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The Effects of Astaxanthin Supplements on Lipid Peroxidation and Antioxidant Status in Postmenopausal Women

Yoo Kyung Kim and Jong-Hee Chyun[§]

Department of food & nutrition, Inha University, 253 Yonghyundong, Namgu, Incheon 402-751, Korea

In postmenopausal women, the incidence of cardiovascular disease(CVD) is common and there is growing evidences that astaxanthin has a strong antioxidant capacity and plays a beneficial role in the prevention of CVD. However, current data are not sufficient to determine the effect of astaxanthin on improving lipid profiles and antioxidant capacity in human. In this study, 15 healthy postmenopausal women were divided into 3 groups and given astaxanthin supplements of 0, 2 or 8mg/day for 8 weeks. Blood samples were taken before and after 4 and 8 weeks of astaxanthin supplementation for analysis of serum total choelsterol, LDL-cholesterol, HDL-cholesterol, triglyceride, plasma TBARS, total antioxidant status(TAS) and urinary 8-isoprostanes. HDL-cholesterol levels in 2mg and 8mg group increased significantly after 8 weeks from 50.6±5.8 to 60.4±7.1mg/dℓ, 44.4±10.7 to 49.4±2.7mg/dℓ respectively ($p<0.05$). In the 2mg group, triglyceride decreased significantly from 171.6±67.4 mg/dℓ to 145.8±5.1mg/dℓ ($p<0.05$). Plasma TBARS level in the 2mg group decreased from 1.42± 0.18nM/mg to 1.13±0.18nM/mg after 8 weeks ($p<0.05$). In the 8mg group, TBARS level decreased significantly from 1.62±0.14nM/mg to 1.13±0.12nM/mg after 8 weeks ($p<0.05$). TAS, as an indicator of lipid peroxidation, increased significantly from 0.85±0.42mM/ℓ to 1.90±0.58mM/ℓ after 8 weeks in the 8mg group ($p<0.05$). Urinary 8-isoprostanes excretion did not decrease significantly with astaxanthin supplementation. In conclusion, it would be helpful for postmenopausal women with common cardiovascular disease to supplement with astaxanthin as an antioxidant.

Key Words : Astaxanthin, Lipid peroxidation, TAS, 8-isoprostanes, Postmenopausal women

INTRODUCTION

Coronary atherosclerosis and cardiovascular disease are less prevalent in women than in age-matched men[1,2]. However, natural and surgical postmenopausal women are more likely to develop coronary artery disease than pre-menopausal women of the same age[3]. A number of studies have examined the prevalence of coronary artery disease in postmenopausal women[4,5].

The presently known cardiovascular risk factors are obesity, hypertension, diabetes, smoking, sedentary life style, etc, which add up to the metabolic syndrome[6]. Considerable research supports the hypothesis that the oxidation of LDL in the blood vessels plays a significant role in the development of atherosclerosis. Evidence of a major role for LDL-cholesterol in cardiovascular disease has mainly been reported, while some aspects of the role of LDL-cholesterol have been disputed[7,8]. The establishment of the important role of LDL cholesterol in cardiovascular disease showed that native LDL particles needed to be oxidative or enzymatically modified in order to be taken up by macrophages and become atherogenic[6,9]. For these reasons, factors that influence the oxidation of LDL lipids have been the subject of several investigations and a series of clinical studies have explored the potential role of antioxidants in cardiovascular functions[10].

In fact, free radical(hydroxyl and peroxyl radicals) and highly reactive forms of oxygen(singlet oxygen) are produced in the body during normal metabolic reactions and processes. Physiological stress, air pollution, tobacco smoke, exposure to chemicals or exposure to ultraviolet light, can enhance the production of such agents. Phagocytes can also generate an excess of free radicals to aid in their defensive degradation of the invader. Free radicals can damage DNA, proteins and lipid membranes. Oxidative damage has been linked to aging, atherosclerosis, ischemia-repofusion injury, age-related muscular degeneration and carcinogenesis [11,12].

