Ascorbic Acid Deficiency Changes Hepatic Gene Expression of Acute Phase Proteins in Scurvy-Prone ODS Rats1,2

Saiko Ikeda,3 Fumihiko Horiö and Atsushi Kakinuma

Laboratory of Nutritional Biochemistry, Department of Applied Biological Sciences, School of Agricultural Sciences, Nagoya University, Nagoya 464–8601, Japan

ABSTRACT The ODS rat (genotype od/od), which has a hereditary defect in ascorbic acid biosynthesis, was used to investigate the effects of ascorbic acid deficiency on the hepatic gene expression of both the positive acute phase proteins, haptoglobin and α1-acid glycoprotein, and the negative acute phase proteins, apolipoprotein A-I and albumin. Male ODS rats (6 wk old, body weight ~140 g) were fed a basal diet containing ascorbic acid (300 mg/kg diet) or a diet without ascorbic acid for 14 d. Ascorbic acid deficiency significantly elevated the serum concentration of haptoglobin and significantly lowered those of apolipoprotein A-I and albumin. The hepatic mRNA levels of haptoglobin and α1-acid glycoprotein in the ascorbic acid–deficient rats were significantly elevated on d 12, and reached 260 (P < 0.05) and 360% (P < 0.01) of respective values in the control rats on d 14. On the contrary, the hepatic mRNA levels of apolipoprotein A-I and albumin in the ascorbic acid–deficient rats were lowered to 68 (P < 0.01) and 71% (P < 0.05) of respective values in the control rats on d 14. Although ascorbic acid deficiency significantly elevated the serum corticosterone concentration on d 14, the changes in mRNA levels of haptoglobin, α1-acid glycoprotein, apolipoprotein A-I and albumin due to ascorbic acid deficiency were not affected by adrenalectomy, as assessed in a separate experiment. The serum concentration of interleukin-6, an inflammatory cytokine that stimulates gene expression of some acute phase proteins, was significantly higher in the ascorbic acid–deficient rats on d 14 than in the control rats. These results suggest that ascorbic acid deficiency causes physiologic changes similar to those that occur in the acute phase response. J. Nutr. 128: 832–838, 1998.

KEY WORDS: • acute phase protein • ascorbic acid • corticosterone • interleukin-6 • ODS rats

The ODS rat (genotype od/od) with a hereditary defect in ascorbic acid biosynthesis (Konishi et al. 1990) is a useful model for investigating the physiologic role of ascorbic acid. This rat cannot synthesize ascorbic acid because of the lack of L-gulono-gamma-lactone oxidase (EC 1.1.3.8), which catalyzes the terminal step of ascorbic acid biosynthesis (Kawai et al. 1992). We recently surveyed serum proteins, whose concentrations are influenced by the ingestion of ascorbic acid in ODS rats, and reported that ascorbic acid deficiency lowered the serum apolipoprotein A-I (Apo A-I1)1 concentration through lowering its mRNA level in liver (Ikeda et al. 1996). We also observed that the serum concentration and hepatic mRNA level of α2u-globulin (Ikeda et al. 1997) were lowered by ascorbic acid deficiency in ODS rats. Lowell et al. (1986) reported that in mice the Apo A-I mRNA level in liver was reduced by the injection of lipopolysaccharide (LPS), which causes inflammatory responses and changes the hepatic gene expression of acute phase proteins. Schreiber et al. (1986) reported that the injection of turpentine, which causes acute inflammation, lowered the hepatic mRNA level of α2u-globulin in rats. From these observations, we hypothesized that ascorbic acid deficiency might lower the hepatic mRNA levels of Apo A-I, and α2u-globulin, as seen in acute inflammation.

The serum concentration of ascorbic acid is decreased in patients with inflammatory diseases such as rheumatoid arthritis (Lunev and Blake 1985, Oberritter et al. 1986) and bronchial asthma (Olusi et al. 1979). Yamaguchi et al. (1995) reported that LPS treatment lowered the ascorbic acid concentration in serum, liver and spleen of ODS rats. Moreover, Winklhofer-Roob et al. (1997) reported that the ascorbic acid concentration in plasma of patients with cystic fibrosis inversely correlated with plasma concentrations of α1-acid glycoprotein (AGP) and interleukin-6 (IL-6). AGP is one of the acute phase proteins, and IL-6 is a major inflammatory cytokine in the acute phase response. These results suggest that ascorbic acid metabolism is altered during inflammation and that ascorbic acid deficiency causes acute phase responses.

