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**NEWSLETTER**  
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## **Association Between Carotenoids and Oral Precancer --Serum Carotenoid Levels of Oral Leukoplakia Cases--**

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### **<Abstract>**

As there is a risk of oral leukoplakia developing into oral cancer, it is regarded as precancer. We measured serum micronutrient levels in oral leukoplakia cases at inhabitant-based medical checkups for oral cancer and found a significant decrease in the serum beta-carotene levels in male cases compared to those of the control group. In the present paper, we will introduce a finding which revealed the possibility that a low serum carotenoid level may be involved in the development of oral precancer.

Many epidemiological and other studies have shown that the development of oral and other cancers is associated with an insufficient intake of fruits and green and yellow vegetables and that such a risk may decrease with an increased level of micronutrients, namely retinol, beta-carotene, and vitamin E, in the serum. Zheng et al. have reported that a high level of carotenoids such as beta-carotene in the serum is strongly associated with a decreased risk of developing oral cancer. In addition, retinoids (eg. retinol) and carotenoids (eg. beta-carotene) have been conventionally used to treat oral precancer. Oral leukoplakia, a type of keratosis which develops on the oral mucous membrane, is a significant disease regarded as precancer because up to 51.5% of cases may develop into cancer. However, there have been no epidemiological studies which compare the levels of anti-oxidative nutrients in the serum of oral leukoplakia cases to those of healthy people. We thus planned an epidemiological study, which is a case-control study on local inhabitants, in order to clarify the relationship between oral leukoplakia and the levels of these micronutrients in the serum.

### **Subjects and Methods**

During the three years from 1995 through 1998, we conducted medical checkups for oral cancer on examinees aged 40 or older of general medical checkups in city T in Aichi Prefecture. The cases were 48 subjects (38 males, aged  $62.4 \pm 10.0$ ; 10 females, aged  $62.1 \pm 10.7$ ) who were found at the checkups to have oral leukoplakia and later tested positive at a confirmatory examination. All participants of the checkups filled out a questionnaire with questions about smoking and alcohol consumption. Blood samples were collected at the checkups, centrifuged, and the serum samples were stored at  $-80^{\circ}\text{C}$ . Four controls for each case were selected out of the group of examinees who proved negative at the checkups after matching for gender and age. The levels of micronutrients, namely retinol, alpha-tocopherol, and carotenoids including zeaxanthin/lutein, cryptoxanthin, lycopene, alpha-carotene, and beta-carotene in the serum were measured by HPLC. The results were statistically analyzed for each gender using the t-test and logistic regression analysis. Data on smoking and alcohol consumption from samples of each group at baseline were examined using the chi-square test.

### **Results**

There was no statistically significant difference in either gender between the cases and controls in the distribution of smokers and alcohol drinkers. The serum micronutrient levels in males are shown in Table 1. The lower levels of beta-carotene and lycopene shown in leukoplakia cases were statistically significant ( $p < 0.005$  and  $p < 0.05$ , respectively). In contrast, higher levels of zeaxanthin/lutein and cryptoxanthin were seen in leukoplakia cases, especially the former, which was

statistically significant ( $p < 0.05$ ). There was no significant difference in the levels of other nutrients. On the other hand, female groups showed no significant difference between cases and controls in the levels of any of the nutrients. The results of the logistic regression analysis in which leukoplakia was a dependent variable and drinking, smoking, and serum carotenoid levels were independent variables showed that beta-carotene may decrease the risk of leukoplakia (odds ratio, 0.16; 95% confidence interval, 0.029-0.866) (Table 2).

### Discussion

The results of the present study conducted through an inhabitant-based epidemiological survey show low levels of beta-carotene and lycopene in the serum of male oral leukoplakia cases. The multivariate

analysis suggests that a high level of beta-carotene in the serum may decrease the risk of developing oral leukoplakia. On the other hand, as serum retinol levels were not shown to be associated with the disease, it is considered that beta-carotene may decrease the risk without being converted to vitamin A. These results suggest that beta-carotene may be a more effective chemical prophylaxis than retinol and alpha-tocopherol, which have conventionally been clinically used for oral cancer. We are thus planning a clinical intervention study on the chemoprophylaxis of oral precancer using mainly beta-carotene.

