

Salonen, J. (2001) Effects of astaxanthin supplementation of men on plasma astaxanthin levels, lipid peroxidation, cytochrome P-450 induction, and oxidative damage of DNA: A double- masked randomized placebo-controlled trial. Unpublished report.

29 August 2001/Tiina Rissanen, Jukka T. Salonen

STUDY REPORT

**EFFECTS OF ASTAXANTHIN SUPPLEMENTATION OF MEN ON PLASMA
ASTAXANTHIN LEVELS, LIPID PEROXIDATION, CYTOCHROME P450 INDUCTION
AND OXIDATIVE DAMAGE OF DNA: A DOUBLE-MASKED RANDOMIZED PLACEBO-
CONTROLLED TRIAL**

Principal Investigator:

Jukka T. Salonen, MD, PhD, MScPH, Professor of Epidemiology, Research Institute of Public Health and Faculty of Medicine, University of Kuopio, Kuopio, Finland (e-mail jukka.salonen@uku.fi, fax 358-17-162936)

Other investigators (in alphabetical order):

Ulf Diczfalusy, MD, Research fellow, Karolinska hospital, Stockholm, Sweden: Measurement of cholesterol oxidation products

Jari Kaikkonen, PhD, Biochemist, Oy Jurilab LTD: Lipid and lipoprotein measurements, Measurements of hydroxy fatty acids

Kristiina Nyysönen, PhD, Clinical Biochemist: Vitamin and fatty acid measurements

Anders Olsson, MD, PhD, Professor of Medicine, Lindköping, Sweden: Inflammation marker measurements

Elina Porkkala-Sarataho, PhD, Biochemist: Oxidation susceptibility measurements

Henrik Poulsen, MD, PhD, Professor of Clinical Pharmacology, Department of Clinical Pharmacology, Rigshospitalet, University Hospital Copenhagen, Denmark: Measurement of DNA oxidative damage marker

Tiina Rissanen, MSc, Nutritionist: Food recordings, statistical analyses, reporting

Kimmo Ronkainen, MPh, Computer analyst: Data management, statistical analyses

Riitta Salonen, MD, PhD, Research Investigator: Medical examinations of subjects

Tomi-Pekka Tuomainen, MD, Research Fellow: Medical examinations, reporting

Sari Voutilainen, PhD, nutritionist: Consultation, reporting

STUDY BACKGROUND

1.1 Atherosclerosis, lipid peroxidation and astaxanthin

Cardiovascular diseases are the leading cause of death and disease globally. Treatment of cardiovascular diseases (CVD) places a heavy burden on the health and economy, and all efforts to limit coronary heart disease are thus of major public health significance.

The traditional risk factors for coronary heart diseases (CHD) are for example smoking, high low density lipoprotein (LDL) cholesterol concentration, lack of exercise, diabetes and elevated blood pressure. These risk factors do not, however, fully explain the variations in CHD risk. One of the widely investigated and accepted hypotheses is the oxidation hypothesis of atherosclerosis. The current opinion is that oxidative modification of LDL plays a key role in the pathogenesis of atherosclerosis (Steinberg et al. 1989, Salonen et al. 1992). It has also been hypothesized that atherosclerosis could be retarded by antioxidant compounds which would prevent the oxidation of LDL.

Lipid peroxidation has an important role in the etiology of many pathological conditions including atherosclerosis (Salonen et al. 1992, 1997, Salonen 1995). The carotenoid astaxanthin has been established to be a powerful antioxidant in vitro (Terao 1989, Miki 1992, Palozza and Krinsky 1992, Nakagawa et al. 1997), far more potent than e.g. beta-carotene. As scavenger of hydroxyl radicals, astaxanthin is more effective than beta-carotene (Rosseau et al. 1992). Astaxanthin has also been shown, in experimental animals, to induce the xenobiotics metabolizing enzyme system, cytochrome P450 (Gradelet et al. 1997) and to reduce oxidative damage to DNA (Gradelet et al. 1998). Oxidative DNA damage is considered a good biomarker for cancer risk in humans (Prieme et al. 1997, Poulsen et al. 1998). The main dietary sources of astaxanthin are crustaceans and other seafood, especially salmonoids. There are, however, no previous randomized placebo-controlled clinical trials testing the effect of astaxanthin supplementation on lipid peroxidation and DNA damage in humans. If the positive effects observed in test tube and animal experiments will be confirmed in humans, this would have a great significance with regard to the value of astaxanthin as an antiatherogenic and anticarcinogenic antioxidant for humans.