Several serum proteins, whose concentrations in serum...
are affected in response to acute inflammation, are called acute phase proteins (Kubser 1982). In rats, the serum concentrations of proteins, including haptoglobin, AGP, α1-antitrypsin, α1-antichymotrypsin, hemopexin, fibrinogen, α2-macroglobulin and α1-cysteine proteinase inhibitor, are increased in acute inflammation. These proteins are called positive acute phase proteins. Interleukin-1 (IL-1), IL-6 and glucocorticoid have been identified as principal mediators of the increased synthesis of hepatic acute phase proteins (Baumann et al. 1989). These mediators act primarily at the level of gene transcription; maximal stimulation requires the combination of these three mediators. On the other hand, the serum concentrations of some proteins such as albumin, Apo A-I and transferrin are lowered during the acute phase response; therefore, these proteins are called negative acute phase proteins (Morrone et al. 1989). The mechanisms of the lowering of their serum concentrations in the acute phase response are unknown.

In this study, using ODS rats, we tried to determine whether ascorbic acid deficiency changes the gene expressions of the positive and negative acute phase proteins in liver as does acute phase inflammation. We also examined the effects of ascorbic acid deficiency on the serum concentrations of corticosterone, a major glucocorticoid in rats, and IL-6.

**MATERIALS AND METHODS**

**Animals and diets.** Male ODS (sd:odl) rats, 5 wk of age, were purchased from Japan Clea (Tokyo, Japan). They were housed in individual wire screen-bottomed cages in the animal colony of Nagoya University and maintained at 24°C with a 12-h light cycle (lights on from 0800 to 2000 h). Rats were allowed free access to water and a purified diet. Composition of the basal diet is shown in Table 1. The dietary addition of 300 mg of ascorbic acid/kg diet is sufficient for maximum growth and prevents the development of scurvy in ODS rats (Horio et al. 1985). All rats were fed the basal diet for 7 d before the start of the experiment and then the experimental diet from d 1 through 14. In this experiment, rats were killed by decapitation between 1000 and 1100 h, and all procedures were performed in accordance with the Animal Experimentation Guides of Nagoya University.

**Experimental procedures.** During the experimental period, rats were fed the basal diet containing 300 mg ascorbic acid/kg diet (control group) or the diet without ascorbic acid (ascorbic acid-deficient group). Four rats from each group were killed on the morning of d 7, 10, 12 and 14 after the start of experiment. The food intake of ascorbic acid-deficient rats began to decrease slightly on d 12. Therefore, rats in the control group were pair-fed the mean amount consumed by rats in the ascorbic acid-deficient group from d 12 to 14.

A second experiment was conducted to examine the effect of adrenalectomy on the changes in the hepatic mRNA levels caused by ascorbic acid deficiency. Six rats were adrenalectomized and another six rats were sham-operated. Subsequently, all rats were allowed free access to water, containing 9 g/L NaCl and 30 g/L glucose, and to the basal diet for 7 d. Then, the rats (three rats each) were fed the basal diet (control group) or the ascorbic acid-free diet (ascorbic acid-deficient group) for 15 d. Rats in the control group were pair-fed with rats in the ascorbic acid-deficient group from d 12 to 15 in both sham-operated and adrenalectomized rats.

In both experiments, blood was collected and serum was prepared by centrifugation at 1500 × g for 10 min. Liver was taken and frozen immediately in liquid nitrogen, and stored at −80°C until used for extraction of RNA and determination of ascorbic acid concentration.

**Determination of liver ascorbic acid concentration.** Liver was homogenized in ice-cold 50 g/L metaphosphoric acid and centrifuged for 10 min at 1600 g. Ascorbic acid concentration in the supernatant was measured with the dinitrophenylhydrazine method (Roe and Kuether 1943) with a modification in which the oxidation of ascorbic acid was accomplished with 2,6-dichlorophenol-indophenol.

