Low Serum \( \alpha \)-Tocopherol Concentrations in Subjects With Various Coronary Risk Factors

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Background Oxidative stress may play an important role in the genesis and development of atherosclerosis. The oxidizability of low-density lipoprotein (LDL) ex vivo is reportedly enhanced in men with an increased risk for developing atherosclerosis, such as those with hypertension, hypercholesterolemia, diabetes mellitus and smoking, as well as in patients with coronary heart diseases. However, the intensity of the oxidative stress in the presence of various kinds of risk factor has not yet been compared.

Methods To examine whether oxidative stress is increased in the presence of various coronary risk factors, serum \( \alpha \)-tocopherol concentrations were determined in 168 apparently healthy subjects recruited from Daioh town (73 men and 95 women, mean age: 42±11 years). The \( \alpha \)-tocopherol concentration (mg/g lipids) was significantly lower in men, older subjects (≥50 years of age), smokers, those with hyper-low density lipoprotein (LDL)-cholesterolemia (C) (≥160 mg/dl), hypertriglyceridemia (TG) (≥150 mg/dl), hyper-high density lipoprotein (HDL)-C (<40 mg/dl), fasting hyperglycemia (>110 mg/dl) and obesity (body mass index ≥25 kg/m²) than in women, younger subjects, nonsmokers, those with normoLDL-C, normoTG, normoHDL-C, fasting normoglycemia and the non-obese. The concentration was not significantly different between hypertensives and nonhypertensives. Multivariate logistic regression analysis showed that the adjusted odds ratios (95% confidence interval) for hyperTG and hypoHDL-C as a factor for the lowest tertile of \( \alpha \)-tocopherol concentration were 6.2 (1.6–24.4, p<0.01) and 6.0 (1.5–22.6, p<0.01), and those of the other risk factors were not significant. The \( \alpha \)-tocopherol concentrations were significantly positively correlated with HDL-cholesterol (C) concentrations (R=0.53) and negatively with TG concentrations (R=−0.53), BMI (R=−0.35), LDL-C concentrations (R=−0.25) and age (R=−0.22).

Conclusion Increased oxidative stress, as shown by decreased \( \alpha \)-tocopherol concentrations, was linked to the presence of various coronary risk factors, among which hyperTG and hypoHDL-C appeared to be most heavily associated with oxidative stress.

Key Words: \( \alpha \)-Tocopherol; Coronary risk factors; HDL-cholesterol; Oxidative stress; Triglyceride

Oxidative modification of lipoproteins has been implicated in the formation of early lesions of atherosclerosis and several lines of evidence support a role for oxidative stress and inflammation in atherogenesis. The oxidizability of low-density lipoprotein (LDL) ex vivo is reportedly enhanced in men with an increased risk for developing atherosclerosis, such as those with hypertension, hypercholesterolemia, diabetes mellitus and smoking, as well as in patients with coronary heart diseases. However, the intensity of the oxidative stress in the presence of various kinds of risk factor has not yet been compared. Vitamin E is the most readily available naturally occurring, lipid-soluble antioxidant, although other antioxidants such as flavonoids are also potent. Vitamin E is consumed as flavonoids are also potent. Vitamin E is consumed as

Methods

Study Patients The studied population comprised town workers in Daioh, Shima county, Mie-prefecture, Japan and staff members of its related institute (Yuyuen) who underwent an annual medical examination in 2002. Of 257 subjects 189 agreed to participate in this study: 6 of these subjects on vitamin E supplementation were excluded and samples from 13 other subjects were not studied because of technical problems related to hemolysis or insufficient serum; 2 subjects with suspected systemic disease or complications were excluded. Finally 168 apparently healthy subjects, comprising 73 men and 95 women with a mean age of 42±11 years (range: 20–73 years), were selected for the study.

Subjects who were under treatment with medications for hypertension (14), hyperlipidemia (3) or diabetes mellitus (1), were included unless complications were present. Patients receiving insulin treatment or with hemoglobin A1C >7.0% were not included, nor were patients receiving antioxidants or lipid-lowering drugs. Written informed consent was obtained from all the participants and the study protocol was approved by the ethics committee of the institute.

Serum Analysis Venous samples were obtained after overnight fasting.

