The effect of dietary supplementation of β-carotene on lipid metabolism in streptozotocin-induced diabetic rats

Jung Sook Seoa,*, Kyeung Soon Leea, Jung Hyun Janga, Zhejiu Quana, Kyung Mi Yangb, Betty Jane Burric

aDepartment of Food and Nutrition, Yeungnam University, Gyeongsan, 712-749, South Korea
bDepartment of Cooking and Nutrition, Daegu Haany University, Gyeongsan, 712-715, South Korea
cWestern Human Nutrition Research Center, Agriculture Research Service, USDA, Davis, CA 95616, USA

Received 31 March 2004; revised 13 August 2004; accepted 29 September 2004

Abstract

Vascular complications such as atherosclerosis hinder the treatment of diabetes. We hypothesized that moderate supplementation with β-carotene might help prevent diabetic vascular complications through its impact on lipid metabolism. Forty Sprague-Dawley rats were fed AIN-76 control diet, or the same diet supplemented with β-carotene (7.2 mg/kg diet) for 3 weeks, then diabetes was induced in half of the rats by streptozotocin. Diabetic and normal rats were fed the experimental diets for 2 more weeks. β-Carotene did not reduce blood glucose in diabetic rats. Plasma triglycerides were increased by diabetes, but reduced by β-carotene. Plasma total cholesterol was increased by diabetes. High-density lipoprotein cholesterol did not differ between groups. However, the atherogenic index of diabetic rats was higher than that of control rats, and β-carotene feeding decreased it. Fecal excretion of cholesterol and coprostanone was decreased by diabetes, and β-carotene tended to increase this excretion. Fecal excretion of bile acid showed similar tendencies, as did neutral steroids. These results suggest that dietary supplementation with β-carotene may reduce plasma triglycerides and other indices of diabetic risk, and thus may decrease the incidence of diabetic vascular complications through the normalization of lipid metabolism in patients with diabetes.

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Keywords: β-Carotene; Triglyceride; Diabetes; Cholesterol; Bile acid
1. Introduction

Diabetes mellitus is a chronic disease associated with serious complications. Accelerated atherosclerosis is common in patients with diabetes mellitus, and this acceleration has been linked to increased lipid peroxidation [1]. It is not certain whether dietary supplementation with antioxidant vitamins can retard or perhaps reverse the oxidative damage associated with diabetes mellitus [2]. However, studies have suggested that increased intake of various antioxidant vitamins reduces the incidence rates of vascular diseases, cancer, and other adverse health outcomes [3]. Patients with insulin-dependent diabetes mellitus were classified among the groups at risk for low vitamin and phytounitrient status [4], and some studies suggest that some degree of supplementation with carotenoids and other antioxidant nutrients may help prevent some long-term complications of diabetes mellitus [5,6].

Whether carotenoids can protect against the vascular disease complications associated with diabetes remains an unsettled question. The relationship is likely to be complex and may vary with the duration and severity of the diabetic complications, as well as the nutritional status of the patient. However, several major studies suggest that high serum carotenoid concentrations are associated with reduced risk for vascular diseases [7,8]. Furthermore, dietary supplementation with β-carotene can normalize low-density lipoprotein (LDL) oxidation and consequently may be of importance in delaying accelerated development of atherosclerosis in patients with diabetes mellitus [9].

Little is known about the association of carotenoids (a diverse group of plant compounds) with diabetes or the reasons for their apparent protective effects against complications of this disease. Most of the research on carotenoids has centered on their well-known antioxidant function, especially because diabetes is a condition characterized by oxidative stress [10]. However, carotenoids have other functions. In particular, β-carotene has an important function as a precursor of vitamin A, and it has a direct impact on cholesterol synthesis [11,12].

Hypercholesterolemia is a major risk factor for atherosclerosis and thus reduction of plasma cholesterol concentration by dietary consumption of carotenoids may reduce the risk of the cardiovascular disease that is a diabetic complication [13]. Carotenoids that are present in plant cells, and cholesterol that is present in animal cells, share the same synthetic pathway. Inhibitors of cholesterol biosynthesis reduce serum cholesterol concentration by enhancing the removal of serum LDL, secondary to activation of the LDL receptors [14]. This impact of carotenoids on cholesterol synthesis and metabolism may be associated with their protective effect on the vascular complications of diabetes.

