Ascorbic Acid Deficiency Elevates Serum Level of LDL-Cholesterol in a Rat Mutant Unable to Synthesize Ascorbic Acid

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Summary  The effect of ascorbic acid deficiency on serum levels of high density lipoprotein- (HDL-), low density lipoprotein- (LDL-), very low density lipoprotein- (VLDL-) and chylomicron-cholesterol was examined in ODS-od/od rat (ODS rat), that is a rat mutant unable to synthesize ascorbic acid. Male ODS rats were fed an ascorbic acid-free diet for 20 days. In another two groups, the diet supplemented with 300 mg ascorbic acid/kg diet was fed either ad libitum (ad libitum control) or in pair-feeding (pair-fed control). Pair-fed rats received the same amount of diet as rats fed the ascorbic acid-free diet. Serum level of total cholesterol in the ad libitum control rats, ascorbic acid-deficient rats, and the pair-fed control rats were 100.1 ± 8.4 mg/dl, 92.8 ± 6.2 mg/dl and 72.2 ± 4.8 mg/dl, respectively. The level of LDL-cholesterol in ascorbic acid-deficient rats was significantly higher than that in the ad libitum control or that in the pair-fed control. The level of HDL-cholesterol in ascorbic acid-deficient rats was lower than that in the ad libitum control, but was not changed as compared with that in the pair-fed control. Ascorbic acid deficiency did not affect serum level of VLDL-cholesterol or chylomicron-cholesterol as compared with those in the controls. These results demonstrate that ascorbic acid deficiency causes the elevation of serum level of LDL-cholesterol both in ad libitum feeding condition and pair feeding condition.  

Key Words  ascorbic acid, ascorbic acid deficiency, ODS rat, cholesterol, LDL-cholesterol, HDL-cholesterol

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ODS-od/od (ODS) rat, which is a rat mutant of the Wistar strain with a hereditary defect in ascorbic acid synthesizing ability, was established by Makino and Katagiri (1) and Mizushima et al. (2). L-Gulonolactone oxidase, which catalyzes the last step of ascorbic acid biosynthesis, is lacking in this rat mutant (3, 4). We (5) have reported that the dietary addition of 300 mg ascorbic acid/kg diet is enough to prevent signs of vitamin C deficiency and to achieve maximum growth in ODS rats.

It has been suggested that ascorbic acid deficiency might be a risk factor in the progress of atherosclerosis. In ascorbic acid-deficient guinea pigs (6–10) and ODS rats (11), hypercholesterolemia is caused due to the depression of bile acid synthesis. Furthermore, we speculate that ascorbic acid deficiency may affect the distribution of lipoprotein cholesterol in serum. We (11) have reported shortly that serum level of high density lipoprotein (HDL)-cholesterol was decreased in ascorbic acid-deficient ODS rats in ad libitum feeding condition. Ascorbic acid deficiency reduces food intake in ODS rats. Therefore, serum levels of lipoprotein cholesterol might be affected by ascorbic acid deficiency via the reduction of food intake. In this study, the effect of ascorbic acid deficiency on the distribution of lipoprotein cholesterol in serum was examined in ad libitum feeding condition or pair-feeding condition. The present report shows that ascorbic acid deficiency in ODS rats causes a significant elevation of serum level of low density lipoprotein (LDL)-cholesterol both in ad libitum feeding condition and pair-feeding condition.

EXPERIMENTALS

Animals and diet. Male ODS rats at eight weeks of age were generously supplied by Aburahi Laboratories, Shionogi Research Laboratories, Shionogi, Shiga. ODS rats were fed a basal diet (Table 1) supplemented with 300 mg ascorbic acid/kg diet for 2 weeks, and thereafter used for experiment. Rats were kept at 24°C with a 12-h cycle of light (from 0800h to 2000h) and dark. All rats were individually housed in wire screen-bottomed cages and were provided food and water ad libitum.

Experimental design. Rats (250–260 g) were divided into three groups of five rats each. Each group was fed a test diet for 20 days. Rats in group A were fed the basal diet supplemented with 300 mg ascorbic acid/kg diet ad libitum. Rats in group B were fed the basal diet (ascorbic acid-free) ad libitum. Rats in group C were fed the basal diet supplemented with 300 mg ascorbic acid/kg diet in pair-feeding. Rats in group C received the same amount of diet as rats fed the ascorbic acid-free diet (group B). On day 20, diet was removed from each cage at 0800 h. The animals were sacrificed by decapitation at 1400 h within a 90-min period. Blood was collected into glass tube, and liver was removed, weighed and stored at −20°C.