1.2. Assessment of lipid peroxidation

The measurement of lipid peroxidation in human samples is complicated. There are no generally applied methods; there is even no consensus about what measurements are the methods of choice (Salonen 2000). The methods we prefer are hydroxy fatty acids, which may be the best indicator of lipid peroxidation in vivo in humans. Hydroxy fatty acids are the first step of peroxidation of 18-carbon fatty acids oleic acid, linoleic acid and linolenic acid.

THE PURPOSE OF THE STUDY

The purpose of the present study was to investigate the effect of supplementation of healthy male volunteers with 8 mg of astaxanthin daily on plasma astaxanthin and other antioxidant status, serum lipids, the susceptibility of the combined fraction of VLDL and LDL to oxidation, lipid peroxidation in vivo, as compared with placebo.

STUDY DESIGN AND SUBJECTS

The subjects of the study were healthy non-smoking male volunteers aged 19-33 years with no severe diseases.

The study was a randomized double-blind trial based on comparison of two groups: one received two 4 mg astaxanthin (Astaxin®) capsules daily in conjunction with two standardized, fat-containing meals and the second group, two identical-looking placebo capsules. The total sample size was 40, and 20 subjects were randomized into each group. The treatment period was 3 months (12 weeks) for each. One subject in the placebo group was disqualified from the statistical analysis because his plasma astaxanthin concentration was high in the baseline measurement.

The subjects were advised to keep their exercise and dietary habits unchanged during the study. Four-day food recording were carried out at the beginning and at the end of the study to monitor any changes in the dietary intake of astaxanthin and other antioxidants.

MEASUREMENTS

- plasma astaxanthin concentration
- serum lipids and other chemical tests to control other factors affecting lipid peroxidation and to estimate changes in the diet during the study, these included serum total, LDL and HDL cholesterol, serum triglycerides,
- copper induced lag-time
- copper induced V-max
- plasma hydroxy fatty acids (measured at Oy Jurilab LTD, www.jurilab.com)
- serum fatty acid profile

RESULTS

There weren't any differences in the diet between the study groups. The changes of serum lipids and lipid peroxidation are shown in table 1. The plasma levels of astaxanthin increased by 18.8 $\mu\text{g/l}$ during the study in the astaxanthin group. Astaxanthin levels were 0.0 $\mu\text{g/l}$ at baseline in both groups and also after supplementation in the placebo group. There were no significant differences in the changes of plasma alpha-tocopherol, retinol, lycopene, beta-carotene, ascorbate, zeaxanthin+lutein, cantaxanthin and cryptoxanthin concentrations between the astaxanthin and placebo groups. There were no significant differences in the changes of inflammation markers (interleukin-2 receptor and interleukin-6) between the study groups.

The levels of plasma 12- and 15-hydroxy fatty acids were reduced statistically significantly in the astaxanthin group ($p=0.048$ and $p=0.047$ respectively) during supplementation but not in the placebo group and the change was almost significantly greater ($p=0.056$) in the A group, as compared with the placebo group. There weren't any other statistically significant differences in serum lipids, lipid peroxidation or serum fatty acid profile between the study groups.

CONCLUSION

The present study indicates that astaxanthin was absorbed well from capsules. In addition in this study we found decreased plasma 15-hydroxy fatty acid production during astaxanthin supplementation. Plasma 15-hydroxy fatty acids are formed by oxidation from the linolenic acids which is the most

ubiquitous fatty acids in plasma and among the most sensitive fatty acids to get oxidized.

In conclusion, the present study suggests that astaxanthin supplementation may decrease in vivo lipid peroxidation in healthy men. As increased lipid peroxidation has been shown to contribute to the development of CVD, this finding suggests that astaxanthin supplementation may play a role in the prevention of CVD.

Table 1. Changes of the main measurements during the study.

Variable	Group	Change				
		Placebo n=19	P for change within group	Astaxanthin n=20	P for change within group	P for difference between groups
Plasma astaxanthin		0.00		+18.79	<0.001	<0.001
Serum total cholesterol (mmol/l)		+0.09	0.474	-0.08	0.437	0.289
Serum HDL cholesterol (mmol/l)		-0.05	0.085	+0.02	0.648	0.149
Serum LDL cholesterol (mmol/l)		+0.17	0.159	-0.04	0.666	0.167
Serum triglycerides (mmol/l)		-0.02	0.773	-0.13	0.395	0.548
Adjusted copper induced lag-time (min)		-2.58	0.149	-1.25	0.465	0.583
Adjusted copper induced V-max (mabs/min)		+0.56	0.166	+0.01	0.989	0.331
Plasma total hydroxy fatty acids (μ mol/l)		-0.20	0.167	-0.14	0.233	0.727
Plasma 8-hydroxy fatty acids (μ mol/l)		+0.01	0.318	-0.01	0.710	0.377
Plasma 9-hydroxy fatty acids (μ mol/l)		+0.03	0.250	-0.01	0.738	0.366
Plasma 10-hydroxy fatty acids (μ mol/l)		+0.01	0.542	-0.02	0.619	0.512
Plasma 11-hydroxy fatty acids (μ mol/l)		+0.00	0.766	-0.02	0.258	0.244
Plasma 12-hydroxy fatty acids (μmol/l)		-0.20	0.128	-0.05	0.048	0.246
Plasma 13-hydroxy fatty acids (μ mol/l)		+0.01	0.727	-0.02	0.555	0.495
Plasma 15-hydroxy fatty acids (μmol/l)		+0.01	0.557	-0.03	0.047	0.056
Plasma 16-hydroxy fatty acids (μ mol/l)		-0.01	0.482	-0.00	0.330	0.549