*We would like to extend our deepest gratitude to our collaborator Professor Yoshinori Ito (Fujita Health University).*

Table.1 Comparison of serum micronutrient levels between cases and controls in Japanese males

Serum nutrients ( $\mu$ mol/l)	Cases(n=38)		Controls(n=152)		t test
	Mean	S.D.	Mean	S.D.	p-value
Retinol	3.188	0.957	3.052	2.450	p=0.589
-Tocopherol	17.595	16.016	17.935	9.901	p=0.901
Carotenoids					
Zeaxanthin/Lutein	7.720	9.606	4.193	6.237	p<0.05
Cryptoxanthin	0.602	0.657	0.439	0.366	p=0.149
Lycopene	0.175	0.202	0.257	0.252	p<0.05
-Carotene	0.208	0.239	0.199	0.309	p=0.872
-Carotene	0.357	0.295	0.555	0.408	p<0.005
Total-cholesterol (mg/dl)	173.4	37.2	188.8	27.6	p<0.05

Table.2 Logistic regression analysis with leukoplakia as the dependent variable for male

Independent variable	Coefficient	S.E.	p-value	Odds ratio	95% C.I.
Drink (occasional)	-0.182	0.592	p=0.856	0.885	0.239-3.283
Drink (daily)	0.041	0.427	p=0.967	1.107	0.447-2.315
Smoking (currently)	0.675	0.606	p=0.500	1.353	0.562-3.255
Zeaxanthin/Lutein	1.723	0.034	p=0.085	1.056	0.993-1.124
Lycopene	0.804	2.698	p=0.421	2.437	0.278-21.348
Beta-Carotene	-2.160	0.137	p<0.05	0.164	0.032-0.846
Total-cholesterol	-1.937	0.008	p=0.053	0.985	0.969-1.000

## Dietary Supplements

Prof. Osamu Igarashi, Ibaraki Christian University

In Japan, dietary supplements have been regarded as merely a type of food. This precluded labeling of the nutritional functions of supplements, allowing only labeling of the contents and their quantities. However, in the U. S. and many other foreign countries, the categorization of vitamins and minerals has been shifted from medicine to food, which has caused some international problems concerning the treatment of supplements, including the problem of tariff barriers. In Japan, in order to promote international cooperation, the matter was examined by a sub-committee of the Ministry of Health and Welfare (MHW) in fiscal year 1999, and the resulting proposals on supplements, including “Foods for Specified Health Uses” which had been approved by the Ministry, were established. In order to realize the proposals, they established a new sub-committee in fiscal year 2000 and have continued discussion. As a result, it was determined that the term “dietary supplement” will not be used; there will be an overall category of “Health Function Foods” which includes two subgroups: “Foods for Specified Health Uses”, which are approved through individual evaluation, and “Nutritional Function Foods”, which must simply meet certain standards. In addition, limitations based on the forms of products were removed, and both subgroups include products in the form of capsules or tablets as well as conventional foods. With respect to Foods for Specified Health Uses, which are approved through individual evaluation, products may carry nutrient contents claim, nutrient function claims, or health claim, among others. As for

Nutritional Function Foods, products may only carry nutrient contents claim and nutrient function claims, not health claim. In the present sub-committee, there was a discussion on what kind of food should fall under each subgroup. It was determined that Foods for Specified Health Uses will be evaluated individually through an examination in a manner similar to that currently in practice, and that Nutritional Function Foods will be limited to vitamins and minerals for the time being. In addition, considering the fact that it is currently not allowed for vitamin K to be used in vitamin supplements, it was determined that the treatment of this vitamin will be discussed later. Among minerals, calcium and iron were approved because intake of these elements tends to be insufficient in many people. However, other trace elements were not approved this time due to the fact that, although their recommended allowances are established, the current intake of these elements is unknown, and many trace elements show toxicity at high doses. The initial draft as of November 1 suggests maximum and minimum amounts for calcium, iron, and 12 types of vitamins in Nutritional Function Foods. All of the maximum amounts are those established for Quasi-drugs which do not require prescription, and the minimum amounts are one third of the recommended allowances (except in the case of niacin, for which the minimum amount of 15mg was obtained by subtracting the recommended allowance from the acceptable upper limit). It has been pointed out that the bases of these figures are

