Distribution of Circulating β -Carotene in Human Plasma Lipoproteins

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Summary We examined the distribution of β -carotene in plasma lipoprotein fractions. In healthy children and adults, LDL contained more β -carotene than did HDL, but in cord blood more β -carotene was found in HDL than in LDL. After the oral administration of β -carotene, its plasma level rose although its distribution in the individual lipoprotein fractions did not change. Among disease conditions associated with hyperlipidemia, the ratio of β -carotene to plasma lipids was highest in anorexia nervosa, while nephrotic syndrome and diabetes mellitus had similar ratios to each other.

Key Words β -carotene, lipoprotein, cord blood, hyperlipidemia, anorexia nervosa, diabetes mellitus, the nephrotic syndrome

Carotenoids including β -carotene in human plasma are of plant origin. Among them, β -carotene forms the chief source of vitamin A (1-3). However, the nutritional role of β -carotene as a vitamin A precursor is actually rather low, and the majority of carotenoids remains in the circulating form after absorption, while a part of it accumulates in the skin as a yellow pigment (4-6). The biological functions of β -carotene in living body are not well known, although epidemiologic evidences of nutritional β -carotene are accumulated in relation to prevention of carcinogenesis (7,8), and the antioxidant activity of carotenoids, especially as a scavenger of singlet oxygen, has recently been focused. In pediatric fields, however, it is well known that a large amount of carotenoids including β -carotene is contained in human milk, especially in colostrum, as compared with cow's milk (9-11), although physiologic role of β -carotene in children is entirely obscure. As a tool to clarify the physiological function of β -carotene, its concentration and distribution in living tissues should be determined. A fat-soluble compound, β -carotene, has to be initially transported in the flow with the chylomicrons after absorption (12,13). With respect to the subsequent steps of transport, there have been few reports to our knowledge. In this study, the distribution of β -carotene in

lipoprotein fractions was investigated in healthy children, and in various other subjects in whom lipid metabolism was affected.

MATERIALS AND METHODS

1. *Subjects.* Experiment I: (1) Healthy group: Eleven healthy children aged from 1–18 years were enrolled. They attended our outpatient clinics either for health checks or with various symptoms (headache, chest pain, nausea, lassitude, and/or motion sickness). All of them had no evidence of any pathology which could affect blood lipid levels. They included seven males and four females. Adult samples were obtained from five laboratory personnel (all males aged from 24–27 years), who were nonsmokers without health problems and were not taking any medication. Cord blood was obtained from the umbilical veins of separated placentas after normal pregnancy and delivery.

(2) Subjects with hyperlipidemia: Patients with nephrotic syndrome (four males and one female, 5 to 12 years old), anorexia nervosa (all females, 13 to 16 years old), and treated insulin-dependent diabetes mellitus (all males, 8 to 12 years old) were also enrolled in this study. The five patients with nephrotic syndrome were receiving therapy with steroids, and all of them showed no proteinuria and no hypoalbuminemia while hyperlipidemia remained (total lipids, 543 ± 35 mg/dl; total cholesterol, 233 ± 32 mg/dl). The six patients with anorexia nervosa were all active stage, whose lipid levels were 528 ± 85 mg/dl in total lipids, and 288 ± 26 mg/dl in total cholesterol. The five patients with insulin-dependent diabetes mellitus were under good or moderate control in their insulin therapy. Their lipid levels were 457 ± 23 mg/dl in total lipids, and 197 ± 19 mg/dl cholesterol. To obtain a wide range of plasma lipid levels, two hypothyroidisms (12 years old and 15 years old) and eight obese children (5 males and 3 females, all more than 10 years old) were enrolled in this study, whose lipid levels were, however, less than 450 mg/dl in total lipids, and less than 230 mg/dl in total cholesterol. Heparinized blood was collected after an overnight fast.

Experiment II: The above-mentioned five laboratory personnel were orally given all-*trans* β -carotene (750 mg/day) together with breakfast for three days. Blood was collected following an overnight fast both before and 24 h after supplementation.

The study protocol was approved by the ethics committee of the hospital, and was only performed after informed consent was obtained from the subjects themselves and/or their parents as was appropriate.