Therefore, antioxidants, such as antioxidant vitamins (vit A, vit C, vit E) and antioxidant enzymes(superoxide dismutase, catalase, glutathione peroxidase), might help to prevent and fight several human disease such as cardiovascular disease and cancer[10,13]. In particular carotenoids, one of the dietary antioxidants, are potent biological antioxidants that can absorb the excited energy of singlet oxygen onto the carotenoid chain, leading to the degradation of the carotenoid molecule but preventing other molecules or tissues from being damaged[14,15]. They can also prevent the chain reactive production of free radicals

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§To whom correspondence should be adressed.

initiated by the degradation of polyunsaturated fatty acids, which can dramatically accelerate the degradation of lipid membranes[16].

Carotenoids are thought to be associated with a number of health benefits and epidemiologic studies have shown an inverse relationship between the presence of various cancers and cardiovascular disease and a high intake of carotenoid containing food, such as fruits and vegetables[17]. Apart from their provitamin A activity, carotenoids exhibit antioxidant properties and can affect cell growth regulation, and modulate gene expression and immune response, all of which are possible mechanisms of relevance[18,19].

Astaxanthin is the main carotenoid pigment found in aquatic animals and is present in many popular seafoods including salmon, trout, red seabream, shrimp, lobster and fish eggs. Also astaxanthin is very good at protecting membrane phospholipids and other lipids against peroxidation. Astaxanthin is closely related to other well-known carotenoids, such as β-carotene, zeaxanthin and lutein, thus it shares many of the metabolic and physiological functions attributed to carotenoids. But it dose not have vitamin A activity and belongs to the xanthophylls groups. Astaxanthin's antioxidant activity has been demonstrated in several studies[20,21]. In some cases, astaxanthin has up to several-fold stronger free radical antioxidant activity than vit E and β-carotene. The scavenging properties of astaxanthin are believed to have a key role in several other areas such as reduction the risk of artherosclerosis and protection against UV-light photooxidation, inflammation, cancer, ulcer's *Helicobacterpylori* infection, aging or age-related diseases, dysfunction of the liver, heart, eye, joints and maintaining prostate health[22,23]. Recent studies with astaxanthin have shown that astaxanthin reduced oxidative stress, lowered the levels of serum lipid peroxides, enhanced cell-mediated immune responses, and inhibited mammary or bladder tumor growth[24].

Thus, we investigated the effects of astaxanthin supplements on lipid peroxidation and antioxidant status in the plasma and urine of postmenopausal women to find out the beneficial effect of astaxanthin supplementation on CVD prevention. Lipid peroxidation was expressed in levels of plasma thiobarbituric acid reactive substance (TBARS). We also evaluated TAS(total antioxidant status) and urinary 8-isoprostanes level as indicators of antioxidant status[25,26].

MATERIALS AND METHOD

1. Subjects and Design

Fifteen healthy non-hysteroctomized postmenopausal women participated in this study. The subjects had not experienced a menstrual period for at least 1 year. All were lifelong nonsmokers, none of them were taking any medication for hormone replacement, steroid drugs or vitamine or mineral supplements. They were divided into 3 groups and were given tablet type astaxanthin supplements(LaHaye Lab. UAS, >99%) of 0mg(placebo), 2mg or 8mg/day every morning before breakfast for 8weeks. Height, weight, body fat and BMI were measured using Inbody(Body composition analyzer, Biospace co., Ltd).

2. Blood and Urine Samples

Blood samples were taken before and after 4 and 8 weeks of astaxanthin supplementation. After the subjects fasted overnight, venous blood samples were collected, separated into plasma or serum and stored at -70℃ until analysis. Serum total cholesterol, HDL-cholesterol and triglyceride were measured using a commercial kit(Young Dong diagnostics Co., Korea) and LDL-cholesterol level was calculated by Friedqwald method[27]. Urine samples were also collected before and after 4 and 8 weeks of astaxanthin supplementation and stored at -70℃ until analysis.

