, coagulated, centrifuged at $1,000\times g$ for 15 min and stored at $-20^\circ C$. At 6 weeks, liver, heart, kidney, testis, and visceral fat were collected. The visceral fat combined epididymal with peritoneal fat pad.

4. Measurement of Serum Lipid Profile

Serum lipid profiles (triglycerides; total cholesterol, Total-C; HDL-cholesterol, HDL-C) were measured using assay kits with 200 μl of serum from Asan Pharm (Seoul, Korea) and a spectrophotometer (CM-3400d, Minolta, Japan).

LDL-cholesterol (LDL-C) and atherogenic index (AI) were calculated with Friedewald Formula (Friedewald and Levey 1972) and AI Formula, respectively, to determine risks of cardiovascular diseases was done with a following formula.

$$LDL-C = Total-C - (HDL-C + Triglyceride / 5)$$

$$Atherogenic\ index = (Total-C - HDL-C) / HDL-C$$

Table 1. Diet composition¹⁾

Ingredient	Amount (g/kg)				
	NC ²⁾	HC	HC-FDP	HC-WEP	HC-EEP
Casein	200.0	200.0	200.0	200.0	200.0
Corn starch	543.5	528.5	528.5	528.5	528.5
Dextrose	100.0	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0	50.0
Tocopherol stripped soybean oil ³⁾	70.0	70.0	70.0	70.0	70.0
Mineral mix ⁴⁾	35.0	35.0	35.0	35.0	35.0
Vitamin mix ⁵⁾	10.0	10.0	10.0	10.0	10.0
Biotin (1 mg/g biotin sucrose mix)	4.0	4.0	4.0	4.0	4.0
Cholesterol		10.0	10.0	10.0	10.0
Cholic acid		5.0	5.0	5.0	5.0
Total	1,012.5	1,012.5	1,012.5	1,012.5	1,012.5
Paprika (%)			7.5	6.4	3.2

¹⁾ Formulated and supplied from Dyets, Bethlehem, PA, according to the recommendations of the AIN,

²⁾ NC, normal control diet; HC, high cholesterol diet; HC-FDP, high cholesterol diet + 7.5% freeze drying paprika; HC-WEP, high cholesterol diet + 6.4% water extract of fresh paprika; HC-EEP, high cholesterol diet + 3.2% ethanol extract of fresh paprika,

³⁾ Contained 0.02% tert-butylhydroquinone, ⁴⁾ AIN-93G mineral mix, ⁵⁾ AIN-93VX vitamin mixture.

5. Serum α -Tocopherol Analysis

Serum α -tocopherol concentration was quantified according to a method of Bieri et al. (1979). One hundred microliter of serum was mixed with 50 μ g of α -tocopherol acetate (Sigma, USA) as an internal standard and extracted with hexane for high performance liquid chromatography (HPLC). Supernatant was collected after centrifuging at 1,000 \times g for 5 min and this procedure was repeated totally three times to obtain final samples. The samples were analyzed by injecting them into HPLC (Beckman, USA) after filtering them with syringes by using membrane filters with 0.45 μ m in diameter and dissolving them with diethyl ether and methanol (1:3) mixed solution. At this time, Pinnacle II C18 (Restek, USA) was used as column, mobile phase was methanol and flow rate was 2.0 ml/min. The analysis was performed at 295 nm.

6. Statistical Analysis

Statistical analysis was done using SPSS program. Data were presented as mean \pm S.E. and the differences among experimental groups analyzed by one-way analysis of variance (ANOVA) with Duncan's multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

1. Body Weight, Food Consumption and Food Efficiency Ratio

The body weights, food consumption, and food efficiency ratios of the rats fed experimental diets for 6 weeks are presented in Table 2. Initial body weight was not significantly different among the groups. The final body weight of the HC group, 462.7 \pm 2.0 g, was increased by 85.2% (212.4 g) after feeding the diet for 6 weeks. The final body weights of the FDP, WEP, and EEP groups increased by 67.4, 68.8, and 65.5%, respectively, compared to their initial weights, and the increased rates were significantly lower than that of the HC group. Food intake was highest in the WEP group as 21.1 g/day, and lowest in the HC group as 20.1 g/day. However, food efficiency ratio, presenting increased weight to food consumption, was the highest in the HC group. Sappanen & Csallany (2002) reported that the weights of rats fed paprika extracts (0.5-1%) were less than those of rats fed 0.5% β -carotene. According to Ikeuchi et al. (Ikeuchi et al. 2007) mice fed a high-fat diet showed lower increased rates of body weight when they were supplemented with astaxanthin (6 mg/kg and 30 mg/kg). The authors suggested that lower rates were observed because astaxanthin inhibited the use of glucose and increased the use of fatty acids, which augmented energy consumption. However, carotenoids contained in paprika, such as capsanthin and capsorubin, are different with astaxanthin. Therefore, the mechanism by which paprika exerts its weight loss effects must be further researched.