2. *Preparation of lipoprotein fractions.* Immediately after 5 ml of heparinized blood was collected, it was centrifuged at $1,500 \times g$ for 10 min to separate the plasma from the cells. Plasma lipoproteins were then fractionated by ultracentrifugation according to the method of Hatch and Lees (16) within 6 h after the isolation of plasma, with adjusting the density to 1.006 and 1.063 with NaBr. Centrifugation was done at 4°C using a Hitachi 65P-7 ultracentrifuge with a rotor

lipids, and triglycerides were measured by the methods of Allain *et al.* (17), Takayama *et al.* (18), and Bucolo *et al.* (19), respectively, using Wako Kits (Wako Chemicals, Tokyo, Japan). Total lipids were estimated by summing the levels of these three major lipids. Free fatty acids were not included in this estimation, because they comprised <4% of the total lipids.

RESULTS

1. Distribution of β -carotene in human lipoprotein fractions

Table 1 shows the β -carotene concentrations in plasma and the individual lipoprotein fractions. The majority of the β -carotene was found in the LDL fraction, which contained 78% of total lipoprotein β -carotene and 64.1% of plasma β -carotene. Since 82% of the β -carotene in plasma was recovered from all the lipoprotein fractions examined, the following studies were undertaken using only the lipoprotein fractions. Table 2 shows the distribution of β -carotene, total cholesterol and free cholesterol in the LDL or HDL fractions of cord blood and children's blood. The percent distribution of β -carotene in LDL or HDL resembled that for total and free cholesterol, although the LDL and HDL levels differed between cord and children's blood. Table 3 shows the β -carotene concentrations in the plasma and individual lipoprotein fractions for cord blood, healthy children and

Table 1. β -Carotene concentrations in plasma and individual lipoprotein fractions.

Plasma	29.8 $\pm$ 3.2 (μ g/dl) (n = 6)
HDL	4.7 $\pm$ 0.5
LDL	19.1 $\pm$ 1.5
VLDL	0.7 $\pm$ 0.2
The sum of lipoproteins	24.5 $\pm$ 2.0

β -Carotene in the sum of lipoproteins was recovered as 82% of plasma β -carotene.

Table 2. Distribution of β -carotene, total or free cholesterol in the LDL and HDL fractions.

	LDL (%)	HDL (%)
Cord blood (n = 18)		
β -Carotene	44.8 $\pm$ 9.7	55.2 $\pm$ 9.7
Total cholesterol	39.9 $\pm$ 8.6	55.1 $\pm$ 8.6
Free cholesterol	43.3 $\pm$ 9.5	52.9 $\pm$ 10.3
Children's blood (n = 13)		
β -Carotene	67.5 $\pm$ 15.6	29.3 $\pm$ 14.9
Total cholesterol	58.8 $\pm$ 7.3	33.6 $\pm$ 7.8
Free cholesterol	62.0 $\pm$ 8.1	25.5 $\pm$ 9.2

Table 3. Distribution of β -carotene in plasma and the individual lipoprotein fractions in cord blood, children, and adults.

	Cord blood ($\mu\text{g/dl}$) ($n=18$)	Children ($\mu\text{g/dl}$) ($n=11$)	Adults ($\mu\text{g/dl}$) ($n=5$)
Plasma	2.1 ± 0.9	20.5 ± 5.4	24.8 ± 5.2
VLDL	0 (0%)	1.4 ± 1.8 (7%)	1.0 ± 0.7 (3%)
LDL	0.9 ± 0.4 (45%)	12.3 ± 4.0 (60%)	19.3 ± 5.7 (78%)
HDL	1.1 ± 0.5 (55%)	6.8 ± 4.3 (33%)	4.5 ± 2.2 (19%)

Fig. 2. Correlation between the percent distribution of β -carotene and that of total cholesterol in LDL and HDL ($n=37$).

adults. The plasma β -carotene concentrations in cord blood was one tenth that in healthy children and adults, probably due to the low plasma lipoprotein concentrations in cord blood, while there was no significant differences between children and adults. Most of the β -carotene was in the LDL fraction in children and adults, while in cord blood more was found in HDL than in LDL.

2. Correlation of β -carotene to lipoprotein lipids

Figure 2 illustrates the correlation of the percent distribution of β -carotene to that of the total cholesterol component in LDL and HDL in children with and without hyperlipidemia ($n=37$). A moderate correlation was observed for both LDL and HDL, although the relationship was more significant for LDL. The correlations between the levels of β -carotene and the individual lipoprotein lipids are shown in Table 4. β -Carotene level correlated significantly with those of all the lipids (total lipids, phospholipids, and total cholesterol), while no correlation was observed with the triglyceride level. Free cholesterol and β -carotene levels were also related to a similar degree (data not shown).