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The LDL-cholesterol (C) concentration was calculated according to the Friedwald equation. Serum \( \alpha \)-tocopherol concentrations were determined using high-performance liquid chromatography and expressed as \( \text{mg/g total lipids} \) (total cholesterol (TC) and triglyceride (TG)).

**Assessment of Coronary Risk Factors**

Smoking status was self-reported: current smoking was defined as \( >5 \) cigarettes/day during the past 3 years. Quitting smoking during the last 2 years classified the subject as a nonsmoker. Hypertension (HT) was defined as a resting blood pressure exceeding 140 (systolic) or 90 (diastolic) mmHg. Body mass index (BMI) was calculated as weight (kg)/height (m)\(^2\).

**Statistical Analysis**

Values are presented as the mean±standard deviation. Comparisons of \( \alpha \)-tocopherol concentrations between the study groups were performed with the unpaired Student’s \( t \)-test. For the multivariate logistic regression, analysis was performed using SPSS Professional Statistics 7.5 for Windows (SPSS Japan Inc) for all the risk factors including age, sex, smoking, LDL-C, TG and high density lipoprotein (HDL)-C concentrations, fasting blood sugar (FBS) concentration, HT and BMI as categorical variables. The concentration of significance was set at \( p<0.05 \).

**Results**

A comparison of the serum \( \alpha \)-tocopherols concentrations between subjects with and without each coronary risk factor is shown in the following tables.
The serum \( \alpha \)-tocopherol concentration was significantly lower in men than in women and was significantly lower in older subjects (\( \geq 50 \) years of age), smokers, subjects with hyperLDL-C (\( \geq 160 \) mg/dl), hyperTG (\( \geq 150 \) mg/dl), hypoHDL-C (<40 mg/dl), fasting hyperglycemia (>110 mg/dl) and in obese subjects (body mass index \( \geq 25 \)). It was not significantly different between subjects with and without HT, although it tended to be lower in the former.

The adjusted odds ratio of men, older age, hyperLDL-C, hyperTG, hypoHDL-C, fasting hyperglycemia and obesity for the lowest tertile of \( \alpha \)-tocopherol concentrations was calculated by multivariate logistic regression analysis (Table 2). The analysis showed that the adjusted odds ratios (95% confidence interval) for hyperTG and hypoHDL-C as factors for the lowest tertile of \( \alpha \)-tocopherol concentrations were 6.2 (1.6–24.4, \( p<0.01 \)) and 6.0 (1.5–22.6, \( p<0.01 \)), although those of the other risk factors were not significant.

The correlation between \( \alpha \)-tocopherol concentrations and the various coronary risk factors was examined (Figs 1–5) and it was found to be significantly positively correlated with HDL-C concentration (\( R=0.53 \)) and negatively with TG concentration (\( R=-0.53 \)), BMI (\( R=-0.35 \)), LDL-C concentration.

Fig 1. Correlation between age and serum \( \alpha \)-tocopherol concentration.

Fig 2. Correlation between serum low-density lipoprotein cholesterol (LDL-C) and \( \alpha \)-tocopherol concentration.

Fig 3. Correlation between serum triglyceride (TG) and \( \alpha \)-tocopherol concentration.

Fig 4. Correlation between serum high-density lipoprotein cholesterol (HDL-C) and \( \alpha \)-tocopherol concentration.

Fig 5. Correlation between body mass index (BMI) and serum \( \alpha \)-tocopherol concentration.
concentrations (R=−0.25) and age (R=−0.22). The TG concentration was significantly negatively correlated with the HDL-C concentration (R=−0.42) and positively with BMI (R=0.42).

Discussion

Low Serum \( \delta \)-Tocopherol Concentrations in the Presence of Various Coronary Risk Factors