Previous study suggested that chronic oral administration of retinol and other retinoids caused elevation of plasma lipid concentrations, especially triacylglycerol concentrations [15]. However, the effects of dietary β-carotene, a carotenoid partially metabolized to retinol, on lipid concentrations in rats or human beings are not well studied. We conducted this study to investigate the effect of dietary supplementation of β-carotene on lipid metabolism in diabetic rats.
2. Methods and materials

2.1. Animals and diets

This study was approved by the Laboratory Animal Care Committee of Yeungnam University. Rats were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Yeungnam University. Forty male Sprague-Dawley rats (Daehan experimental animal center, Korea) weighing 200 to 220 g were housed individually in stainless steel mesh cages in a room at 22°C ± 2°C and 60% to 65% relative humidity with normal 12-hour light-dark cycles. Rats were fed chow diet (Jinyang Co, Korea) and tap water ad libitum for 1 week before the experiment. Casein, vitamin, and mineral mixtures for diets were purchased from Harlan Teklad Company (Madison, Wis).

After 1 week of acclimation, rats were randomly divided into 4 equal groups (n = 10 each). The 4 groups are described in Table 1. The dietary treatment consisted of AIN-76–based diets [16] with or without \( \beta \)-carotene (7.2 mg/kg diet) (Table 2). We chose this level of \( \beta \)-carotene supplementation because it is the recommended intake for rats. Rats were fed diets containing 15% fat. On the 21st day after introduction of these diets, rats in diabetic groups received an intramuscular injections of streptozotocin (STZ; Sigma, St Louis, Mo; 45 mg/kg BW in 0.1 M citrate buffer, pH 4.5) to induce diabetes. The other rats received an injection of citrate buffer without STZ. Diabetic and normal rats were returned to their diets for 2 more weeks (for a total of 5 weeks on their respective diets). Table 1 represents the experimental design related to the dietary supplementation of \( \beta \)-carotene and the STZ injection.

All animals were killed 14 days after the STZ injection. Blood was drawn from the abdominal aorta into heparinized syringes, and plasma was obtained by centrifugation at 1400 \( \times \) g at 4°C for 15 min. Livers were perfused with cold normal saline, then excised. After excision, livers were blotted dry, weighed, then frozen in liquid nitrogen. Feces were collected for the last 2 days of the experiment. Collected samples were stored at −80°C for analysis.

2.2. Biochemical analysis

Plasma glucose in rats was determined by the glucose oxidase method using a commercially available enzymatic kit. Serum total cholesterol and triglyceride concentrations were assayed enzymatically using commercial kits. Atherogenic index (AI) was calculated by the equation: (total cholesterol – HDL-cholesterol) / HDL-cholesterol [17]. Liver was homogenized and

<table>
<thead>
<tr>
<th>Group (n = 10)</th>
<th>Basal diet</th>
<th>( \beta )-Carotene (mg/kg diet)</th>
<th>STZ (mg/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>7.2</td>
<td>–</td>
</tr>
<tr>
<td>CD</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>BD</td>
<td>+</td>
<td>7.2</td>
<td>+</td>
</tr>
</tbody>
</table>

C indicates control diet (no \( \beta \)-carotene); B, \( \beta \)-carotene–supplemented control diet; CD, control diet (no \( \beta \)-carotene, STZ-induced diabetes); BD, \( \beta \)-carotene–supplemented control diet (STZ-induced diabetes).

\(^a\) Streptozotocin (45 mg/kg BW) was injected to rats intramuscularly.
lipids were extracted with chloroform/methanol (2/1 vol/vol) according to the method of Folch et al [18]. Total cholesterol and triglyceride concentrations in hepatic lipid were assayed enzymatically using commercial kits. All kits were supplied by the Embiel Company (Gunpo, Korea).

2.3. Measurement of fecal excretion of neutral steroids and bile acid

Fecal concentrations of neutral steroids and bile acid were determined by the method of Crowell and Macdonald [19]. Feces were freeze-dried for 48 hours and ground into fine powder. Lipids in feces were saponified by autoclaving at 120°C for 1 hour with KOH solution and neutral sterols were extracted twice with ethyl ether. The supernatant was evaporated with a rotary evaporator (Buchi, Switzerland) and dried under a nitrogen stream. Portions of the residue were dissolved in chloroform and then injected into a gas chromatograph (HP5890, Hewlett Packard, Palo Alto, Calif) fitted with a Supelco SAC-5 capillary column (30 m × 0.25 mm × 0.25) with flame-ionization detection. Oven temperature was 200°C for 5 min, then 250°C at 5°C/min and 300°C at 2°C/min. Helium gas was used as a carrier. Cholesterol and coprostanone comigrated in the method, so results are the sum of cholesterol plus coprostanone. 5α-Cholestane was used as the internal standard.