Analytical methods. Agarose gel electrophoresis of serum. Agarose gel electrophoresis was carried out by using Corning Universal Film (Corning, Palo Alto, Calif., U.S.A.). Lip-
Table 1. Composition of the basal diet (ascorbic acid-free).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>(%)</th>
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<tbody>
<tr>
<td>Casein(^1)</td>
<td>30.0</td>
</tr>
<tr>
<td>Mineral mixture(^2)</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture(^3)</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose powder(^4)</td>
<td>4.0</td>
</tr>
<tr>
<td>Starch: Sucrose (2:1)</td>
<td>56.8</td>
</tr>
<tr>
<td>Retinyl palmitate (mg/kg)</td>
<td>6.67</td>
</tr>
<tr>
<td>Ergocalciferol (µg/kg)</td>
<td>100</td>
</tr>
<tr>
<td>DL-α-Tocopheryl acetate (mg/kg)</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^1\) Crude casein (85% protein, Grade I, Katayama Chemical, Osaka). 
\(^2\) AIN-76 mineral mixture (12). The basal diet contains the following (mg/kg diet): Ca, 5,200; P, 4,000; K, 3,600; Na, 1,020; Mg, 500; Mn, 54; Fe, 35; Cu, 6; Zn, 30; I, 0.2; Se, 0.2; Cr, 2.0; Cl, 1,560; sulfate, 1,000.

\(^3\) The basal diet contains the following (mg/kg diet): thiamin-HCl, 10; riboflavin, 10; nicotinic acid, 50; Ca-pantothenate, 40; pyridoxine-HCl, 5; folic acid, 0.4; menadione, 1; biotin, 0.2; vitamin B-12, 0.04; inositol, 200; ascorbic acid was not included (13).

\(^4\) Crystalline cellulose, AVICEL type FD-101, Asahi Chemical, Tokyo.

oprotein-cholesterol was stained by cholesterol dehydrogenase method (Cholest-A, Nippon Chemiphar, Tokyo), and densitometry was carried out at 570 nm by using Dual-Wavelength TLC Scanner CS-910 (Shimadzu, Kyoto).

**Preparation of lipoproteins.** Lipoproteins in serum were separated into four fractions, that is chylomicrons, very low density lipoprotein (VLDL), LDL, and HDL, with ultracentrifugation according to the method of Hatch and Lees (14). Lipoproteins were separated at 16° C in a Hitachi 65P-type ultracentrifuge with type RP55 rotor. Aliquots of 3.6 ml of serum were measured into ultracentrifuge tubes, and 1.8 ml of density 1.006 solution (0.195 M NaCl) was layered over the surface. The tubes were capped and centrifuged for 30 min at 19,000 rpm (26,000 × g). A fraction that was floated to the top of the tube was defined as chylomicrons and collected. Further separations of lipoproteins were carried out according to the method of Hatch and Lees. The density classes of other lipoproteins were as follows: VLDL, \(d < 1.006 \text{ g/ml} \); LDL, \(d = 1.006-1.063 \text{ g/ml} \); HDL, \(d > 1.063 \text{ g/ml} \). Total cholesterol in serum or in each fraction of serum lipoproteins was measured by an enzymatic colorimetric method (Monotest cholesterol, Chod-PAP. method, Boehringer Mannheim GmbH, Mannheim, Germany). The protein content in each fraction of serum lipoproteins was determined according to Lowry et al. (15).

**Liver cholesterol.** Liver that had been stored at \(-20^\circ \text{C}\) was homogenized with chloroform: methanol (2:1). A portion of this extract was dried, and used for the measurement of the concentration of hepatic cholesterol by the same method as that in serum cholesterol.

**Liver ascorbic acid.** Liver that had been stored at \(-20^\circ \text{C}\) was homogenized...
with ice-cold 5% metaphosphoric acid and centrifuged for 10 min (1,600×g). Ascorbic acid in the supernatant was measured by the dinitrophenylhydrazine method (16).

Statistical analysis. Statistical significance of the difference between values was analyzed by one-way analysis of variance (ANOVA). When the effect of treatment (effect of ascorbic acid or pair-feeding) was significant, Duncan's multiple range test (17) was performed.

