Note

 β -Carotene 15,15'-Dioxygenase Activity in Streptozotocin-Induced Diabetic Rats

Takao MORINOBU and Hiroshi TAMAI

Department of Pediatrics, Osaka Medical College, Osaka 569–8686, Japan

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Summary We measured retinol levels and β -carotene 15,15'-dioxygenase activity in rats with streptozotocin-induced diabetes mellitus to assess the relationship between the disease and the conversion of β -carotene to retinol. The plasma retinol level was significantly lower in diabetic rats than in control rats, but the hepatic retinol level was significantly higher than the control. The hepatic dioxygenase activity, but not that of the intestinal mucosa, was significantly lower in diabetic rats than in control rats. The hepatic dioxygenase activity showed a significant negative correlation with the hepatic retinol levels. The results suggest the disturbed secretion of retinol from the liver and suppression of hepatic dioxygenase activity by the retinol increased in the liver in diabetic rats.

Key Words diabetes mellitus, vitamin A, β -carotene 15,15'-dioxygenase

It is known that the plasma β -carotene level is high and the retinol level is low in diabetes mellitus (1, 2). In a recent study, rats with streptozotocin (STZ)-induced diabetes were found to have low plasma vitamin A concentrations but high hepatic vitamin A levels, and this discrepancy was related to a defect in the transport of vitamin A from the liver (3). Impaired conversion of β -carotene to vitamin A was also suggested because cutaneous signs of carotenemia were observed in diabetic patients before insulin therapy (1, 4). β -Carotene 15,15'-dioxygenase (EC 1.13.11.21) is an enzyme found in the intestines, liver, and other organs which converts β -carotene to retinal (5, 6). Although this enzyme activity has previously been found to respond to the vitamin A status of rats, there has been no assessment of the relationship between diabetes mellitus and β -carotene dioxygenase activity. In this study, we measured vitamin A levels and β -carotene 15,15'-dioxygenase activity in STZ-diabetic rats to investigate the relationship between diabetes and the conversion of β -carotene to retinol.

Materials and Methods

Subjects. Male Wistar rats (8 wk old) were obtained from the animal center and were housed in stainless steel cages in a well-ventilated room. After 2 wk on a standard diet (containing 10,000 IU vitamin A per kg) and free access to water, the rats were divided into two groups: 8 rats with streptozotocin-induced diabetes (STZ group) and 6 age-matched control rats (control group).

Induction of diabetes. Diabetes was created by a single injection of STZ (50 mg/kg) dissolved in citrate buffer (pH 4.5), which was given into a tail vein.

Control rats were injected with the same volume of citrate buffer. At 2 d after STZ injection, a diabetic state was confirmed by assessing urine glucose with commercial enzymatic test strips (Bayer Sankyo Co.). All rats received food and water ad libitum until the time of sacrifice.

Sample collection. At 4 wk after STZ injection, all of the rats were sacrificed by exsanguination under diethyl ether anesthesia after being fasted overnight. Samples of blood, liver, and intestinal mucosa were obtained. After measurement of the blood glucose and hemoglobin A_{1c} (HbA_{1c}) levels, the plasma was stored at -70°C with protection from the light until analysis. Liver tissue and intestinal mucosa were homogenized at 0°C in five volumes of 50 mM HEPES-KOH buffer (pH 7.4) containing 1.15% (w/v) KCl, 1 mM ethylenediaminetetraacetic acid, and 0.1 mM dithiothreitol (DTT). The post mitochondrial supernatants were harvested after centrifugation at $10,000\times g$ for 30 min. To make an enzyme preparation, an aliquot of the supernatant was applied to a 1.5×5.5 cm Sephadex G-25M column (7) and the protein fraction was stored at -70°C until use. HbA_{1c} was measured using a latex agglutination immunoassay (8).

Analysis of vitamin A and β -carotene 15,15'-dioxygenase activity. The vitamin A concentrations in plasma and the supernatant of liver tissue homogenate were assayed by HPLC (9). To measure free retinol in the liver, the supernatants were extracted without saponification. The protein concentrations of the supernatants and the enzyme preparations were determined by the method of Bradford (10) using bovine serum albumin as the standard. β -Carotene 15,15'-dioxygenase activity was measured by the method of During et al. (7). The standard reaction mixture for the assay of β -carotene dioxygenase activity contained $15\text{ }\mu\text{M}$ all-*trans* β -carotene, 0.1 M Tricine-KOH buffer (pH 8.0), 0.5 mM

Table 1. Body weight, HbA_{1c} level, blood glucose level, vitamin A status and β -carotene 15,15'-dioxygenase activity in the STZ group and control group.