Table 2
Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3.5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
<td>400</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
<td>100</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix. a</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix. b</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a Mineral mixture according to AIN-76.
b Vitamin mixture according to AIN-76.

Fig. 1. Effect of dietary supplementation of β-carotene on plasma glucose in normal and diabetic rats. Values shown are mean ± SD (n = 10). Values with the same superscript letter are significantly different (P < .05).
The amount of total fecal bile acids was determined enzymatically with a diagnostic bile acids kit (Sigma).

2.4. Statistical analysis

Results are presented as means and standard deviations. Group means were compared by Duncan’s multiple range test after preliminary analysis of variance and differences were considered statistically significant at $P < .05$. All statistical tests were performed using the computer software program SPSS for Windows (SPSS, version 10.0, Chicago, Ill) for statistics and data analyses.

3. Results

Plasma concentration of glucose was significantly increased in the diabetic groups (CD and BD). Dietary supplementation with $\beta$-carotene did not reduce the blood glucose in diabetic rats (Fig. 1).

Plasma lipid profiles are summarized in Table 2. Plasma concentrations of triglyceride were increased greatly in diabetic rats, but reduced by $\beta$-carotene feeding ($P < .05$). Plasma concentrations of total cholesterol were also increased by induction of diabetes. Dietary supplementation of $\beta$-carotene tended to reduce these levels, but the decreases were not significant. Plasma concentrations of high-density lipoprotein cholesterol (HDL-C) did not differ between groups. However, AI of diabetic rats was higher than that of control rats, and $\beta$-carotene feeding decreased it (Table 3).

### Table 3
Effect of dietary supplementation of $\beta$-carotene on plasma concentrations of triglyceride, total cholesterol, HDL-C, LDL-C, and AI in normal and diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglyceride (mg/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>73.5 ± 8.23$^c$</td>
<td>79.5 ± 10.35$^b$</td>
<td>25.4 ± 4.25$^{NS}$</td>
<td>2.13 ± 0.20$^c$</td>
</tr>
<tr>
<td>CD</td>
<td>126.4 ± 18.52$^a$</td>
<td>110.3 ± 15.32$^a$</td>
<td>23.5 ± 5.25</td>
<td>3.69 ± 0.26$^a$</td>
</tr>
<tr>
<td>B</td>
<td>70.7 ± 10.53$^c$</td>
<td>82.5 ± 8.92$^b$</td>
<td>24.5 ± 4.26</td>
<td>2.17 ± 0.20$^c$</td>
</tr>
<tr>
<td>BD</td>
<td>96.4 ± 22.51$^b$</td>
<td>100.5 ± 16.3$^a$</td>
<td>23.7 ± 5.27</td>
<td>3.42 ± 0.17$^b$</td>
</tr>
</tbody>
</table>

Values shown are mean ± SD (n = 10). Values with the same superscript letter within the column are not significantly different ($P < .05$).

AI = (total cholesterol − HDL-C) / HDL-C.

### Table 4
Effect of dietary supplementation of $\beta$-carotene on hepatic concentrations of triglyceride and total cholesterol in normal and diabetic rats (mg/g liver)

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglyceride</th>
<th>Total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>23.5 ± 2.54$^{b,c}$</td>
<td>10.0 ± 1.93$^c$</td>
</tr>
<tr>
<td>CD</td>
<td>36.3 ± 3.56$^a$</td>
<td>20.0 ± 1.83$^a$</td>
</tr>
<tr>
<td>B</td>
<td>21.3 ± 2.65$^c$</td>
<td>10.3 ± 1.04$^c$</td>
</tr>
<tr>
<td>BD</td>
<td>26.5 ± 1.72$^b$</td>
<td>16.0 ± 1.32$^b$</td>
</tr>
</tbody>
</table>

Values shown are mean ± SD (n = 10). Values with the same superscript letter within the column are not significantly different ($P < .05$).
Hepatic concentrations of triacylglycerol were increased in diabetic groups, but \( \beta \)-carotene feeding reduced the value to some extent \((P < .05)\). Hepatic concentrations of total cholesterol showed the similar tendency to the triglyceride level (Table 4).