not clear, and that the way by which the maximum amounts of these nutrients in Quasi-drugs not requiring prescription are determined has some problems. It is likely that these figures will be changed through negotiation with foreign countries and future review of Quasi-drugs not requiring prescription. Table 2 shows the maximum possible amounts of vitamins and minerals for Nutritional Function Foods. Furthermore,

the subcommittee established the examination procedures through which vitamins and minerals used exclusively as food additives in Health Function Foods may be approved (a somewhat easier examination than that required for conventional food additives) as well as establishing guidelines for the application, evaluation, and labeling of Foods for Specific Health Uses.

Table 1 Health Function Foods

Foods for Specified Health Uses (individual evaluation)	Nutrient contents claim, Nutrient function claims, Health claim, Warning
Nutritional Function Foods (certain standards)	Nutrient contents claim, Nutrient function claims, Warning

Table 2 Maximum possible amounts of vitamins and minerals for Nutritional Function Foods  
(October 24, 2000)

Vitamin A	2,000 IU	Vitamin D	200 IU
Vitamin E	150 mg	Vitamin B1	25 mg
Vitamin B2	12 mg	Niacin	15 mg
Vitamin B6	10 mg	Folic acid	200 µg
Vitamin B12	60 µg	Biotin	500 µg
Vitamin C	1,000 mg	Calcium	600 mg
Iron	10 mg		

Other vitamins and minerals were not established.

Minimum amount = less than one third of RDA

**Serum Vitamin C Concentration was Inversely Associated with Subsequent 20-Year Incidence of Stroke in a Japanese Rural Community  
(Tetsuji Yokoyama et al., Stroke, Oct.2000:2287-2294)**

Epidemiological evidence suggests that vitamin C may decrease the risk of stroke. The purpose of the present study was to examine the association of serum vitamin C concentration with the subsequent incidence of stroke.

Study District: Shibata located in the northern part of Niigata Prefecture, Japan  
 Subjects: aged 40 years or older (880 men and 1241 women)  
 No one in the cohort had been taking vitamin supplements.  
 Study period: follow up for 20 years (1977-1997)

Results: 196 incident cases of all stroke, including 109 cerebral infarctions and 54 hemorrhagic strokes, were documented.  
 Strong inverse associations were observed between serum vitamin C concentration and all stroke, cerebral infarction, and hemorrhagic stroke. In addition, weak but significant inverse correlations were observed between serum vitamin C concentration and blood pressure.

**Hazard Ratios of Stroke Incidence According to Serum Vitamin C Concentration Adjusted for Age and Sex**

Serum Vitamin C level ( μ mol/L)	All Stroke (95% CI)	Cerebral infarction (95% CI)	Hemorrhagic stroke (95% CI)
40	1	1	1
41-51	0.93 (0.64-1.36)	0.71 (0.42-1.19)	0.89 (0.43-1.83)
52-63	0.72 (0.48-1.08)	0.59 (0.34-1.02)	0.75 (0.36-1.55)
64	0.59 (0.38-0.93)	0.51 (0.28-0.92)	0.45 (0.19-1.11)

## Oxidative Stress and Age-Related Macular Degeneration

(Stephen Beartty et al., *Survey of Ophthalmology* Vol.45 No.2, 115-134)

Although age-related macular degeneration (AMD) is a cause of blindness in developing countries, it has not been fully understood. Oxidation stress, in which reactive oxygen species cause damage to cells, causes many diseases, especially age-related diseases. Reactive oxygen species, including free radicals, hydrogen peroxide, and singlet oxygen, are metabolites of oxygen. As the retina contains a high level of polyunsaturated fatty acids and is exposed to ultraviolet rays, its oxygen consumption is high and apt to be affected by oxidation stress. In in-vitro studies, photochemical damage to the retina is caused by oxidation stress, which can be prevented by anti-oxidative vitamins, namely vitamins A, C, and E. In addition, it has been shown that lipofuscin is produced through oxidation damage to the exterior of the photoreceptors and that the pigment itself is photosensitive.