**Determination of serum interleukin-6 concentration.** An ELISA-dependent cell line, B9 (Aarden et al. 1987), was kindly provided by Y. Kumazawa of Kitasato University. IL-6 in serum was quantitated by measuring its activity in proliferation of B9 cells according to the method of Sharma et al. (1992). Two thousand cells in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) with 5 × 10^4 units/L penicillin, 50 mg/L streptomycin, 50 μmol/L 2-mercaptoethanol and 10% fetal bovine serum were added to serial dilutions of rat serum in 96-well microtiter plates. The cells were incubated for 72 h and their proliferation was measured by using a Cell Counting Kit (Wako Pure Chemical Industries, Osaka, Japan). Recombinant human IL-6 (Boehringer Mannheim Biochemica, Tokyo, Japan) was used as a standard in this assay.

**Determination of serum corticosterone concentration.** Serum corticosterone concentration was determined fluorometrically according to a modification (Gibbs 1970) of the method of Silber et al. (1958).

**Immunoblot analysis.** For the detection of haptoglobin and Apo A-I, serum (0.1 μL) was subjected to SDS-PAGE on a 10 g/L acrylamide-gel, and the proteins in the gel were transferred onto polyvinyldene difluoride membranes (Immobilon transfer membranes, Nihon Millipore, Tokyo, Japan). The filters were blocked for 1.5 h with PBS containing 30 g/L skim milk powder and incubated for 1.5 h with either goat anti-human haptoglobin antisera (Cappel Product, Organon Teknika, Durham, NC) or rabbit anti-rat Apo A-I antisera (Ikeda et al. 1996), which had been diluted 1:500 with PBS containing 0.5 g/L Tween-20 and 10 g/L skim milk powder. The filter was then washed twice with PBS containing 0.5 g/L Tween-20 and incubated for 1.5 h with either peroxidase-conjugated sheep anti-goat immunoglobulin G antibody or peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody. The filter was washed twice with PBS and autographed with the ECL Western blotting detection system (Amersham, Tokyo, Japan). Quantification of each protein on the band was performed with the densitometer.

For the detection of albumin, serum (0.01 μL) was subjected to SDS-PAGE on a 10 g/L acrylamide-gel and the proteins in the gel were transferred onto the polyvinylidene difluoride membranes as described above. The filters were blocked for 1.5 h with PBS containing 30 g/L skim milk powder and incubated for 1.5 h with either goat anti-human haptoglobin antisera (Cappel Product, Organon Teknika, Durham, NC) or rabbit anti-rat Apo A-I antisera (Ikeda et al. 1996), which had been diluted 1:500 with PBS containing 0.5 g/L Tween-20 and 10 g/L skim milk powder. The filter was then washed twice with PBS containing 0.5 g/L Tween-20 and incubated for 1.5 h with either peroxidase-conjugated sheep anti-goat immunoglobulin G antibody or peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody. The filter was washed twice with PBS and autographed with the ECL Western blotting detection system (Amersham). Quantification of each protein on the band was performed with the densitometer.

For the quantification of each protein, we had determined the quantitative range in which the intensity of the band was proportional to the volume of serum loaded as follows: haptoglobin, 0.01–0.5 μL; Apo A-I, 0.01–0.2 μL; albumin, 0.005–0.02 μL. In each analysis,

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**Table 1**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tr>
<td>Casein</td>
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<tr>
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</tr>
<tr>
<td>Cornstarch</td>
<td>408.5</td>
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<tr>
<td>Ascorbic acid</td>
<td>0.3</td>
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</table>

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1 AIN93-MX (AIN 1993).
2 AIN93-VX (AIN 1993).
3 AVICEL type FD-101, Asahi Chemical Industry, Osaka, Japan.
4 Amylalpha-CL, Chuoshokuryo, Aichi, Japan.
the band detected was single and no band was detected with non-immune serum of rabbit.