Fecal excretion of coprostanol was not different among treatment groups (Fig. 2). Fecal excretion of the sum of cholesterol and coprostanone was decreased by diabetes and \( \beta \)-carotene feeding tended to increase it, but not significantly (Fig. 3). Fecal excretion of bile acid showed similar tendencies with that of the neutral steroids. Fecal excretion of bile acid was decreased by diabetes and \( \beta \)-carotene feeding tended to increase it to some extent (Fig. 4).

4. Discussion

Diabetes mellitus often leads to generalized vasculopathy. Cardiovascular disease is the most prevalent and detrimental complication of diabetes mellitus [20]. The incidence of cardiovascular mortality in diabetic subjects without a clinical history of previous cardiac events is as high as the incidence in nondiabetic subjects with a history of myocardial infarction [21].

Until now the studies on diabetic complications concentrated on their relation to oxidative stress. In studies on diabetes mellitus and antioxidants such as vitamin E and carotenoids, their
antioxidative functions on diabetes were usually the only functions studied. However, these nutrients have other important functions. Specifically, carotenoids also have a direct impact on cholesterol synthesis [12,13]. Therefore, changes in carotenoids might influence either cholesterol or other compounds that effect cholesterol synthesis, storage, or metabolism.

In this experiment, we found the effect of dietary supplementation of β-carotene induced changes on lipid metabolism in STZ-induced diabetic rats. Specifically, plasma concentrations of triacylglycerol were greatly increased in diabetic rats, but reduced by β-carotene feeding. In addition, as shown in Table 3, plasma concentrations of total cholesterol were increased when we induced diabetes. Although it was not significant, dietary supplementation of β-carotene tended to reduce total cholesterol level. When we looked at cholesterol subsets, we found that the AI (calculated using total cholesterol and HDL-C) was increased by diabetes, but β-carotene feeding alleviated this increase significantly.

Vitamin A and its derivatives cause hypertriglyceridemia with high levels of intake [15], but β-carotene is known to be relatively nontoxic and generally does not elevate serum vitamin A concentrations above normal levels [22]. Few studies have measured the effects of β-carotene on lipid metabolism, and these studies are contradictory. One study showed that high intakes of β-carotene had no effect on serum lipid concentrations in human beings [23]. However, Tsai et al [24] observed that dietary β-carotene reduced serum total cholesterol and LDL cholesterol (LDL-C) concentrations in spontaneously hypertensive rats. They also showed that supplementation with β-carotene resulted in significant reductions in total, LDL, and very low-density lipoprotein triacylglycerol concentrations. These results are consistent with ours and seem to suggest that dietary β-carotene may play a role in altering abnormal lipid metabolism in diabetic rats.

Hyperglycemia contributes directly to atherosclerosis by inducing endothelial changes [25]. We found that β-carotene feeding did not reduce plasma glucose elevated by diabetes. However, hyperglycemia is just one of the reasons why people with type 2 diabetes have vascular complications. In addition to the hyperglycemia, type 2 diabetes and its precursor state of insulin resistance commonly occur in a metabolic syndrome whose symptoms include hypertension, atherogenic dyslipidemia, and a procoagulant state [26]. Therefore, the decrease of AI by β-carotene feeding can be an important factor for the prevention of diabetic vascular complications.
Our results are consistent with other reports. For example, Uchida et al [27] reported fecal sterol excretion and the cholesterol/sitosterol ratio were decreased in diabetic rats. They also demonstrated positive correlations between cumulative serum cholesterol level and the atheromatous lesion area. Their findings suggest that alteration of bile acid metabolism coupled with increases in cholic acid synthesis in diabetic rats enhanced cholesterol absorption producing significant hypercholesterolemia, which in turn led to the development of atheromatous lesions.