REFERENCES

- Gradelet S, Astorg P, Le Bon AM, Berges R, Sushet M. Modulation of aflatoxin B1 carcinogenicity, genotoxicity and metabolism in rat liver by dietary carotenoids: evidence for a protective effect of CYP1A inducers. *Cancer Lett* 1997;114:221-3.
- Gradelet S, Le Bon AM, Berges R, Sushet M, Astorg P. Dietary carotenoids inhibit aflatoxin B1-induced liver preneoplastic foci and DNA damage in the rat: role of the modulation of aflatoxin B1 metabolism. *Carcinogenesis* 1998;19:403-11.
- Kaikkonen J, Nyssönen K, Porkkala-Sarataho E, Poulsen HE, Metsä-Ketelä T, Hayn M, Salonen JT. Effect of oral coenzyme Q10 supplementation on the oxidation resistance of human VLDL + LDL fraction: absorption and antioxidative properties of oil and granula based preparation. *Free Rad Biol Med* 1997;22: 1195-202.
- Miki W. Recent advances in carotenoid studies--metabolisms and bioactivities. *J Nutr Sci Vitaminol Tokyo* 1992; Spec No: 469-72.
- Nakagawa K, Kang SD, Park DK, Handelman GJ, Miyazawa T. Inhibition of beta-carotene and astaxanthin of NADPH-dependent microsomal phospholipid peroxidation. *J Nutr Sci Vitaminol Tokyo* 1997;43:345-55.
- Nyssonen K, Porkkala E, Salonen R, Korpela H, Salonen JT. Increase in oxidation resistance of atherogenic serum lipoproteins following antioxidant supplementation: a randomized double-blind placebo-controlled clinical trial. *Eur J Clin Nutr* 1994;48:633-42.
- Nyssonen K, Poulsen HE, Hayn M, Agerbo P, Porkkala-Sarataho E, Kaikkonen J, Salonen R, Salonen JT. Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation. *Eur J Clin Nutr* 1997;51:154-63.
- Palozza P, Krinsky NI. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch Biochem Biophys* 1992;297:291-5.
- Poulsen HE, Prieme H, Loft H. Role of oxidative DNA damage in cancer initiation and promotion. *Eur J Cancer Prev* 1998;7:9-16.
- Prieme H, Loft S, Nyssonen K, Salonen JT, Poulsen HE. No effect of supplementation with vitamin E, ascorbic acid or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7, -dihydro-2' -deoxyguanosine excretion in human. *Am J Clin Nutr* 1997;65:503-7.
- Rousseau EJ, Davison AJ, Dunn B. Protection by beta-carotene and related compounds against oxygen-mediated cytotoxicity and genotoxicity: implications for carcinogenesis and anticarcinogenesis. *Free Radic Biol Med* 1992; 13:407-33.
- Salonen JT. Epidemiological studies on LDL oxidation, pro- and antioxidants and atherosclerosis. In: *Free Radicals, Lipoprotein Oxidation and Atherosclerosis*. Bellomo G, Finardi G, Maggi E, Rice-Evans C (eds). Richelieu Press, London 1995.
- Salonen JT. Markers of oxidative damage and antioxidant protection: Assessment of LDL oxidation. *Free Rad Res* 2000; 33: S41-46.
- Salonen JT, Nyssonen K, Salonen R, Porkkala-Sarataho E, Tuomainen T-P, Diczfalusy U, Björkhem I. Lipoprotein oxidation and progression of carotid atherosclerosis. *Circulation* 1997;95:840-5.
- Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyssonen K, Palinski W, Witztum JL. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 1992;339:883-7.
- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915-24.
- Terao J. Antioxidant activity of beta-carotene-related carotenoids in solution. *Lipids* 1989;24:659-61.