RESULTS

Figure 1 shows body weight gain of rats during the 20-day course. Body weight in group B began to decrease on day 14 due to ascorbic acid deficiency. The growth rate of rats in group C was similar to that in group B.

Table 2 shows the effect of ascorbic acid deficiency on body weight gain, food intake, liver weight, liver concentration of ascorbic acid, and liver concentration of cholesterol. Body weight gain, for 20 days, of rats in group B or C was lower than that in group A. Liver weight (g/100 g body weight) was lower in group B or C than that in group A. A marked reduction in liver concentration of ascorbic acid was observed in group B as compared with that in group A or C. Liver concentration of cholesterol in group B was slightly but significantly higher than that in group A or C.

Table 3 shows the effect of ascorbic acid deficiency on serum concentration of total, chylomicron-, VLDL-, LDL-, and HDL-cholesterol. Serum concentration of total cholesterol in group B was not different from that in group A. The concentration of serum cholesterol in group A or group B was higher than that in group C. There were no changes in the concentrations of chylomicron- or VLDL-cholesterol among these three groups. However, serum concentration of LDL-cholesterol in group B was higher than that in group A, and also higher than

![Fig. 1. Effect of ascorbic acid deficiency on body weight gain ODS rats.](image)
Table 2. Effect of ascorbic acid deficiency on body weight gain, food intake, liver weight, liver concentration of ascorbic acid and cholesterol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dietary ascorbic acid (mg/kg)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding pattern</td>
<td>Ad libitum</td>
<td>Ad libitum</td>
<td>Pair-feeding</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>269.3±16.0</td>
<td>267.0±7.8</td>
<td>267.3±15.4</td>
<td></td>
</tr>
<tr>
<td>Body weight gain for 20 days (g)</td>
<td>55.5±7.4s</td>
<td>35.0±12.8s</td>
<td>39.0±2.0s</td>
<td></td>
</tr>
<tr>
<td>Food intake for 20 days (g)</td>
<td>330.9±9.8s</td>
<td>288.3±9.4s</td>
<td>290.5±5.2s</td>
<td></td>
</tr>
<tr>
<td>Liver weight (g/100 g body weight)</td>
<td>4.15±0.10s</td>
<td>3.52±0.12s</td>
<td>3.63±0.22s</td>
<td></td>
</tr>
<tr>
<td>Liver ascorbic acid (μg/g)</td>
<td>112±10b</td>
<td>4.0±2.0a</td>
<td>102±10b</td>
<td></td>
</tr>
<tr>
<td>Liver cholesterol (mg/g)</td>
<td>2.22±0.06a</td>
<td>2.54±0.12b</td>
<td>2.31±0.18a</td>
<td></td>
</tr>
</tbody>
</table>

1Values are means ± SEM for 5 rats. Data were analyzed by one-way analysis of variance and Duncan's multiple range test. Means within a line not followed by the same superscript letter are significantly different (p<0.05).

Table 3. Effect of ascorbic acid deficiency on serum levels of total, chylomicron-, VLDL-, LDL-, and HDL-cholesterol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dietary ascorbic acid (mg/kg)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding pattern</td>
<td>Ad libitum</td>
<td>Ad libitum</td>
<td>Pair-feeding</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>100.1±8.4b</td>
<td>92.8±6.2b</td>
<td>72.2±4.8a</td>
<td></td>
</tr>
<tr>
<td>Chylomicron-cholesterol (mg/dl)</td>
<td>7.4±0.6</td>
<td>7.2±1.2</td>
<td>6.5±1.2</td>
<td></td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>2.8±0.4</td>
<td>2.6±0.4</td>
<td>3.0±0.4</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>19.5±0.4b</td>
<td>28.3±5.6c</td>
<td>10.4±1.6a</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>67.4±6.2b</td>
<td>53.3±5.0a</td>
<td>46.5±4.2a</td>
<td></td>
</tr>
</tbody>
</table>

1Serum level of total cholesterol was measured directly. The sum of chylomicron-, VLDL-, LDL-, and HDL-cholesterol in each group was as follows (mg/dl): group A, 97.0±4.2; group B, 91.4±5.6; group C, 66.4±5.6. 2Values are means ± SEM for 5 rats. Data were analyzed by one-way analysis of variance and Duncan's multiple range test. Means within a line not followed by the same superscript letter are significantly different (p<0.05).