3. Lipid Peroxidation and Antioxidant Status

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances(TBARS) in plasma using the method of Yagi[28]. A hundred $\mu\ell$ volume of plasma was suspended in distilled water followed by thiobarbituric acid-acetic acid reagent and then immersed in boiling water for 30 min. After cooling, the pink color was extracted in n-butanol, and measured at 531nm. 1,1',3,3'-tetramethoxypropane was used as the standard and results were expressed as nmol/㎖ plasma.

Total antioxidant status(TAS) is one of the methods for measuring antioxidant status in the body. TAS was assessed using a TAS method kit(Randox Laboratories Ardmore, UK). This is based on the activity of the free radical ferrylmyoglobin, which is formed by the oxidation of metmyoglobin by H_2O_2. Ferrylmyoglobin interacts with the ABTS®(2,2'-Azino-di-[3-ethylbin-zthiazoline sulphonate]) to form a blue-green chromophore radical. ABTS® absorbance was determined by UV-visible spectrophotometer at 600nm and was expressed as nmol/l[29].

The concentration of 8-isoprostanes (8-isoPGF$_{2\alpha}$) in the urine samples was analyzed using a commercial-available enzyme immunoassay kit(Oxis Bioxytech 8-isoprostane Assay. Cat No. 21019D) and analyzed using ELISA(ELx 800G, Bio-TeK Inst. USA). The absorbance was determined at 650nm and was expressed as pg/mg creatinine[30].

4. Statistical Analysis

The data were analyzed using the SPSS program and results were expressed in terms of means and standard deviations. Statistical comparisons between experimental

Table 1. General characteristics and anthropometric measurements of subjects

	astaxanthin supplementation levels(mg/day)			
	0mg	2mg	8mg	total[ns]
age(yr)	54.8±2.4[1)]	51.0±1.2	53.4±2.1	53.1±2.4
height(cm)	156.9±3.4	157.2±4.7	158.5±4.0	157.5±3.8
weight(kg)	55.9±5.3	58.7±1.9	59.6±9.7	58.1±6.2
body fat(%)	27.9±2.1	29.2±5.9	29.3±4.3	28.1±3.6
BMI[2)]	22.6±1.8	23.7±1.2	23.7±3.1	23.3±2.1

1) values are mean ±SD
2) BMI(body mass index) = weight(kg)/height(m)^2
ns: not significantly different among 3 groups

periods in the serum lipid, TBARS, TAS, and urinary 8-isoprostanes were made using paired *t*-test. Significance was defined at the 0.05 level of confidence.

RESULTS AND DISCUSSION

1. General Characteristics of Subjects

General characteristics of the postmenopausal women are given in Table 1. The mean age of the women was 53.1 years, and the range was 51-58 years. Mean height and weight of the subjects were 157.5 cm and 58.1 kg respectively. Mean body fat was 28.1 % and BMI was 23.3. There were no significant differences in age, height, weight, body fat and BMI among subjects in the 3 groups.

2. Serum Lipids Profiles

The data presented in Table 2 shows the significant difference in triglyceride and HDL-cholesterol levels. HDL-cholesterol level in the 2mg group increased significantly from 50.6±5.8 mg/dl to 56.4±8.3 mg/dl after 4 weeks, and increased to 60.4±7.1 mg/dl after 8 weeks of suppletation ($p<0.05$). HDL-cholesterol level in the 8 mg group increased significantly from 44.4±10.7 mg/dl to 49.4±2.7 mg/dl after 8weeks of astaxanthin supplementation ($p<0.05$).

In the case of triglyceride, the serum level of the 2 mg group decreased significantly from 139.2±51.4 mg/dl to 94.2±24.5 mg/dl after 8 weeks ($p<0.05$). In the 8 mg group, the triglyceride level increased from 171.6±67.4 mg/dl to 216.8±97.5 mg/dl after 4 weeks, and decreased to 145.8±5.1 mg/dl after 8 weeks. However, the changes were not significant.