Northern blot analysis. Total RNA was extracted from liver and other tissues by the method of Chomczynski and Sacchi (1987) and subjected to Northern blot analysis. Twenty micrograms of the extracted RNA was separated by electrophoresis on 10 g/L agarose gel containing 66 g/L formaldehyde; 40 mmol/L 3-(N-morpholino)-propanesulfonic acid buffer (pH 7.0), 10 mmol/L sodium acetate and 1 mmol/L EDTA. RNA was denatured by heating at 55°C for 15 min in 3-(N-morpholino)propanesulfonic acid buffer containing 500 g/L formamide and 66 g/L formaldehyde. The electrophoresis buffer was 40 mmol/L 3-(N-morpholino)-propanesulfonic acid (pH 7.0), containing 10 mmol/L sodium acetate and 1 mmol/L EDTA. After electrophoresis, RNA was transferred directly onto a nitrocellulose membrane (Hybond, Amersham) in 1X SSC (1X SSC is 150 mmol/L NaCl and 15 mmol/L sodium citrate, pH 7.0). The membrane was then baked at 80°C for 2 h.

cDNA probes were labeled with [32P]dCTP by using a labeling system kit (Multiprime, Amersham). Hybridization with the probe (0.9 MBq/L of [32P]-labeled cDNA) was carried out overnight at 42°C in a solution containing 500 g/L formamide, 5X SSC, 5X Denhardt’s solution, 10 g/L SDS, 50 mmol/L sodium phosphate (pH 6.5) and 0.5 g/L denatured salmon sperm DNA. The membrane was washed twice with 2X SSC containing 1 g/L SDS for 15 min at room temperature, and twice with 0.1X SSC containing 1 g/L SDS for 15 min at 50°C. The washed membrane was subjected to autoradiography and the radioactivities on the bands were quantitated with Bioimage Analyzer System (BAS 2000II, Fuji Photographic Film, Kanagawa, Japan). Each value was normalized to the apolipoprotein E (Apo E) mRNA level.

cDNA clones. cDNA encoding rat AGP (Ricca and Taylor 1981) was synthesized by using the polymerase chain reaction. The upstream and downstream primers for AGP were 5'-CCGAGTTCGGCA- TGCGCCAGCATG-3' (nucleotide 26–52) and 5'-CCTCAGGATCCCTTGTGCCTCC-3' (inverse complement of nucleotide 633–655), respectively. Total RNA (10 μg) from male rat liver was reverse transcribed into cDNA by incubating with 0.25 μg of the downstream primer, 20 mmol/L MgCl2, 200 U M-MLV reverse transcriptase (SUPERSCRIPTII, Gibco BRL, Life Technologies, Tokyo, Japan), 0.5 mmol/L dNTP, 0.01 mmol/L diithiothreitol, 0.1 mol/L Tris-HCl (pH 8.3) in a final volume of 20 μL at 42°C for 2 h. The two primers (each 0.1 μmol) were added to a standard polymerase chain reaction mixture (final volume, 100 μL) containing the synthesized cDNA as template. The major reaction product (650 bp) was isolated and its DNA sequence was determined by the dideoxy chain-terminating method (Sanger et al. 1977).

The cDNA clone for rat haptoglobin (Marinkovic and Baumann 1990) was purchased from American Type Culture Collection (Rockville, MD). cDNA clones for rat Apo A-I (Haddad et al. 1986), rat Apo E (McLean et al. 1983) and rat albumin (Iwatsuki et al. 1987) were kindly provided by S. Kato of Tokyo University, J. M. Taylor of Gladstone Foundation Laboratories (San Francisco, CA) and K. Nakamura of Nagoya University, respectively.

Statistical analysis. Values in the text are means ± SEM. Mean values obtained for the control and the ascorbic acid–deficient groups were compared using Student’s t test when variances of each group were equal. When variances of each group were unequal, significance of differences was determined using Welch’s test (Aspin 1949, Trickett et al. 1956). Differences with a P-value < 0.05 were regarded as significant. In the experiment to examine the effect of adrenalectomy on the changes in the hepatic mRNA levels by ascorbic acid deficiency, data were analyzed by a two-way ANOVA. The significance of F-values for ascorbic acid deficiency effect and adrenalectomy effect as well as interaction effect of the two components (ascorbic acid deficiency × adrenalectomy) is shown in each table and figure. When the F-value was significant (P < 0.05), the mean value of each group was compared using Student’s t test or Welch’s test (Aspin 1949, Trickett et al. 1956).

