Plasma β-Carotene, Retinol, and α-Tocopherol Levels in Relation to Glycemic Control of Children with Insulin-Dependent Diabetes Mellitus

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Summary Plasma β-carotene, α-tocopherol and retinol were measured in 15 female and 5 male children with insulin-dependent diabetes mellitus (IDDM), and the correlations with plasma hemoglobin A1c (HbA1c) and fructosamine were analyzed. Twelve female and 8 male children served as age-matched controls. The plasma β-carotene and α-tocopherol levels of the IDDM children were significantly higher than those of the control children, but there were no differences in plasma retinol or total lipid levels. The plasma β-carotene level, β-carotene/retinol ratio and β-carotene/total lipids ratio each showed significant correlations with serum HbA1c and fructosamine in all subjects studied. Similarly, the plasma α-tocopherol level and α-tocopherol/total lipids ratio were correlated with these indexes of glycemic control. These findings suggest certain mechanisms may exist to prevent lipid peroxidation and vascular complications in IDDM patients.

Key Words α-tocopherol, β-carotene, retinol, diabetes mellitus, HbA1c

There is much evidence to show that oxidative cell injury caused by free radicals contributes to the development of various complications of diabetes mellitus such as angiopathy (1-3). Both vitamin E and β-carotene are important fat-soluble antioxidants, which protect lipoproteins and the vascular endothelium from oxidative injury that may occur in diabetic patients (4, 5). Therefore, it is important to assess the serum antioxidant vitamin concentrations in diabetic patients. Serum levels of vitamin A were reported to be reduced in children with insulin-dependent diabetes mellitus (IDDM) (6). Impaired conversion of β-carotene to vitamin A was suggested because cutaneous signs of elevated serum carotene are common in diabetic patients before insulin therapy (7, 8). Serum vitamin E levels have also

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been studied in diabetic patients by several investigators and were found to be increased (9) despite elevated lipid peroxidation (10). To our knowledge, however, the precise mechanisms underlying the increased serum levels of antioxidant vitamins have not yet been well explained.

Based on the above findings, this study was undertaken for patients with IDDM in order to determine the plasma vitamin A, β-carotene and vitamin E levels and to understand their regulatory mechanisms.

SUBJECTS AND METHODS

The IDDM patients enrolled in this study attended the Summer Camp for Diabetic Children at Sasayama (Osaka Kuruminokai) in 1993. Their details are summarized in Table 1. The diabetic group consisted of 15 females (12.6 ± 3.2 y, mean ± SD) and 5 males (11.2 ± 2.2 y, mean ± SD). The duration of diabetes ranged from 4.5 to 3.7 y. The insulin requirement ranged from 0.73 to 3.5 U/kg, with a mean of 1.06 U/kg body weight. None of the diabetic patients had any complications, such as cataract, retinopathy or nephropathy, and they had no evidence of other systemic diseases including malabsorption syndrome. The BMI was 15.5 ± 2.5 kg/m² in males and 18.8 ± 3.5 kg/m² in females. The dietary guidelines were based on the current recommendations of the Japanese Diabetes Association. None of the subjects received any vitamin supplements.

Twelve female (11.6 ± 2.4 y, mean ± SD) and 8 male outpatients (11.1 ± 2.0 y, mean ± SD) served as age-matched controls. The BMI was 16.2 ± 1.2 kg/m² in males and 16.9 ± 2.8 kg/m² in females. They had been referred to our clinic with the diagnosis of headache or autonomic dysfunction, and had no metabolic abnormalities or malnutrition on biochemical testing.

As shown in Table 1, there were no differences in age or BMI which could have influenced plasma vitamin concentrations between the sexes or between IDDM and control children. Therefore, the combined values for both sexes were used in analysis of the correlations between indexes of glycemic control and plasma vitamin levels. This study complied with the code of ethics of the World Medical Association (Declaration of Helsinki), and informed consent was obtained from all of the

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<th>Table 1. Subjects enrolled in the study.</th>
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<td><strong>IDDM</strong></td>
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Values represents the mean ± SD.
Before the morning insulin injection, overnight fasting blood samples were collected into tubes containing EDTA-2Na by venipuncture and were centrifuged to separate the plasma. The samples were frozen at $-80^\circ$C until analysis. Plasma $\beta$-carotene (11), retinol (12) and $\alpha$-tocopherol (13) levels were analyzed by high-performance liquid chromatography (HPLC) using electrochemical and fluorescence detection. HbA1c was determined by an autoanalyzer (Hi-Auto A1c: HA-8121; Kyoto Dai-ichi Kagaku, Kyoto, Japan) using cation exchange HPLC (14). Fructosamine was determined by an autoanalyzer (COBAS MIRA PLUS; F. Hoffman-La Roche, Basel, Switzerland) using a colorimetric procedure. Total plasma lipids were estimated by summing the three major lipids (cholesterol, triglycerides and phospholipids), which were measured by the methods of Allain et al (15), Bucolo and David (16) and Takayama et al (17), respectively.

Data are presented as the mean $\pm$ SD. Results were analyzed by Student’s $t$-test (unpaired). Pearson’s correlation coefficients were calculated to evaluate correlations between variables, and $p<0.05$ was considered significant.

RESULTS

Plasma $\beta$-carotene, retinol, and $\alpha$-tocopherol levels in IDDM patients and control children

The plasma levels of $\beta$-carotene, retinol and $\alpha$-tocopherol as well as total lipids in the IDDM patients and control children are shown in Table 2. Plasma $\beta$-carotene

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<th>Table 2. Plasma vitamin and lipid levels in IDDM patients and controls.</th>
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Values represents the mean $\pm$ SD.
* $p<0.01$ compared to control.