Table 2. Changes of body weights, food intakes, and food efficiency ratio (FER) in rats fed with high cholesterol diets containing paprika

	NC ¹⁾	HC	HC-FDP	HC-WEP	HC-EEP
Initial body weight (g)	243.2±1.0 ²⁾	250.3±5.4	248.9±2.7	251.9±1.9	249.3±2.0
Final body weight (g)	429.4±4.5 ^{b3)}	462.7±2.0 ^a	416.4±2.0 ^{cd}	425.2±3.6 ^{bc}	412.6±4.3 ^d
Weight gain ratio (%)	76.6±2.0 ^{ab}	85.2±4.1 ^a	67.4±1.9 ^b	68.8±1.1 ^b	65.5±2.1 ^b
Weight gain (g/day)	4.4±0.1 ^b	5.1±0.1 ^a	4.0±0.1 ^c	4.1±0.1 ^{bc}	3.9±0.1 ^c
Food intake (g/day)	20.3±0.3 ^{bc}	20.1±0.2 ^c	20.8±0.2 ^{ab}	21.1±0.1 ^a	20.5±0.1 ^{abc}
FER ⁴⁾	0.2±0.0 ^b	0.3±0.0 ^a	0.2±0.0 ^c	0.2±0.0 ^c	0.2±0.0 ^c

¹⁾ NC, normal control diet; HC, high cholesterol diet; HC-FDP, high cholesterol diet + 7.5% freeze drying paprika; HC-WEP, high cholesterol diet + 6.4% water extract of fresh paprika; HC-EEP, high cholesterol diet + 3.2% ethanol extract of fresh paprika,

²⁾ Values are mean ± S.E. (n=5),

³⁾ Means with the different letters in the same row are significantly different ($p<0.05$) by Duncan's multiple range test,

⁴⁾ Food efficiency ratio (FER) = Weight gain (g/day)/Food intakes (g/day).

2. Effect of Paprika Supplementation in Serum Lipid Profile

Higher triglyceride concentrations following a cholesterol-rich diet are considered to be attributed to increased activation of lipogenic enzymes caused by increases in serum cholesterol levels (Steinberg et al. 1989). In all groups, the average serum triglyceride concentration was 65.4±5.7 mg/dl before eating the

experimental diet (Fig. 1). After 6 weeks the paprika fed groups had significantly lower triglyceride levels than the HC group. Among them, the HC-EEP group showed the most effective inhibition of increased triglyceride concentration by presenting the lowest level of 75.0±2.7 mg/dl. Serum cholesterol was reduced from about 280.3±7.0 mg/dl to 98.7±3.8 mg/dl in the HC-EEP group. In HC group, the concentrations of total cholesterol was significantly higher than other groups, while its concentrations was significantly lower in paprika fed groups than HC group. The increase of total cholesterol concentrations by high cholesterol diet was similar with the result of Kim et al. (1999).

The HDL-C concentrations of all the paprika groups were significantly higher than that of the HC group, and those of the HC-WEP group (52.0±1.8 mg/dl) and the HC-EEP group (51.9±2.1 mg/dl) were higher than that of the FDP group (47.1±2.7 mg/dl) (Table 3). In terms of LDL-C concentration to induce atherosclerosis, levels for FDP, WEP, and EEP were 36.5±2.2, 65.8±6.1, and 30.3±3.4 mg/dl, respectively, and these levels were significantly lower than that of the HC group (216.0±7.6 mg/dl). Paprika supplementation was found to inhibit the production of LDL-C by 3-7 times (Table 3). Aizawa and Inakuma (2009) reported that the administration of purified capsanthin for 2 weeks resulted in significant increases in plasma HDL-C concentrations. The results of the present research are in agreement with their data. An explanation is that capsanthin has an HDL-C concentration-raising effect on plasma, and the potential to increase cholesterol efflux to HDL particles by increasing apoA5 levels and/or the enhancement of LCAT activity. Xanthophylls