that in group C. The ratio of cholesterol to protein (mg/mg) in LDL fraction of each group was as follows: group A, 0.56±0.04; group B, 0.57±0.06; group C, 0.46±0.02. This ratio in group A or group B was higher than that in group C. Serum concentration of HDL-cholesterol in group B was lower than that in group A, but not changed as compared with that in group C. The sum of chylomicron-, VLDL-, LDL- and HDL-cholesterol in each group was as follows: group A, 97.0±4.2 mg/dl; group B, 91.4±5.6 mg/dl; group C, 66.4±5.6 mg/dl. The recovery of lipoprotein cholesterol (chylomicron- plus VLDL- plus LDL- plus HDL-cholesterol/total cholesterol in serum measured directly) in each group was 96.9% in group A, 98.5% in group B and 92.0% in group C, respectively.

Figure 2 shows electrophoretograms of the lipoproteins on agarose gel, and
**Fig. 2.** Agarose gel electrophoresis of lipoproteins from ad libitum control rats (lanes 1, 2), ascorbic acid-deficient rats (lanes 3, 4), and pair-fed control rats (lanes 5, 6). Lipoprotein-cholesterol was stained by cholesterol dehydrogenase method.

**Fig. 3** shows its densitometric patterns at 570 nm. β-Lipoprotein cholesterol was increased in group B as compared with that in group A or group C (Fig. 2), and a higher intensity of β-lipoprotein cholesterol was observed in group B than that in group A or group C (Fig. 3).

**DISCUSSION**

These results demonstrate that ascorbic acid deficiency causes the elevation of serum level of LDL-cholesterol both in ad libitum feeding condition and in pair-feeding condition. The recovery of lipoprotein cholesterol during the course of ultracentrifugation in the pair-fed control was lower than that in the ad libitum control or ascorbic acid-deficient group. Even if the differences in the recovery of lipoprotein cholesterol among these groups were taken into consideration, these results indicated that serum level of LDL-cholesterol in ascorbic acid-deficient group was higher than that in the pair-fed control. Serum concentration of total cholesterol in the control rats in pair-feeding condition was lower than that in the control group in ad libitum feeding condition. The distribution of lipoprotein cholesterol in serum was not changed remarkably between these two control groups.

It has been reported (6–9) that ascorbic acid deficiency in guinea pigs caused a marked elevation of serum cholesterol and an accumulation of cholesterol in liver due to the suppression of cholesterol biotransformation into bile acids. We (18) also observed that an acute deficiency of ascorbic acid caused the elevation of serum cholesterol and the reduction of bile acid synthesis when ODS rats were fed a cholesterol-containing diet. Although ODS rats were fed a diet without cholesterol
in the present study, it is supposed that ascorbic acid deficiency might change the distribution of lipoprotein cholesterol in serum via the reduction of bile acid synthesis. In addition, ascorbic acid deficiency might affect the metabolism of lipoprotein cholesterol, mainly LDL-cholesterol, directly. Ginter and his coworkers (19) observed that ascorbic acid deficiency lowered the catabolic rate of LDL in guinea pigs. Aulinskas et al. (20) reported that the number of LDL receptors of cultured arterial smooth muscle cell was decreased in the absence or the low concentration of ascorbic acid in the culture medium. The present results indicated that ascorbic acid deficiency increased the serum level of LDL-cholesterol but did not change remarkably the ratio of cholesterol to protein in LDL fraction. We speculate that ascorbic acid deficiency might decrease the uptake of LDL by liver and extrahepatic tissues or affect the secretion of LDL by liver.

Recently, several studies have suggested that ascorbic acid deficiency in ODS rats causes atherogenic changes in serum lipids and aortic endothelial cells. Kono et al. (21, 22) reported that ascorbic acid deficiency decreased serum level of HDL-cholesterol without affecting serum activity of lecithin:cholesterol acyltransferase in ODS rats. Noguchi et al. (23) observed the abnormality of aortic endothelial cells and high level of serum lipoperoxide in ascorbic acid-deficient ODS rats.

In conclusion, this study suggested that ascorbic acid deficiency might change
the distribution of lipoprotein cholesterol in serum with significant increase in the level of LDL-cholesterol. The effect of ascorbic acid deficiency on the uptake of LDL by some tissues or the synthesis of LDL in liver remains to be clarified.

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REFERENCES