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and \( \alpha \)-tocopherol levels were significantly higher in the IDDM patients than in control children, but there were no significant differences in plasma retinol or total lipid levels. Even when the plasma \( \beta \)-carotene and \( \alpha \)-tocopherol levels were divided by plasma total lipids, the ratios for the IDDM patients were significantly higher than those for control children.

**Correlations between plasma vitamins and plasma total lipids**

Combined values for both sexes were used to compare IDDM patients and control children. Plasma \( \alpha \)-tocopherol, but not \( \beta \)-carotene, was significantly correlated with plasma total lipids, as shown in Fig. 1.

**Correlations between \( \beta \)-carotene and HbA1c or fructosamine**

Plasma \( \beta \)-carotene, the \( \beta \)-carotene/retinol ratios and the \( \beta \)-carotene/total lipids ratio were significantly correlated with the plasma HbA1c level, as shown in Fig. 2. Similar results were obtained for plasma fructosamine levels.

**Correlations between \( \alpha \)-tocopherol and HbA1c or fructosamine**

The plasma \( \alpha \)-tocopherol level and \( \alpha \)-tocopherol/total lipids ratio were significantly correlated with HbA1c and fructosamine, as shown in Fig. 3.

**Correlations in the IDDM patients**

Similar significant correlations of plasma \( \beta \)-carotene, \( \beta \)-carotene/retinol ratio and plasma \( \alpha \)-tocopherol with HbA1c and fructosamine were observed when only the diabetic patients were assessed (data not shown).
Fig. 2. Correlation between plasma β-carotene and index of glycemic control. BC, β-carotene; Ret, retinol; TL, total lipids. Plasma β-carotene, β-carotene/retinol and β-carotene/total lipid ratios significantly (p<0.05) correlate with HbA1c and fructosamine. Open circles represent the IDDM children and closed circles represent control subjects.
DISCUSSION

Previous studies have shown low plasma retinol levels in IDDM patients (18) and an increase of retinyl esters (19) in both IDDM and NIDDM patients, which have been explained by decreased mobilization of vitamin A from the liver, reduced hepatic stores of this vitamin or decreased conversion of carotene to retinol at the intestinal level. Skin involvement (xanthodermia) is observed in severe diabetes, especially in children, before commencement of insulin therapy (7, 8). The excessive intake of green-yellow vegetables containing carotene is not the only cause, because diabetic patients may show carotenemia even without such a high intake of these vegetables.

In this study, although the plasma retinol level in our IDDM patients did not differ from that in the control subjects, the plasma β-carotene level was significantly higher in the IDDM patients, even when it was corrected by total plasma lipids.
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This may be explained by the shorter duration of disease or the enrollment of younger subjects than in our previous study (18). There were also no complications in our patients, such as diseases of the liver, kidney or thyroid, which can influence the transport of vitamin A.

The plasma β-carotene level and β-carotene/retinol ratio were significantly correlated with HbA1c and fructosamine levels, suggesting that poor glycemic control in IDDM patients was associated with the elevation of β-carotene. The mechanism of elevation of β-carotene is not lipid-dependent and is considered to involve impairment of the enzyme converting β-carotene to retinol. Such an explanation may be supported if the action of this enzyme depends on insulin. In fact, it has been demonstrated in rats that the release of retinol from the liver is influenced by insulin (20). Therefore, another possibility is reduced hepatic utilization of β-carotene in insulin-deficient IDDM patients.

α-Tocopherol is an important fat-soluble antioxidant vitamin in the lipid bilayers of biomembranes. Previous studies have indicated that a higher plasma α-tocopherol level is associated with high plasma lipid levels (21) or occurs in patients with diabetic nephropathy (22). This study showed that plasma α-tocopherol was significantly higher in IDDM patients than in control children, even after adjusting for total plasma lipids. Plasma α-tocopherol also correlates with glycemic control, as does plasma β-carotene. One possible explanation is the excessive intake of vegetables and another is the effect of diabetic nephropathy (22). However, excessive intake of vitamin E-rich food was ruled out by history taking, and our IDDM patients did not have proteinuria or nephropathy. The other possibility is an increased uptake of dietary α-tocopherol by the elevation of hepatic α-tocopherol-transfer protein (αTTP) (23–25), which regulates the body content of α-tocopherol. This assumption is supported by previous reports of a high vitamin E level in the livers of rats with streptozotocin (STZ)-induced diabetes (26) and of increased αTTP expression and mRNA in the diabetic rat liver (unpublished data).

Increases in plasma β-carotene and α-tocopherol levels may be advantageous as antioxidants in IDDM patients, but appear to be insufficient to prevent oxidative injury. This may represent a mechanism for the prevention of peroxidation, although we did not determine the plasma level of thiobarbituric acid reactive substances. This assumption is supported by the detection of increased oxidative stress in the plasma (9), low-density lipoprotein (4) and erythrocytes of diabetic patients (6, 10), and by the improved action of insulin after the administration of pharmacologic doses of vitamin E to diabetic patients (27). When peroxidation by free radicals in IDDM patients overcomes the defensive antioxidants, complications like angiopathy might occur.

In this present study, we observed that elevated plasma β-carotene and α-tocopherol levels were associated with poor glycemic control. Certain mechanisms may operate to elevate these antioxidant vitamins, but further studies are needed to understand the precise metabolic processes involved and the free radical reactions in IDDM.
REFERENCES


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