Absorption and Excretion of Ascorbic Acid Alone and in Acerola (Malpighia emarginata) Juice: Comparison in Healthy Japanese Subjects

Eriko UCHIDA, Yoshitaka KONDO, Akiko AMANO, Shingo AIZAWA, Takayuki HANAMURA, Hitoshi AOKI, Kenichi NAGAMINE, Takeshi KOIZUMI, Naoki MARUYAMA, and Akihito ISHIGAMI

Research and Development Division, Nichirei Foods Inc.; Chiba 261–8545, Japan; Molecular Regulation of Aging, Tokyo Metropolitan Institute of Gerontology; Tokyo 173–0015, Japan; Cellular Genetics, Graduate School of Science and Engineering, Tokyo Metropolitan University; Tokyo 192–0397, Japan; and Research and Development Division, Research and Development Center, Nichirei Bioscience Inc.; Tokyo 189–0003, Japan.

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It has been suggested that some food components, such as bioflavonoids, affect the bioavailability of ascorbic acid in humans. Since little is known in Japan about the effective intake of this dietary requirement, we tested young Japanese males after the ingestion of commercial ascorbic acid or acerola (Malpighia emarginata DC.) juice to compare the quantities absorbed and excreted. Healthy Japanese subjects received a single oral dose of ascorbic acid solution (50, 100, 200 or 500 mg) and received distilled water as a reference at intervals of 14 d or longer. All subjects were collected blood and urine until 6 h after ingestion and evaluated for time-dependent changes in plasma and urinary ascorbic acid levels. Predictably, the area under the curve (AUC) values in plasma and urine after ingestion increased dose-dependently. Next, each subject received diluted acerola juice containing 50 mg ascorbic acid. Likewise, their plasma and urinary ascorbic acid concentrations were measured. In plasma, the AUC value of ascorbic acid after ingestion of acerola juice tended to be higher than that from ascorbic acid alone. In contrast, the urinary excretion of ascorbic acid at 1, 2 and 5 h after ingestion of acerola juice were significantly less than that of ascorbic acid. These results indicate that some component of acerola juice favorably affected the absorption and excretion of ascorbic acid.

Key words  ascorbic acid; acerola; vitamin C

Ascorbic acid (vitamin C) is vitally important for human health, as widely reported and reviewed by Weber et al. Among its nutritive attributes, ascorbic acid has numerous metabolic functions that are largely dependent on its potent reducing properties. Additionally, ascorbic acid acts as a co-factor in reactions catalyzed by several metal-dependent oxygenases, e.g., Cu⁺-dependent mono-oxygenases including peptidylglycine α-amidating mono-oxygenase involved in peptide hormone synthesis, dopamine β-hydroxylase involved in norepinephrine synthesis, and Fe²⁺/α-ketoglutarate-dependent dioxygenases including prolyl and lysyl hydroxylases involved in collagen synthesis. Others include 6-N-trimethyllysine dioxygenase and γ-butyrobetaine dioxygenase involved in carnitine synthesis, and asparaginyl hydroxylase, which modifies hypoxia-inducible factor. Ascorbic acid depletion induces scurvy with such symptoms as dry skin, fatigue and bleeding. Moreover, ascorbic acid has non-enzymatic reductive activity in chemical reactions. That is, ascorbic acid’s strong anti-oxidant function is evident as its ability to scavenge superoxide radicals in intracellular and extracellular reactions. Many animals can synthesize ascorbic acid in vivo; however, others such as humans and guinea pigs have lost the ability to make ascorbic acid because of mutations in the l-gulono-γ-lactone oxidase gene, which is essential for ascorbic acid synthesis in vivo. Therefore, animals without the enzyme activity of l-gulono-γ-lactone oxidase must obtain ascorbic acid from dietary sources.

Acerola (Malpighia emarginata DC.) is a fruit found throughout Central America and within the northern part of South America. This fruit is well known to be one of the best natural sources of ascorbic acid and has become extremely popular among health-conscious people. Besides ascorbic acid, acerola contains functional ingredients such as carotenoids, γ-amino butyric acid (GABA) and polyphenols. As for polyphenols, acerola was found to contain cyanidin-3-α-O-rhamnoside and pelargonidin-3-α-O-rhamnoside as anthocyanins, quercitin (quercetin-3-α-O-rhamnoside), hyperoside (quercetin-3-β-O-galactoside) and kaempferol glycosides as flavonols, and astilbin and proanthocyanidin.