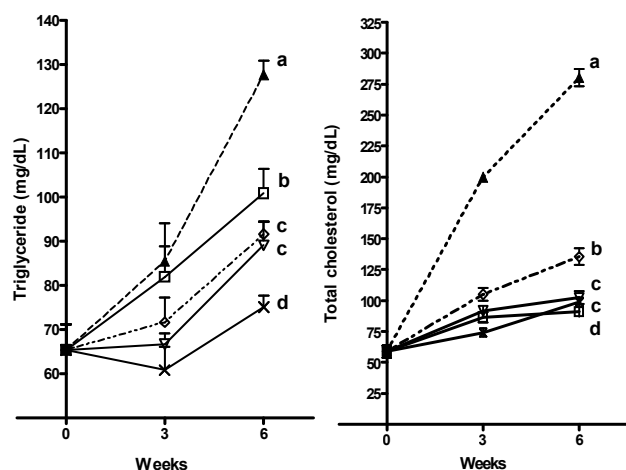


Fig. 1. Levels of triglyceride and total cholesterol in the serum of rats fed with high cholesterol diets containing paprika for 6 weeks.

-□-; normal control diet -▲-; high cholesterol diet, -▽-; high cholesterol diet+7.5% freeze drying paprika, -◇-; high cholesterol diet+6.4% water extract of fresh paprika, -×-; high cholesterol diet+3.2% ethanol extract of fresh paprika.

^{a-d} Values with different superscript indicate significant difference ($p<0.05$) between the groups by Duncan's multiple range test

Table 3. Levels of HDL-cholesterol, LDL-cholesterol and α -tocopherol in the serum and the weight of visceral fat in rats fed with high cholesterol diets containing paprika

	HDL-cholesterol (mg/dl)	LDL-cholesterol ¹⁾ (mg/dl)	AI ²⁾	α -Tocopherol (μ mol/l)	Visceral fats (g/100 g body weight)
NC ³⁾	44.6 \pm 0.7 ^{4)bs)}	26.4 \pm 1.7 ^d	1.0 \pm 0.0 ^c	30.2 \pm 0.8 ^a	5.0 \pm 0.1 ^a
HC	38.8 \pm 0.9 ^c	216.0 \pm 3.4 ^a	6.2 \pm 0.2 ^a	27.7 \pm 0.3 ^{bs2)}	5.1 \pm 0.2 ^a
HC-FDP	47.1 \pm 1.2 ^b	36.5 \pm 1.0 ^c	1.2 \pm 0.0 ^c	29.1 \pm 0.5 ^{ab}	3.7 \pm 0.2 ^b
HC-WEP	52.0 \pm 0.9 ^a	65.8 \pm 1.5 ^b	1.6 \pm 0.1 ^b	30.0 \pm 0.8 ^a	4.0 \pm 0.1 ^b
HC-EEP	51.9 \pm 0.8 ^a	30.3 \pm 2.7 ^c	0.9 \pm 0.1 ^c	30.5 \pm 0.6 ^a	4.0 \pm 0.2 ^b

¹⁾ LDL cholesterol = Total cholesterol - (HDL cholesterol + Triglyceride/5),

²⁾ AI (Atherogenic index) = (Total cholesterol - HDL-cholesterol) / HDL-cholesterol,

³⁾ NC, normal control diet; HC, high cholesterol diet; HC-FDP, high cholesterol diet + 7.5% freeze drying paprika; HC-WEP, high cholesterol diet + 6.4% water extract of fresh paprika; HC-EEP, high cholesterol diet + 3.2% ethanol extract of fresh paprika,