RESULTS

Body weight, liver weight and hepatic ascorbic acid concentration of control and ascorbic acid–deficient rats. On d 7, 10, 12 and 14, the body weight and the liver weight did not differ in the control and ascorbic acid–deficient groups (Table 2). No signs of scurvy were observed in any rats of the ascorbic acid–deficient group during the course of the experiment. Throughout the experiment, the hepatic concentration of ascorbic acid in the ascorbic acid–deficient group was significantly lower than that in the control group.

Serum concentrations of haptoglobin, apolipoprotein A-I and albumin in control and ascorbic acid–deficient groups. Ascorbic acid deficiency did not affect the serum concentrations of haptoglobin, Apo A-I and albumin until after d 12 (Fig. 1). On d 14, the haptoglobin concentration in the ascorbic acid–deficient group was significantly higher than that in the control group, whereas those of Apo A-I and albumin were significantly lower than in the control group. We could not measure the serum concentration of AGP because an appropriate anti-rat AGP antiserum was not available.

Hepatic mRNA levels of haptoglobin, α1-acid glycoprotein, apolipoprotein A-I and albumin in control and ascorbic acid–deficient groups. The serum mRNA levels of haptoglobin, Apo A-I and albumin were significantly lower than in controls on d 12, and on d 14 they reached 260 and 360% of the respective control values (Fig. 2B). On d 14, the hepatic mRNA levels of Apo A-I and albumin in the ascorbic acid–deficient group were significantly lowered to 68 and 71% of the respective control values.

Serum concentrations of interleukin-6 and corticosterone in control and ascorbic acid–deficient groups. The serum concentrations of IL-6 (Fig. 3A) and corticosterone (Fig. 3B) in the control and ascorbic acid–deficient groups on d 7, 10 and 12 were not different, whereas the concentrations in the ascorbic acid–deficient group on d 14 were 400 and 460%, respectively, of those in the control group.

Body weight, liver weight, hepatic ascorbic acid concentration and serum corticosterone concentration of adrenalectomized and sham-operated rats. The initial body weight of the adrenalectomized rats was lower than that of the sham-operated rats (Table 3). No signs of scurvy were observed in any rats of the ascorbic acid–deficient group during the experiment, and the final body weight of rats was not affected by either ascorbic acid deficiency or adrenalectomy. In the sham-operated and the adrenalectomized rats, the hepatic ascorbic acid concentration in the ascorbic acid–deficient group was significantly lower than that in the respective control group. Adrenalectomy did not affect the hepatic ascorbic acid concentration. Although the serum corticosterone concentrations of sham-operated and adrenalectomized rats were not significantly different by post hoc test, no adrenal glands were found in any adrenalectomized rats when they were killed on d 15.

Hepatic mRNA levels of haptoglobin, α1-acid glycoprotein, apolipoprotein A-I and albumin in sham-operated and adrenalectomized rats fed control or ascorbic acid–deficient diets. Adrenalectomy did not affect the hepatic level of haptoglobin mRNA (Fig. 4). In both the sham-operated and adrenalectomized rats, the haptoglobin mRNA levels in the ascorbic acid–deficient groups were significantly higher than those in the respective control groups. The hepatic level of AGP mRNA was lowered by adrenalectomy, and in the sham-operated rats, the AGP mRNA level in the...
ascorbic acid–deficient group was significantly higher than that in the control group. In the adrenalectomized rats, the AGP mRNA level in the ascorbic acid–deficient group tended to be greater (P = 0.053) than in the control group. The hepatic level of Apo A-I mRNA was elevated by adrenalectomy, and in the sham-operated rats, the level in the ascorbic acid–deficient group was significantly lower than that in the control group. Adrenalectomy did not affect the hepatic level of albumin mRNA. Ascorbic acid deficiency had a significant effect on the albumin mRNA level but post hoc testing did not identify significantly different means.

DISCUSSION

In this study, the hepatic concentration of ascorbic acid was significantly lower in ODS rats fed the ascorbic acid–free diet than in the control rats fed the ascorbic acid–containing diet from d 7 to 14. No differences in body weight or relative liver weight were observed.