Total cholesterol and LDL cholesterol levels in the 2 mg group and the 8 mg groups did not change significantly, as in the placebo group (0 mg). But total cholesterol in the 2 mg group decreased slightly from 199.0±28.1 mg/dl to 183.6±48.9 mg/dl after 8 weeks of astaxanthin supplementation. Although astaxanthin supplementation studies are rare, it is well known that other carotenoids reduce LDL oxidation[17)].

3. Plasma TBARS and Total Antioxidant Status

Plasma TBARS in the 2 mg group decreased significantly from 1.42±0.18 nM/ml to 1.13±0.18 nM/ml after 8 weeks ($p<0.05$, Table 3). Also, in the 8mg group, plasma TBARS decreased significantly from 1.62±0.14 nM/ml to 1.13±0.12 nM/ml after 8 weeks ($p<0.05$). Because there have been an insufficient number of studies of astaxanthin supple- mentation in human, the results could not be

Table 2. Serum lipid levels of postmenopausal women before and after astaxanthin supplementation with different levels.

(mg/dl)

	astaxanthin supplementation levels(mg/day)								
	0mg			2mg			8mg		
	0wk	4wks	8wks	0wk	4wks	8wks	0wk	4wks	8wks
Total cholesterol	184.2±43.9[1)]	190.4±18.2	202.8±17.7	199.0±28.1	192.8±41.4	183.6±48.9	193.6±29.7	189.8±17.8	198.0±14.7
Triglyceride	189.0±57.7	217.2±29.7	182.4±54.9	139.2±51.4	130.4±48.5	94.2±24.5[c]	171.6±67.4	216.8±97.5	145.8±5.1
HDL cholesterol	42.2±9.3	45.6±7.0	49.0±9.0	50.6±5.8	56.4±8.3[a]	60.4±7.1[c]	44.4±10.73	44.4±6.5	49.4±2.7[b]
LDL cholesterol	110.0±31.9	112.0±12.6	123.4±9.8	112.2±19.3	106.2±30.5	112.8±19.7	116.0±23.3	114.4±16.1	115.6±14.2

1) values are mean ±SD
a : significantly different by paired t-test at $p<0.05$(0-4weeks)
b : significantly different by paired t-test at $p<0.05$(4-8weeks)
c : significantly different by paired t-test at $p<0.05$(0-8weeks)

Table 3. Effect of astaxanthin supplementation on lipid peroxidation and antioxidant status in postmenopausal women

	astaxanthin supplementation levels(mg/day)								
	0mg			2mg			8mg		
	0wk	4wks	8wks	0wk	4wks	8wks	0wk	4wks	8wks
TBARS (nM/㎖)	1.28±0.19[1)]	1.23±0.15	1.43±0.17	1.42±0.18	1.29±0.19	1.13±0.18[c]	1.62±0.14	1.33±0.15	1.13±0.12[c]
TAS (mM/l)	1.43±0.62	1.39±0.29	0.93±0.64	0.93±0.59	1.33±0.46	1.76±0.26	0.85±0.42	1.38±0.65	1.90±0.58[c]
8-isoprostanes (pg/mg creatinine)	3.59±2.42	3.02±1.12	2.40±1.46	3.02±1.12	2.79±1.40	2.55±0.73	3.41±2.01	2.40±0.88	2.80±1.17

1) values are mean ±SD
a : significantly different by paired t-test at p<0.05(0-4weeks)
b : significantly different by paired t-test at p<0.05(4-8weeks)
c : significantly different by paired t-test at p<0.05(0-8weeks)

compared with other data on the subject. In animal study, Hiroshi et al.[23)] reported that administration of astaxanthin(100 mg/kg, 4days) in the female C57BL/6 and DBA/2 mice significantly reduced the TBARS levels of the liver (p<0.001). Chew et al.'s study[24)] also reported that TBARS was significantly lower in mice fed 0.4 % astaxanthin for 3 weeks as compared to unsupplemented mice (p<0.05). Tumor TBARS was 15.5 nM/g before feeding dietary astaxanthin and it had decreased significantly by 8.8 nM/g after 3 weeks of supplementation. These studies support our findings of reducing plasma TBARS with astaxanthin supplementation.