	STZ (n=8)	Control (n=6)
Body weight before treatment (g)	308 $\pm$ 9	312 $\pm$ 7
Body weight 4 wk after treatment (g)	229 $\pm$ 41**	385 $\pm$ 24
HbA _{1c} (%)	9.1 $\pm$ 1.7**	3.2 $\pm$ 0.1
Glucose (mg/dL)	389 $\pm$ 172*	134 $\pm$ 14
Plasma retinol (μ g/dL)	26.9 $\pm$ 8.4**	44.3 $\pm$ 7.6
Hepatic retinol (μ g/g protein)	36.3 $\pm$ 12.2**	8.7 $\pm$ 2.2
Hepatic retinyl palmitate (mg/g protein)	5.1 $\pm$ 2.7	3.0 $\pm$ 0.9
Hepatic dioxygenase activity (pmol/mg protein/h)	74.6 $\pm$ 13.2*	89.9 $\pm$ 11.1
Intestinal dioxygenase activity (pmol/mg protein/h)	42.4 $\pm$ 21.4	62.1 $\pm$ 58.6

Values represent the mean $\pm$ SD.
* $p < 0.05$ compared for control group, ** $p < 0.01$ compared for control group.

DTT, 0.15% Tween 40, 4 mM sodium cholate, 0.1 mM α -tocopherol, 15 mM nicotinamide, and an enzyme sample (less than 0.5 mg protein) in a total volume of 0.2 mL. The enzymic reaction was conducted at 37°C for 30 min and was terminated by adding 50 μ L of 37% (w/w) formaldehyde. Then the mixture was further incubated at 37°C for 10 min. After acetonitrile (500 μ L) was added, the mixture was mixed thoroughly, placed on ice for at least 5 min, and then centrifuged at 10,000 $\times g$ at 4°C for 10 min. The supernatant was directly subjected to HPLC to determine the retinal concentration (7).

Statistical analysis. Results are expressed as the mean $\pm$ SD, and the significance of differences was estimated by Student's *t*-test. Correlations were calculated using StatView ver. 4.0 software (Abacus Concepts, Inc. USA). Probability values of less than 0.05 were considered significant.

Results and Discussion

At four wk after STZ injection, the body weight of diabetic rats was significantly decreased, while that of control rats was markedly increased. The body weight at 4 wk was significantly lower in the STZ group than in the control group (Table 1). Blood glucose and HbA_{1c} levels were markedly elevated in the STZ group compared with the control group. These findings showed that the STZ-treated rats had severe diabetes. The plasma retinol level was significantly lower in diabetic rats than in control rats, but the hepatic retinol level was significantly higher in the diabetic rats than in the control rats (Table 1). In contrast, we found no significant difference of hepatic retinylpalmitate levels between diabetic rats and control rats. Previous studies have shown low plasma retinol levels and increased retinylester levels in patients with insulin-dependent diabetes mellitus (IDDM), which has been explained by decreased mobilization of vitamin A from the liver, reduced hepatic stores, or decreased intestinal conversion of carotene to retinol (11–13). It is also known that plasma RBP levels are lower in IDDM patients than in normal controls (11). With regard to the discrepancy of retinol levels between the plasma and liver, Tuitoeck ex-

Fig. 1. Correlation between hepatic retinol level and β -carotene 15,15'-dioxygenase activity in the two groups. Open circles represent the control group and closed circles represent the STZ group.

plained this finding as a result of defective secretion of vitamin A from the liver (3) as well as low plasma RBP levels in STZ diabetic rats (14). In diabetic patients, urinary loss of zinc, which was necessary to synthesize RBP, was reported to be increased (15, 16). Thus, zinc deficiency may be involved in vitamin A deficiency in diabetes mellitus. Although various possibilities have been suggested regarding the relationship between vitamin A metabolism and diabetes, the exact reason why plasma retinol and RBP levels are reduced in diabetes is not well known yet.

β -Carotene 15,15'-dioxygenase is a very important enzyme in the metabolism of β -carotene and vitamin A, and is also known to exist in the intestinal mucosa, liver, brain, and fat. In this study, we found a lower hepatic β -carotene dioxygenase activity in diabetic rats which had higher free-retinol levels, although intestinal dioxygenase activity showed no significant difference between the two groups (Table 1). Moreover, the hepatic dioxygenase activity showed a significant negative correlation with the hepatic retinol level. The dietary intake of vitamin A, β -carotene, and fat is known to affect

intestinal β -carotene dioxygenase activity (17–19). van Vliet et al. reported that low vitamin A intake results in higher intestinal β -carotene dioxygenase activity, while supplementation of the diet with β -carotene results in a decrease of dioxygenase activity (17). In contrast to intestinal dioxygenase activity, liver dioxygenase activity did not vary with the dietary retinylpalmitate level, so they suggested that hepatic β -carotene dioxygenase activity might be induced by an increase of β -carotene in the liver (17, 20). From our finding of an increased hepatic retinol level and decreased hepatic dioxygenase activity in the present study, the hepatic dioxygenase activity seems to be suppressed by an increase of retinol in the liver, although it might also have been affected by STZ or the diabetic state. Further investigations are required to clarify whether there is an altered retinol demand and/or disturbed β -carotene metabolism in diabetes.

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