Although there are reports on the effectiveness of a high level of alpha-tocopherol in the plasma, the association between AMD and dietary intake and the serum level of anti-oxidative vitamins is not evident. It has been considered that macular pigments also protect the retina against oxidation damage by absorbing ultraviolet rays and eliminating reactive oxygen species. Many presumed risk factors of AMD (female gender, lens density, smoking, and decline of vision) are associated with a shortage of macular pigments. In addition, the results of an eye disease case-control study show that a high level of lutein and zeaxanthin in the plasma is associated with a decreased risk of developing neovascular AMD. Although not yet proven, it is considered that the accumulation of oxidation stress causes the development of AMD.

### Micronutrient and AMD

#### Carotenoids

	Dietary Carotenoids and AMD	Serum Carotenoids and AMD
EDCC	Highest quintile of carotenoid intake: associated with a 43% reduction in risk for AMD (odds ratio:0.57; confidence interval: 0.35-0.92; P=0.02 )	Reported a significantly decreased risk of neovascular AMD associated with medium and high serum levels of carotenoids compared with those in the low group* ( n=421 )
Beaver Dam Eye Study	Intake of -, -carotene and pro-vitamin A carotenoids was associated with a significantly reduced risk of large drusen at 5 years follow-up	Low levels of lycopene were associated with increased risk of AMD ( n=167 )

\*<sup>1</sup>: Serum Carotenoids= Lutein + zeaxanthin + -carotene + -carotene + cryptoxanthin + lycopene

### Vitamin C

	Dietary Vitamin C and AMD	Plasma Vitamin C and AMD
NHANES	Diet containing high quantities of foodstuffs rich in vitamins A and C was negatively associated with AMD	
EDCC	Reported protective effect associated with high intake of oral vitamin C	Low plasma levels of vitamin C were associated with increased risk of AMD, but high levels were not found to be protective

### Vitamin E

	Dietary Vitamin E * and AMD	Plasma Vitamin E and AMD
BDES	Reported a significantly increased risk of large macular drusen associated with the lowest quintile of past dietary intake of vitamin E	
EDCC	Found no significant inverse association between dietary intake of vitamin E and neovascular AMD	Found no significant associations between plasma levels of vitamin E and AMD
POLA		Fasting blood levels of vitamin E showed a weak negative association with AMD

\* Dietary intake of vitamin E is very difficult to estimate, because the long-term consumption of oils, in which the  $\alpha$ -tocopherol concentration varies considerably, is difficult to determine by questionnaire.

### Vitamin A

	Dietary Vitamin A and AMD	Plasma Vitamin A and AMD
NHANES	Reported a 40% reduction in risk for AMD in persons who consumed foods rich in vitamin A at least once per day, as compared with those who ate these foods less than once per week	
POLA		With respect to plasma retinol and AMD, the POLA study found no significant association

EDCC: Eye Disease Case Control Study

BDES: Beaver Dam Eye Study

NHANES: National Health and Nutrition Examination Survey

POLA: Pathologies Oculaires Liees a l'age Study

## **AHA Dietary Guidelines**

### **Revision 2000: A Statement for Healthcare Professionals From the Nutrition Committee of the American Heart Association**

*( Ronald M. Krauss, MD et.al., Circulation 102, 2296-2311 )*

#### **Overview and Summary**

The AHA has a long-standing commitment to the promotion of lifestyle practices aimed at preventing the development or recurrence of heart and blood vessel diseases and promoting overall well-being. An important component of this mission has been the provision of dietary guidelines for the American population that are based on the best available scientific evidence. The present statement formulates the core elements of population-wide recommendations for cardiovascular disease prevention and treatment that are supported by decades of research. This revised statement also provides a summary of a number of important ancillary issues, including those for which the scientific evidence is deemed insufficient to make specific recommendations.