Haptoglobin is one of the positive acute phase proteins, and Apo A-I and albumin are negative acute phase proteins. We previously reported that ascorbic acid deficiency lowered the serum concentration of Apo A-I through lowering its mRNA level in liver (Ikeda et al. 1996). We found in this study that ascorbic acid deficiency elevated the serum haptoglobin concentration and lowered those of Apo A-I and albumin. The changes in serum concentrations of these proteins coincided with changes in their hepatic mRNA levels. AGP is also one of the major positive acute phase proteins synthesized in liver and its gene expression is controlled in the same way as the haptoglobin gene. Ascorbic acid deficiency elevated its mRNA level in liver. It has been reported that the injection of the inflammatory agents, LPS or turpentine, to mice or rats elevated the haptoglobin and AGP mRNA levels and reduced the Apo A-I and albumin mRNA levels in liver (Lowell et al. 1986, Schreiber et al.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Deficient</th>
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<tr>
<td><strong>Initial body weight, g</strong></td>
<td>142 ± 6</td>
<td>142 ± 5</td>
</tr>
<tr>
<td><strong>Final body weight, g/kg body wt</strong></td>
<td>189 ± 6</td>
<td>189 ± 5</td>
</tr>
<tr>
<td><strong>Relative liver weight, g/kg body wt</strong></td>
<td>55.9 ± 1.0</td>
<td>53.5 ± 1.8</td>
</tr>
<tr>
<td><strong>Hepatic ascorbic acid, nmol/g</strong></td>
<td>909 ± 17</td>
<td>176 ± 17*</td>
</tr>
<tr>
<td><strong>Haptoglobin</strong></td>
<td>1.5 52.0</td>
<td>3.8 43.5</td>
</tr>
<tr>
<td><strong>Apo A-I</strong></td>
<td>1.0 41.2</td>
<td>0.8 32.0</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>2.0 47.8</td>
<td>1.0 41.2</td>
</tr>
<tr>
<td><strong>AGP</strong></td>
<td>1.0 41.2</td>
<td>0.8 32.0</td>
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</table>

1 Values are means ± SEM, n = 4. From d 12 to 14, the control group was pair-fed the amount consumed by the ascorbic acid–deficient group. Means that are significantly different from controls are denoted: *P < 0.001, t test; **P < 0.01, Welch’s test.

**FIGURE 2** Effects of ascorbic acid deficiency on hepatic mRNA levels of haptoglobin, α1-acid glycoprotein (AGP), apolipoprotein A-I (Apo A-I) and albumin in control and ascorbic acid–deficient rats on d 7, 10, 12 and 14. A) Northern blot analyses of total RNA from liver of rats in the control (C) and the ascorbic acid–deficient (D) groups. B) The hepatic mRNA levels of haptoglobin, AGP, Apo A-I, albumin or apolipoprotein E (Apo E).

**FIGURE 1** Effects of ascorbic acid deficiency on serum concentrations of haptoglobin, apolipoprotein A-I (Apo A-I) and albumin in control and ascorbic acid–deficient rats on d 7, 10, 12 and 14. A) Immunoblot analyses of sera from rats in the control (C) and ascorbic acid–deficient (D) groups were performed as described in Materials and Methods. B) The serum concentrations of haptoglobin, Apo A-I and albumin in the control and the ascorbic acid–deficient groups. Values are means ± SEM, n = 4. Values are a percentage of the mean of the control group on d 14. *Significantly different (P < 0.05) from the control group by t test.
Hepatic ascorbic acid deficiency elevated the serum IL-6 concentration in control and ascorbic acid–deficient rats. (A) Serum IL-6 concentration in the control and the ascorbic acid–deficient groups. The serum level of IL-6 was quantified by measuring its activity to proliferate B9. (B) Serum corticosterone concentration in the control and the ascorbic acid–deficient groups. Values are means ± SEM, n = 4. Means that are significantly different from controls are denoted: *P < 0.05, t test; **P < 0.001, t test.