Table 4. Correlations between plasma β -carotene and plasma lipid levels.

	<i>r</i> (<i>n</i> = 37)	<i>p</i>
Total lipid	0.53	<0.01
Phospholipid	0.54	<0.01
Total cholesterol	0.60	<0.01
Triglyceride	—	—

Table 5. Distribution of β -carotene and total cholesterol in plasma and the individual lipoprotein fractions before and after administration of β -carotene.

β -Carotene	Before (<i>n</i> = 5)	After
Plasma (μ g/dl)	24.8 $\pm$ 2.9	75.7 $\pm$ 11.5
HDL (μ g/dl)	4.7 $\pm$ 0.5 (19 $\pm$ 3%)	14.4 $\pm$ 1.2 (19 $\pm$ 4%)
LDL (μ g/dl)	19.3 $\pm$ 1.5 (78 $\pm$ 5%)	59.0 $\pm$ 4.3 (78 $\pm$ 6%)
VLDL (μ g/dl)	0.7 $\pm$ 0.1 (3 $\pm$ 1%)	2.3 $\pm$ 0.2 (3 $\pm$ 1%)
Total cholesterol	Before (<i>n</i> = 5)	After
Plasma (mg/dl)	18.9 $\pm$ 2.9	20.3 $\pm$ 3.8
HDL (mg/dl)	4.7 $\pm$ 0.3 (25 $\pm$ 2%)	5.1 $\pm$ 0.4 (25 $\pm$ 2%)
LDL (mg/dl)	13.0 $\pm$ 1.0 (69 $\pm$ 5%)	14.0 $\pm$ 1.2 (69 $\pm$ 4%)
VLDL (mg/dl)	1.1 $\pm$ 0.2 (6 $\pm$ 1%)	1.2 $\pm$ 0.2 (6 $\pm$ 1%)

The percent distribution of β -carotene and total cholesterol to lipoproteins did not change after orally administered β -carotene.

3. Changes of lipoprotein β -carotene and distribution after administration of β -carotene

The plasma β -carotene level was increased about 3-fold above baseline after administration of β -carotene to the five healthy volunteers. The distribution is shown in Table 5. The distribution of β -carotene in the individual lipoproteins was not changed after administration. In addition, no changes in lipids occurred after β -carotene administration.

4. Distribution of β -carotene in lipoprotein fractions under hyperlipidemic conditions

The distributions of β -carotene and the individual lipids are shown in Fig. 3. The distribution of β -carotene in the individual lipoprotein fractions was closest to that in total cholesterol for all the diseases tested. The plasma β -carotene was highest in patients with anorexia nervosa, although the extent of hyperlipidemia in anorexia nervosa and the nephrotic syndrome was comparable. β -Carotene levels were similar in diabetes mellitus and the nephrotic syndrome, although hyperlipide-

Fig. 3. Distribution and concentration of β -carotene, total cholesterol, triglycerides, phospholipids, and total lipids in normal controls and patients with various hyperlipidemic states. The circle areas represent the relative concentration of β -carotene and various lipids between control and disease conditions, respectively. The numbers in the circle represent the distribution in each lipoprotein fractions. The numbers under the circle also represent the concentrations of β -carotene and individual lipids in plasma.

mia was less severe in diabetes mellitus. Since the extent of hypercarotenemia and hyperlipidemia varied between these conditions, Table 6 shows the ratios of β -carotene to total plasma lipids. The ratio of β -carotene to total lipids was 3-fold greater in anorexia nervosa than in the healthy children, while it was only 1.7-fold greater in diabetes mellitus and the nephrotic syndrome. Similar results were observed regarding the ratios of β -carotene to total cholesterol and phospholipids.

DISCUSSION

The vast majority of the plasma β -carotene was found in the lipoprotein

Table 6. Ratios of β -carotene to total lipids, total cholesterol, and phospholipids in various disease states.