#### Three principles underlie the current guidelines:

- There are dietary and other lifestyle practices that all individuals can safely follow throughout the life span as a foundation for achieving and maintaining cardiovascular and overall health.
- Healthy dietary practices are based on one's overall pattern of food intake over an extended period of time and not on the intake of a single meal.
- The guidelines form a framework within which specific dietary recommendations can be made for individuals based on their health status, dietary preferences, and cultural background.

The guidelines are designed to assist individuals in achieving and maintaining:

#### **A Healthy Eating Pattern Including Foods From All Major Food Groups**

Major guidelines:

- Consume a variety of fruits and vegetables and grain products, including whole grains.
- Include fat-free and low-fat dairy products, fish, legumes, poultry, and lean meats.

#### **A Healthy Body Weight**

Major guidelines:

- Match intake of energy (calories) to overall energy needs; limit consumption of foods with a high caloric density and/or low nutritional quality, including those with a high content of sugars.
- Maintain a level of physical activity that achieves fitness and balances energy expenditure with energy intake; for weight reduction, expenditure should exceed intake.

#### **A Desirable Blood Cholesterol and Lipoprotein Profile**

Major guidelines:

- Limit the intake of foods with a high content of saturated fatty acids and cholesterol.
- Substitute grains and unsaturated fatty acids from vegetables, fish, legumes, and nuts.

## **Guidelines for the General Population**

**<Achieve and maintain a healthy eating pattern that includes foods from each of the major food groups.>**

### **a. General Principles**

Eating adequate amounts of essential nutrients, coupled with energy intake in balance with energy expenditure, is essential to maintain health and to prevent or delay the development of cardiovascular disease, stroke, hypertension, and obesity. Individual foods as well as foods within the same food group vary in their nutrient content. No one food contains all of the known essential nutrients. Eating foods from each of the different food groups helps ensure that all nutrient needs are met. The AHA strongly endorses consumption of a diet that contains a variety of foods from all the food categories and emphasizes fruits and vegetables; fat-free and low-fat dairy products; cereal and grain products; legumes and nuts; and fish, poultry, and lean meats. Such an approach is consistent with a wide variety of eating patterns and lifestyles.

Portion number and size should be monitored to ensure adequate nutrient intake without exceeding energy needs. The AHA recommends that healthy individuals obtain an adequate nutrient intake from foods. Vitamin and mineral supplements are not a substitute for a balanced and nutritious diet designed to emphasize the intake of fruits, vegetables, and grains. As discussed in subsequent sections, excessive food intake, especially of foods high in saturated fat, sugar, and salt, should be avoided.

### **b. Specific Guidelines**

#### **1) Consume a variety of fruits and vegetables; choose 5 or more servings per day.**

The AHA strongly endorses the consumption of diets that include a wide variety of fruits and vegetables throughout the day, both as meals and snacks. Fruits and vegetables are high in nutrients

and fiber and relatively low in calories and hence have a high nutrient density. Dietary patterns characterized by a high intake of fruits and vegetables are associated with a lower risk of developing heart disease, stroke, and hypertension.

Habitually consuming a variety of fruits and vegetables (especially those that are dark green, deep orange, or yellow) helps ensure adequate intakes of micronutrients normally present in this food group. Fruits and vegetables also have a high water content and hence a low energy density. Substituting foods of low energy density helps to reduce energy intake and, as discussed below, may assist in weight control. To ensure an adequate fiber intake, as described below, whole fruits and vegetables rather than juice are recommended.