Achim et al.[31)] said that among the carotenoid-rich vegetable products, tomato juice consumption for 2 weeks reduced lipid peroxidation in healthy men. During the tomato juice intervention, plasma TBARS decreased by 12% from 1.62±0.49 nM/㎖ to 1.40±0.36 nM/㎖ after consumption (p<0.05).

Total antioxidant status(TAS) is an overall indicator of the antioxidant status of an individual. As the value increases, the antioxidant defense against free radical reaction increases[25)]. In this study, serum TAS in the 2 mg group increased from 0.93±0.59 mM/L to 1.76±0.26 mM/L after 8 weeks, although this increase was not significant. In the 8 mg group, however, TAS increased significantly from 0.85±0.42 mM/L to 1.90±0.58 mM/L after 8 weeks (p<0.05). The rate of increase was higher than that seen in postmenopausal women in Choi et al.'s study[32)] which looked at the effects of isoflavone supplementation. In the Choi's study, serum TAS increased significantly from 1.36± 0.16 mM/L to 1.43± 0.16 mM/L after 12 weeks of 200 mg isoflavone supplementation in postmenopausal women (p<0.05). Beacause TAS level roughly doubled in 8 weeks in our study, it is thought that astasxanthin supplementation is more potent than isoflavone supplementation for improving total antioxidant status. Miller et al.[33)] reported that the average plasma TAS of European was 1.30-1.77 mM/L.

4. Urinary 8-isoprostanes Concentration

Urinary 8-isoprostanes concentration tended to decrease in all groups, although the reduction was not significant(Table 3). The levels of urinary 8-isoprostanes in the 2 mg group were 3.02±1.12 pg/mg creatinine before supplementation, 2.79±1.40 pg/mg creatinine after 4 weeks, and 2.55±0.73 pg/mg creatinine after 8 weeks. In the 8 mg group, urinary 8-isoprostanes concentration decreased from 3.41±2.01 pg/mg creatinine to 2.40±0.88 pg/mg creatinine after 4 weeks and to 2.80±1.17 pg /mg creatinine after 8 weeks. But the placebo group also showed a reduction from 3.59±2.42 pg/mg creatinine to 2.40±1.46 pg/mg creatinine after 8 weeks. Therefore, we could not find any clear-cut effect of astaxanthin supplementation on urinary 8-isoprostanes reduction. In Eva et al.' study[34)], however, the urinary 8-isoprostanes level was significantly lower in vitamin E supplemented rats compared to that of the control rats (p<0.05).

8-Isoprostane, a chemically stable end product of arachidonic acid belonging to the F2-isoprostanes, has been found to reflect oxidative stress and lipid peroxidation *in vivo*[26)]. Thus, increased plasma and urinary levels of 8-isoprostane have been reported in human atherosclerosis and the presence of F2-isoprostanes has also been noted in oxidized LDL cholesterol. Moreover, in apoprotein E (apoE) knockout mice, decreased atherosclerosis has been found after suppression of F2-isoprostanes formation with vitamin E, suggesting that these F2-isoprostanes are not only markers but also potential mediators in atherogenesis[35,36)].

CONCLUSION

In this study, we observed that astaxanthin supplementation in postmenopausal women was somewhat effective on reducing lipid peroxidation and increasing antioxidant status. Astaxanthin supplementation did not

significantly change serum total cholesterol and LDL-cholesterol levels in postmenopausal women. However, serum HDL-cholesterol levels increased significantly and triglyceride levels decreased with the astaxanthin supplementation. Plasma TBARS levels decreased significantly with astaxanthin supplementation.

An indicator of the antioxidant status of the body, the level of TAS increased significantly with astaxanthin supplementation.

Urinary 8-isoprostanes concentration tended to decrease with the astaxanthin supplementation. However, reducing urinary 8-isoprostanes concentration had no clear-cut effect.

Therefore, it would be helpful for postmenopausal women with common cardiovascular disease to use astaxanthin as a dietary antioxidant. However, further research including large-scale randomized clinical trials are required to determine whether astaxanthin has any other antioxidant benefit in postmenopausal women.

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