	β -Carotene/ total lipids ($\mu\text{g}/\text{mg}$)	β -Carotene/ total cholesterol ($\mu\text{g}/\text{mg}$)	β -Carotene/ phospholipid ($\mu\text{g}/\text{mg}$)
Healthy children ($n = 11$)	0.057	0.142	0.138
Anorexia nervosa ($n = 6$)	0.169	0.309	0.464
Diabetes mellitus ($n = 5$)	0.102	0.237	0.291
Nephrotic syndrome ($n = 5$)	0.100	0.232	0.289

The ratios of β -carotene to total lipids and each individual lipid were calculated as μg of β -carotene to mg of lipids per 100 ml of plasma.

fractions, with the largest part being in the LDL fraction in children and adults and in the HDL fraction in cord blood, both of which are the major plasma lipoprotein fractions at these ages. The plasma level of β -carotene was closely correlated with the levels of plasma total cholesterol, phospholipids, and total lipids, while it was poorly correlated with the triglyceride level, because triglycerides are variably partitioned in the individual lipoprotein fractions. The distribution of β -carotene in LDL and HDL was also coincident with that of both total and free cholesterol, as shown in Table 2. This indicates that β -carotene resides not only in the outer region but also in the inner or core region of the lipoproteins, because esterified cholesterol is known to be located at lipoprotein core. Cornwell *et al.* (14) administered oral β -carotene (120 and 200 mg) to human subjects and reported that an increase of the β -carotene level occurred equally in all lipoprotein fractions up to 24 h, and that it then shifted to the LDL fraction during the period from 24 to 72 h. In our study of the effects of oral β -carotene (750 mg/day for 3 days), the lipoprotein distribution of β -carotene was not altered by treatment. Our finding of an unchanged distribution in all lipoprotein fractions after administration seems consistent with the early phase distribution in Cornwell's study. Both studies indicate that the distribution depends on the global partitioning of lipids among the lipoproteins. Thus, β -carotene appears to be absorbed, transported, and distributed during the production of various lipoproteins. This is the same as the lipoprotein distribution of tocopherol (20), probably because of the lack of specific binding proteins for both β -carotene and tocopherol.

Diseases associated with hypercarotenemia include diabetes mellitus, hypothyroidism, panhypopituitarism, anorexia nervosa, liver dysfunction, and the nephrotic syndrome (21). Considering that such conditions may also relate to hyperlipidemia, the relationship between hyperlipidemia and β -carotene was examined with respect to the lipoprotein distribution of β -carotene. The diseases studied were anorexia nervosa, diabetes mellitus, and the nephrotic syndrome in which the extent of hyperlipidemia and hypercarotenemia are varied. The lipoprotein distribution of β -carotene in these hyperlipidemic conditions was similar to that of total cholesterol-

ol. However, the extent of the hypercarotenemia did not reflect that of the hyperlipidemia in these three conditions. In anorexia nervosa and the nephrotic syndrome, the extent of the hyperlipidemia was similar, but hypercarotenemia was greater in the former disease than in the latter. The extent of hypercarotenemia was similar between the nephrotic syndrome and diabetes mellitus, but hyperlipidemia was more marked in the former. When the ratio of β -carotene to total lipids was examined, this was the largest in anorexia nervosa, followed by diabetes mellitus and nephrotic syndrome at a similar level, and was the smallest in the normal subjects. This finding suggests that hypercarotenemia in the disease states was not completely explainable on the basis of the extent of hyperlipidemia alone. Hypercarotenemia may appear in response to a variety of mechanisms besides hyperlipidemia, including excessive dietary intake, impaired conversion of β -carotene to vitamin A, and the decreased catabolism of β -carotene (21). In diabetes mellitus, hypercarotenemia has been reported to be related to restricted dietary habits, since some diabetics increase their intake of β -carotene-containing foods while restricting their carbohydrate intake (22). An impaired capacity to convert β -carotene to vitamin A has also been found in the isolated intestinal mucosa of alloxan-induced diabetic rats. Anorexia nervosa showed the most severe hypercarotenemia among the hyperlipidemic states examined in our study. In this condition, a high-carotene diet cannot be held responsible. Klinefelter (23) has observed that there was no correlation between the cholesterol level and the dietary intake in these patients, and proposed that hypercholesterolemia may be related to the decreased catabolism of cholesterol and may account for some cases of hypercarotenemia in anorexia nervosa. Another explanation for the hypercarotenemia in anorexia nervosa may be defective conversion of β -carotene to vitamin A. In our experience of patients with anorexia nervosa, low plasma retinol levels are observed in association with low levels of retinol binding proteins, reflecting their poor nutritional status. However, this finding does not adequately explain the poor conversion to retinol in anorexia nervosa, because low retinol levels associated with low levels of retinol binding protein are found in various nutritional deficiency states, including protein and zinc deficiency. Further studies are required to resolve the mechanism producing hypercarotenemia in various disease states.

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