#### **2) Consume a variety of grain products, including whole grains; choose 6 or more servings per day.**

Grain products provide complex carbohydrates, vitamins, minerals, and fiber. Dietary patterns high in grain products and fiber have been associated with decreased risk of cardiovascular disease. Foods high in starches (polysaccharides; eg, bread, pasta, cereal, potatoes) are recommended over sugar (monosaccharides and disaccharides). Foods that are sources of whole grains as well as nutrient-fortified and enriched starches (such as cereals) should be major sources of calories in the diet.

Soluble fibers (notably  $\beta$ -glucan and pectin) modestly reduce total and LDL cholesterol levels beyond those achieved by a diet low in saturated fat and cholesterol. Additionally, dietary fiber may promote satiety by slowing gastric emptying and helping to control calorie intake and body weight. Grains, vegetables, fruits, legumes, and nuts are good sources of fiber. Although there are insufficient data to recommend a specific target for fiber intake, consumption of the recommended portions of these foods can result in an intake of 25 g per day.

## **Achieve and maintain a desirable blood cholesterol and lipoprotein profile.**

### **b. Specific Guidelines**

#### **<Substitute grains and unsaturated fatty acids from fish, vegetables, legumes, and nuts.>**

Limiting the intake of saturated and *trans*-fatty acids requires the substitution of other nutrients unless there is a need to reduce total energy intake. Reductions of LDL cholesterol are generally similar with substitution of carbohydrate or unsaturated fat for saturated fat. In addition, certain soluble fibers (eg, oat products, psyllium, pectin, and guar gum) reduce LDL cholesterol, particularly in hypercholesterolemic individuals. A recent meta-analysis concluded that for every gram increase in soluble fiber from these sources, LDL cholesterol would be expected to decrease by an average of 2.2 mg/dL.

However, in the absence of weight loss, diets high in total carbohydrate (eg, > 60% of energy) can lead to elevated triglyceride and reduced HDL cholesterol, effects that may be associated with increased risk for cardiovascular disease. These changes may be lessened with diets high in fiber, in which carbohydrate is derived largely from unprocessed whole foods and may be more extreme with consumption of monosaccharides (particularly fructose) than with oligosaccharides or starch.

These metabolic effects do not occur with substitution of monounsaturated or polyunsaturated fat (eg, from vegetable oils) for saturated fat. As described further below, diets enriched in unsaturated fatty acids rather than carbohydrate may be of particular benefit in modulating the atherogenic dyslipidemia characterized by reduced HDL cholesterol, elevated triglycerides, and small dense LDL. This dyslipidemia is commonly found in individuals with insulin resistance and type 2 diabetes mellitus. Although it is not proven that diet-induced

changes in these lipid parameters have direct effects on cardiovascular disease risk, diets relatively high in unsaturated fatty acids offer a reasonable option to high-carbohydrate diets in optimizing the metabolic profile in patients who are susceptible to these lipoprotein changes.

A growing body of evidence indicates that foods rich in  $\omega$ -3 polyunsaturated fatty acids, specifically EPA and DHA, confer cardioprotective effects beyond those that can be ascribed to improvements in blood lipoprotein profiles. The predominant beneficial effects include a reduction in sudden death, decreased risk of arrhythmia, lower plasma triglyceride levels, and a reduced blood-clotting tendency. There is some evidence from epidemiological studies that another  $\omega$ -3 fatty acid,  $\alpha$ -linolenic acid, reduces risk of myocardial infarction and fatal ischemic heart disease in women. Several randomized controlled trials recently have demonstrated beneficial effects of both  $\alpha$ -linolenic acid and marine  $\omega$ -3 fatty acids on both coronary morbidity and mortality in patients with coronary disease. Because of the beneficial effects of  $\omega$ -3 fatty acids on risk of coronary artery disease as well as other diseases such as inflammatory and autoimmune diseases, the current intake, which is generally low, should be increased. Food sources of  $\omega$ -3 fatty acids include fish, especially fatty fish such as salmon, as well as plant sources such as flaxseed and flaxseed oil, canola oil, soybean oil, and nuts. At least 2 servings of fish per week are recommended to confer cardioprotective effects.