Little is known about the mechanisms by which \(\beta\)-carotene is absorbed and transported in the blood or about the metabolic pathways where \(\beta\)-carotene and cholesterol might interact. As yet, the mechanism of the hypolipidemic effect of dietary \(\beta\)-carotene is not known. However, \(\beta\)-carotene and cholesterol share a common synthetic pathway, and \(\beta\)-carotene is transported in blood associated with chylomicron, very low-density lipoprotein, and LDL remnants. Carotenoid absorption is generally thought to involve disruption of the food matrix, dispersion into lipid emulsions, and solubilization into mixed bile salt micelles [28]. There are several areas of cholesterol metabolism that carotenoids might influence, possibly by a competitive mechanism. The regulation of blood cholesterol levels is achieved by homeostasis between cholesterol absorption, synthesis, and LDL fractional catabolism [29]. A reduction in intestinal cholesterol absorption prevents the accumulation of cholesterol in the liver. Because the expression of LDL receptor is controlled by feedback inhibition of intracellular cholesterol, reductions in hepatic cholesterol accumulation in turn stimulate the production of more high-affinity LDL receptors [30]. This results in an increase in clearance of cholesterol from the circulation by LDL receptor and thus lowers blood cholesterol [31]. This regulation may be less effective in diabetes because of their abnormal lipid metabolism. Half of the cholesterol eliminated from the body is excreted into the feces after conversion of bile salts [32]. Conversion of cholesterol to bile acids is the major pathway of cholesterol elimination and it accounts for about 50% of daily cholesterol excretion. Cholesterol 7\(\alpha\)-hydroxylase, the rate-determining enzyme in the conversion of cholesterol to bile acids, is mainly regulated by feedback inhibition of bile acids reabsorbed from the intestine [33]. The increase in the excretion of bile acids and cholesterol seems to activate cholesterol 7\(\alpha\)-hydroxylase, which can enhance the conversion of liver cholesterol to bile acids for excretion. This leads to a decrease in hepatic cholesterol content, which in turn stimulated LDL receptor expression and lowered blood cholesterol level. Thus, a mechanism of hypocholesterolemic action may be due to the promotion of cholesterol and bile acids excretion [34].

There are many studies on the relationships between antioxidants and vascular diseases [35,36]. The LDL oxidation hypothesis of atherosclerosis led to extensive investigation on the possible role of antioxidants against LDL oxidation and atherosclerosis. These studies showed that oxidation of LDL leads to its aggregation. This has perhaps led to an overemphasis on the role of carotenoids as antioxidants in disease prevention studies. Carotenoids have other important functions. Thus, Fuhrman et al [11] reported that dietary consumption of nutrients rich in polyphenols led to attenuation in the development of atherosclerotic lesions, secondary to their inhibitory effects on LDL oxidation and aggregation [11]. Fuhrman et al [13] concluded in their study on the effect of carotenoids on macrophage cholesterol metabolism that dietary supplementation of carotenoids may act
not as antioxidants, but as moderate hypcholesterolemic agents secondary to their inhibitory effect on macrophage 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis [16]. They clearly demonstrated that macrophage enrichment with \( \beta \)-carotene resulted in the suppression of cellular cholesterol synthesis and increased macrophage LDL receptor activity. Thus, carotenoids may be as hypcholesterolemic agents: the mechanism by which carotenoids could lower cholesterol and influence cholesterol-related metabolites has a solid biochemical basis.

In this study our relatively low supplementation of \( \beta \)-carotene resulted in small differences associated with changes in cholesterol metabolism. These changes might have been larger, if we had used higher, less physiological concentrations of carotenoids in our study, or supplemented for a longer time. It is difficult to engender a stable diabetic condition in rats, harsher treatments often cause death in the animals. Diabetic response of rats is very different according to the duration of diabetes, STZ treatment, and animal age. We were able to engender a stable diabetic condition in rats by this relatively mild treatment and believe that this stable condition is more representative of diabetes in human beings than the more severe diabetic conditions that might have produced larger treatment effects.

We hypothesize that \( \beta \)-carotene supplementation might have increased bile acid excretion and reduced cholesterol concentrations because plasma or liver cholesterol would be used to maintain the bile acid pool. An alternative hypothesis is that \( \beta \)-carotene influenced bile acid binding within the small intestine, disrupted micelle formation, which led to a reduced ability to solubilize cholesterol (as well as monoglycerides and fatty acids) and consequently, reducing cholesterol absorption. Increased bile acid excretion represents another mechanism by which a reduction in cholesterol can be produced [35]. Our results suggest that dietary supplementation of \( \beta \)-carotene to normal diet of patients with diabetes might reduce the incidence of diabetic vascular complications through the improvement of their abnormal lipid metabolism, not by control of plasma glucose.

**Acknowledgment**

This research was supported by a grant (01-PJ1-PG3-22000-0064) from the Good Health R&D Projects, Ministry of Health and Welfare, R.O.K.

**References**