In rats, LPS treatment elevated the serum concentrations of corticosterone and IL-6 (Schobitz et al. 1993), decreased the hepatic content of cytochrome P-450 (Khatsenko et al. 1985, Rikans et al. 1985, Odumosa 1982) and decreases the hepatic content of cytochrome P-450 (Horio et al. 1993), and induced the expression of heme oxygenase-1 gene in liver of ODS rats and guinea pigs, ascorbic acid deficiency also elevates the serum concentration of corticosterone (Horio et al. 1993), and decreases the hepatic content of cytochrome P-450 (Horio et al. 1985, Odumosa 1982) and decreases the hepatic content of cytochrome P-450 (Horio et al. 1985, Rikans et al. 1978). We also observed that ascorbic acid deficiency induced the expression of heme oxygenase-1 gene in liver of ODS rats (unpublished data). In this study, we demonstrated that ascorbic acid deficiency elevated the serum IL-6 concentration in ODS rats. However, the mechanisms responsi-

1986, Sharma et al. 1992). We also observed that the injection of LPS elevated the hepatic mRNA levels of haptoglobin, α1-acid glycoprotein (AGP), and lowered the hepatic mRNA levels of Apo A-I and albumin in ODS rats fed an ascorbic acid–containing diet. Taken together with these observations, ascorbic acid deficiency seems to cause changes in these hepatic mRNA levels that are similar to those caused by acute inflammation.

In rats, LPS treatment elevated the serum concentrations of corticosterone and IL-6 (Schobitz et al. 1993), decreased the hepatic content of cytochrome P-450 (Khatsenko et al. 1985), and induced the expression of heme oxygenase-1 gene in liver (Yamaguchi et al. 1995). On the other hand, in ODS rats and guinea pigs, ascorbic acid deficiency also elevates the serum concentration of corticosterone (Horio et al. 1985, Odumosa 1982) and decreases the hepatic content of cytochrome P-450 (Horio et al. 1985, Rikans et al. 1978). We also observed that ascorbic acid deficiency induced the expression of heme oxygenase-1 gene in liver of ODS rats (unpublished data). In this study, we demonstrated that ascorbic acid deficiency elevated the serum IL-6 concentration in ODS rats. However, the mechanisms responsi-

![Graph A](https://example.com/graphA.png)

**FIGURE 3** Effect of ascorbic acid deficiency on serum concentrations of interleukin-6 (IL-6) and corticosterone in control and ascorbic acid–deficient rats. (A) Serum IL-6 concentration in the control and the ascorbic acid–deficient groups. The serum level of IL-6 was quantified by measuring its activity to proliferate B9. (B) Serum corticosterone concentration in the control and the ascorbic acid–deficient groups. Values are means ± SEM, n = 4. Means that are significantly different from controls are denoted: *P < 0.05, t test; **P < 0.001, t test.

![Graph B](https://example.com/graphB.png)

**FIGURE 4** Effects of adrenalectomy on hepatic mRNA levels of haptoglobin, α1-acid glycoprotein (AGP), apolipoprotein A-I (Apo A-I) and albumin in control and ascorbic acid–deficient rats. A) Northern-blot analyses of total RNA from liver of rats in the control (C) and the ascorbic acid–deficient (D) groups in both sham-operated and adrenalectomized rats. Total RNA (20 μg) isolated from liver was separated on a 10% agarose gel containing 66 g/L formaldehyde. RNA in the gel was transferred to a Hybond N+ membrane and hybridized with 32P-labeled cDNA of haptoglobin, AGP, Apo A-I, albumin or apolipoprotein E (Apo E). B) The hepatic mRNA levels of haptoglobin, AGP, Apo A-I and albumin in the control and the ascorbic acid–deficient groups in both sham-operated (Sham) and adrenalectomized (Adex) rats. These four mRNA levels were normalized by the Apo E mRNA level. Bars indicate means, and the vertical line on the top of the bar indicates ± SEM, n = 3. Values are presented as a percentage of the mean of each control group in the sham-operated rats. A significant effect by two-way ANOVA was as follows: haptoglobin mRNA, ascorbic acid effect (P < 0.01); AGP mRNA, ascorbic acid effect (P < 0.01) and adrenalectomy effect (P < 0.01); Apo A-I mRNA, ascorbic acid effect (P < 0.05) and adrenalectomy effect (P < 0.01); Albumin mRNA, ascorbic acid effect (P < 0.01). Means that are significantly different from controls are denoted: *P < 0.05, t test; **P < 0.01, t test; ***P < 0.001, t test. Means that are significantly different from sham-operated rats are denoted: $P < 0.05, t test; $P < 0.01, t test; $$P < 0.001, t test.