The relation between \( \delta \)-tocopherol status and various coronary risk factors has not been clarified in apparently healthy subjects. The results of the present study clearly demonstrated that \( \delta \)-tocopherol concentrations were low in the presence of various coronary risk factors, including male gender, increased age, smoking, dyslipidemia, glucose intolerance, and obesity, which was consistent with the hypothesis that subjects with coronary risk factors have increased oxidative stress leading to low \( \delta \)-tocopherol concentrations, and that enhanced oxidative stress causes augmented lipid oxidation and the development of atherosclerotic lesions or coronary artery disease. Tobacco smoke contains a vast amount of oxygen free radicals.\(^{15,16}\) Recent evidence suggests that endothelium-dependent vascular relaxation is impaired in hypercholesterolemia, and increased degradation of endothelium-derived NO by the superoxide anion in hypercholesterolemia may be an important contributing factor in the progression of atherosclerosis.\(^{6,8,17}\) Also, hyperglycemia is known to stimulate the production of reactive oxygen species by several pathways.\(^{18–20}\) Administration of antioxidant vitamin C has been reported to restore acute hyperglycemia-induced impairment of endothelium-dependent vasodilation.\(^{21,22}\) Obesity, and in particular central fat distribution, is associated with increased cytosolic TG stores in nonadipose tissues such as muscles, liver and pancreatic \( \beta \)-cells and this is accompanied by elevated concentrations of cytosolic long-chain acyl-coenzyme A esters, which inhibit mitochondrial adenine nucleotide translocators, resulting in an intramitochondrial ADP deficiency.\(^{23,24}\) Such an ADP deficiency is a potent stimulator of mitochondrial oxygen free radical production.\(^{25}\) Öhrvall et al reported that serum \( \delta \)-tocopherol concentration was inversely correlated to abdominal adiposity.\(^{26}\) In contrast, in the present study the \( \delta \)-tocopherol concentrations were not significantly different between subjects with and without HT observed at the time of the examination, although several recent studies have shown that certain forms of genetic or acquired HT are associated with oxidative stress and that animals with those types of HT respond favorably to antioxidant therapy.\(^{4,7,9}\) The link between HT and oxidative stress may manifest itself in limited situations. Endogenous antioxidant \( \delta \)-tocopherol appears to play an important role in the prevention of excessive increases in oxidative stress. Whether synthetic vitamin E supplementation therapy is effective in the prevention of such increases in oxidative stress remains to be determined and needs to be further investigated, as other antioxidative systems might also be exhausted in such conditions.

Oxidative Stress in Elevated TG and Reduced HDL-C Concentrations

Among the various coronary risk factors both TG and HDL-C concentrations were most strongly related to serum \( \delta \)-tocopherol concentration. Previous studies have shown that production of reactive oxygen species was increased in exclusively hyperTG patients\(^{27,28}\) and a positive correlation between total TG and leukocyte reactive oxygen species production has been reported.\(^{29}\) A smaller LDL particle size with lower \( \delta \)-tocopherol content is commonly observed in hyper TG patients and LDL particle size and particle antioxidant content are reported to be inversely correlated with oxidative susceptibility.\(^{30,31}\) HyperTG is known to be frequently associated with hypoHDL-C and also obesity. Indeed, in the present study subjects the TG concentrations were significantly negatively correlated with HDL-C concentration and positively with BMI. Although the precise mechanisms by which TG enhances reactive oxygen species production needs to be clarified, correction of such metabolic disorders by lifestyle modifications, including diet, smoking cessation and exercise, may be effective in reducing oxidative stress and preventing the development of coronary artery disease. Parks et al reported reduced oxidizability concomitant with increased contents of \( \delta \)-tocopherol and \( \beta \)-carotene during a treatment program that included intensive exercise therapy, stress management and a low-fat diet in patients with coronary artery disease.\(^{31}\) The protective effect of HDL against atherosclerosis could be linked to its participation in the reverse transport of cholesterol from the arterial wall, and recently an antioxidant effect of HDL has been postulated.\(^{32}\) This antioxidant effect preventing LDL oxidation, which is believed to be central to the initiation and progression of atherosclerosis, relates to its enzymes, primarily paraoxonase.\(^{33}\) Paraoxonase in HDL also inhibits oxidation of HDL itself and preserves its function.\(^{34}\) The positive correlation between \( \delta \)-tocopherol and HDL-C concentrations demonstrated in the present study indicates that HDL is important as an endogenous antioxidative protective substance and the HDL-C concentration could be an inverse marker for oxidative stress.

Conclusions

Oxidative stress, as shown by decreased serum \( \delta \)-tocopherol concentration, was found in apparently healthy subjects with various coronary risk factors including male gender, higher age, smoking, high serum concentrations of LDL-C, TG and FBS, low HDL-C concentration, and obesity. Both the TG and HDL-C concentrations seemed to be strongly related to increased oxidative stress.

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References

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