⁴⁾ Values are mean \pm S.E. (n=5),

⁵⁾ Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

work as more effective antioxidants in oxidation responses caused by free radicals or active oxygen of HDL-C as compared to hydroxycarbone carotenoids (Ojima et al. 1993, Boey et al. 1992). Therefore, the high HDL-C levels of the paprika fed groups are considered to have followed the high antioxidant activities of capsanthin and capsorubin in the paprika with high affinity for HDL-C. In terms of LDL-C, the paprika fed groups had significantly lower LDL-C levels than the HC group. In terms of LDL-C, the paprika fed groups had significantly lower LDL-C levels than the HC group. Fuhrman et al. (1997) found that when 6 healthy males ate 60 mg/day of lycopene from tomatoes for 3 months their serum LDL-C concentrations were reduced by 14%, and suggested that the decrease was due to the inhibited activation of HMG-CoA reductase related to cholesterol synthesis. The inhibition of LDL-C oxidation following β -carotene supplementation in the diets of humans and animals was reported to result from β -carotene's effective elimination of free radicals, with β -carotene locating in cellular membranes and inside lipoproteins (Allard et al. 1994, Niki et al. 1995). Therefore, carotenes from paprika distributed in LDL-C are thought to decrease serum cholesterol concentration by a similar mechanism. AI is closely related to the occurrence of cardiovascular diseases including arteriosclerosis (Kang et al. 2004). The HC group had the highest AI value at 6.2 \pm 0.5, but the values of the paprika fed groups were greatly decreased. The HC-WEP group, with high triglycerides and LDL-C, showed a significantly higher AI value (1.6 \pm 0.1) compared to the other paprika fed groups ($p < 0.05$). The HC-EEP group (0.9 \pm 0.2) and HC-FDP group

(1.2 \pm 0.1) had significantly lower AI values than the HC-WEP group. As a result, paprika supplementation would be expected to have effects in reducing the risk of arteriosclerosis.

3. Effect of Paprika Supplementation in Serum α -Tocopherol

Increases in serum α -tocopherol concentrations after vitamin A and β -carotene supplementation are known to be attributed to the inhibition of oxidation and lipid peroxidation of LDL-C caused by antioxidants (Jang et al. 2002). The serum α -tocopherol level of the HC group was 27.7 \pm 0.3 μ mol/l after feeding it a tocopherol-stripped AIN-93G diet, and was significantly lower than the levels of the HC-EEP group (30.5 \pm 0.6 μ mol/l) and HC-WEP group (30.0 \pm 0.8 μ mol/l) (Table 3, $p < 0.05$). In Nam's results (1994), when fish oil with high polyunsaturated fatty acid supplemented to rats fed high fat diets, plasma α -tocopherol concentrations reduced for inhibiting autooxidation of PUFA. The finding that the α -tocopherol concentrations of the paprika fed groups were higher than that of the HC group is considered to have resulted from antioxidant activity of carotenoid contained in paprika by inhibiting the oxidation of triglycerides, total cholesterol, and LDL-C after consuming the high cholesterol diet. Paprika feeding was considered to inhibit the oxidation of serum lipids leading to decreases in α -tocopherol status. In fact, LDL-C and α -tocopherol had a slightly negative correlation ($r = -0.54$, $p < 0.05$). Nicolle et al. (2003) showed that total cholesterol concentrations in the plasma of rats were decreased after feeding freeze dried carrot powder, and plasma α -tocopherol concentrations of the carrot powder fed group were higher than those of the

control group, which did not receive carrot powder. Therefore, the consumption of α -tocopherol inside the body was considered to be inhibited by paprika feeding.

Fat tissue weights around the liver, heart, and testis were not significantly different among the feeding groups, but in the HC-EEP group the weight around the kidneys was significantly higher. Visceral fat weight was highest in the HC group at 5.1 ± 0.2 g, and those of the HC-FDP, HC-WEP and HC-EEP groups were 3.7 ± 0.2 , 4.0 ± 0.1 , and 4.0 ± 0.2 g, respectively and compared to that of the HC group (Table 3). The accumulation of visceral fat was significantly reduced in the paprika fed groups compared to the HC group. This result is somewhat similar to that of Ikeuchi et al. (2007) reporting that astaxanthin feeding reduced increased abdominal weight caused by a high fat diet, but it did not cause significant differences in weights of the kidney, spleen, or heart.

SUMMARY

In conclusion, rats were fed diets containing either NC, HC, HC-FDP, HC-WEP, or HC-EEP for 6 weeks. At the 6th week, increases in body weight and visceral fat were lower in the paprika fed groups as compared to the HC group. Serum levels of triglycerides, total cholesterol, low density lipoprotein cholesterol, and atherogenic index values were significantly lower in the paprika diet fed groups than the HC group ($p < 0.05$). In particular, the lipid lowering effects in the HC-EEP group were superior among the paprika fed groups. Also, serum α -tocopherol levels were lower in the control group compared to the paprika fed groups. The supplementation of paprika may exert lipid lowering effects and saving effect of α -tocopherol in the serum of high-cholesterol diet fed rats. However, it should be noted that the results are based on very small sample numbers and a short experimental period.

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