**TABLE 3**

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<th>Sham-operated</th>
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<td>Control</td>
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<td>Initial body weight, g</td>
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<td>Relative liver weight, g/kg body wt</td>
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<td>Hepatic ascorbic acid, nmol/g</td>
<td>801 ± 45</td>
<td>70 ± 51</td>
<td>813 ± 39</td>
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<tr>
<td>Serum corticosterone, nmol/L</td>
<td>2.5 ± 1.0</td>
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<td>1.2 ± 0.4</td>
</tr>
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</table>

1Values are means ± SEM, n = 3. From d 12 to 15, the control group was pair-fed the amount consumed by each ascorbic acid–deficient group. Data were analyzed by a two-way ANOVA. The significance of F-values for ascorbic acid effect and adrenalectomy effect as well as interactive effect of the two components (ascorbic acid × adrenalectomy) is shown. When the F-value was significant (P < 0.05), the mean value of each group was compared using Student's t test or Welch's test. 2Significantly different (P < 0.05) from the respective sham-operated rates by Student's t test. 3Significantly different (P < 0.01) from the respective control group by Welch's test. NS, not significant, P ≥ 0.05.
able for these effects of ascorbic acid deficiency are not clear. The pathogenesis of these abnormalities due to ascorbic acid deficiency might be clarified by elucidating how ascorbic acid deficiency causes the acute phase response.

Although the hepatic concentration of ascorbic acid in the ascorbic acid−deficient group was extremely low even on d 7, the change in the hepatic mRNA levels of haptoglobin, AGP, Apo A-I and albumin of the deficient group were not observed before d 12. This result suggests that ascorbic acid itself does not directly regulate these mRNA levels. The expression of the haptoglobin gene is stimulated by glucocorticoid and inflammatory cytokines such as IL-6 and IL-1, and the haptoglobin gene has glucocorticoid responsive sequences (not the consensus sequence) and two types of IL-6 responsive elements in its upstream region (Bau- mann et al. 1990, Marinkovic and Baumann 1990). The AGP gene has a glucocorticoid responsive element, two types of IL-6 responsive elements and an IL-1 responsive element in its upstream region (Won and Baumann 1990). In this study, the serum corticosterone concentration in the ascorbic acid−deficient group on d 14 was 450% of the control group. To determine the mechanism of action of ascorbic acid deficiency in the stimulation of the expression of haptoglobin and AGP genes, we examined the effects of adrenalectomy on the ascorbic acid deficiency−induced changes in the hepatic mRNA levels of acute phase proteins. The results with adrenalectomized rats showed that the ascorbic acid deficiency−induced changes in the hepatic mRNA levels of haptoglobin, AGP, Apo A-I and albumin were also observed in the adrenalectomized rats. These results suggest that the elevation of the serum glucocorticoid concentration by ascorbic acid deficiency is not responsible for the change in the hepatic mRNA levels of positive and negative acute phase proteins during ascorbic acid deficiency.

The serum concentration of IL-6 in the ascorbic acid−deficient group tended to be greater than that in the controls by d 12 and had reached 400% of that in the control group on d 14. Because IL-6 induces the hepatic synthesis of many acute phase proteins, including haptoglobin and AGP, the elevation of serum IL-6 concentration by ascorbic acid deficiency might contribute to the change in the hepatic mRNA levels of haptoglobin and AGP. However, it remains unclear whether the high serum IL-6 concentration is due to an increase in its synthesis or to the stimulation of its secretion. What kind of cells produce or secrete IL-6 in response to ascorbic acid deficiency should be investigated. This study clearly demonstrates for the first time that ascorbic acid deficiency changes the gene expression of acute phase proteins in liver and increases the serum concentrations of corticosterone and IL-6, as does acute inflammation. These results suggest that ascorbic acid deficiency causes symptoms similar to those caused by acute inflammation.

ACKNOWLEDGMENT

The authors are very grateful to Y. Kumazawa (School of Science, Kitasato University, Kanagawa, Japan) for supplying the IL-6 dependent cell line, B9.

LITERATURE CITED


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