In this study, we measured the time-dependent changes of the plasma and urinary ascorbic acid levels after a single oral ingestion by healthy Japanese males, because little information about ascorbic acid bioavailability is available in this population. Moreover, we compared the pharmacokinetics in healthy subjects given ascorbic acid alone to that from acerola juice, one of its natural sources.

MATERIALS AND METHODS

Acerola Juice  Acerola juice was prepared from the frozen mature fruit procured from Nichirei do Brazil (Recife, Brazil). The frozen acerola fruit was defrosted and squeezed by using a juice extractor (GP-E1503, GREEN POWER Co., Ltd., South Korea) and then filtered (No. 5C, Toyo Advantec Co., Tokyo, Japan).

Study Design  This study protocol was approved by the Human Subjects Committee of the Tokyo Metropolitan Institute of Gerontology. All subjects gave their written informed consent.

Healthy Japanese males volunteered as subjects and ranged in age from 22 to 26 years with an average of 24 ± 1 years. Only subjects who were non-smokers and did not take high-dose vitamin C supplements were included. All subjects

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* To whom correspondence should be addressed.  e-mail: ishigami@tmig.or.jp
received the same limited diet for three days before each test to control their blood level of ascorbic acid.

To measure the absorption and excretion of ascorbic acid alone, all subjects fasted overnight before ingesting a single dose of either 50, 100, 200 or 500 mg and distilled water as a reference. For this crossover experimental design, the ascorbic acid solution of all doses contained 100 ml distilled water. Each experiment was carried out at intervals of 14 d or longer. The blood was collected from each subject in tubes containing ethylenediaminetetraacetic acid (EDTA) at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 h after the oral dose. The blood was immediately centrifuged at 1700 \( g \) for 15 min at 4 \( ^\circ \)C and the supernatant obtained was used as plasma. Urine samples were collected and their volume recorded every 1 or 2 h after the oral dose. The overall collection time was 6 h.

The same and more subjects volunteered for the acerola juice ingestion study. After an overnight fast, each subject ingested 100 ml acerola juice diluted with water containing 50 mg ascorbic acid. Plasma and urine collecting schedules were the same as above.

**Measurement of Ascorbic Acid** Total ascorbic acid was measured by using high-performance liquid chromatography (HPLC)-electrochemical detection as described previously. The plasma was mixed with 4.5 volumes of 3% metaphosphoric acid (MPA) (Wako Pure Chemical, Osaka, Japan) and centrifuged at 21000 \( g \) for 10 min at 4 \( ^\circ \)C and the supernatants obtained were stored as plasma. Urine samples were collected and their volume recorded every 1 or 2 h after the oral dose. The overall collection time was 6 h.

Creatinine levels in urine were measured with a Creatinine Test Wako kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer’s instructions, and ascorbic acid levels in urine were normalized by creatinine value.

**Statistical Analysis** The experimental results are displayed as means \( \pm \) S.E.M. The significant differences were calculated by Student’s \( t \) test.

**RESULTS**

**Subjects’ Characteristics** The initial anthropometric and hematological characteristics of subjects in this study appear in Table 1. All hematological components were within normal levels.

**Absorption of Ascorbic Acid in Plasma** Oral ascorbic acid ingestion was measured in the plasma of all fasting subjects, as shown in Fig. 1A. The means of fasting plasma ascorbic acid concentrations were 32.9 \( \pm \) 1.1 \( \mu \)M. We measured the ascorbic acid amounts of the three day’s prescribed diets. These were 34.3, 14.3 and 11.3 mg, respectively. Diets control for 3 and overnight fasting provided enough conditioning to ascertain accurate ascorbic acid levels in plasma.

Thereafter, upon oral ingestion of 50, 100, 200 and 500 mg, maximal concentrations of ascorbic acid \( (C_{\text{max}}) \) increased dose-dependently, as expected, to 41.1 \( \pm \) 4.7, 53.9 \( \pm \) 4.8, 55.2 \( \pm \) 5.8 and 63.1 \( \pm \) 4.0 \( \mu \)M, respectively. By contrast, in case of not receiving an ascorbic acid, the plasma ascorbic acid concentration had no notable change (Fig. 1). The time intervals to reach maximal ascorbic acid concentrations \( (T_{\text{max}}) \) were also longer dose-dependently as the interval lengthened from 1.5 to 3 h. Values for area under the curve \( (\text{AUC}) \) are shown in Fig. 1B. The slope rose most sharply at between 50 and

![Fig. 1](image-url)  
(A) Plasma Time–Concentration Curves for Fasting Subjects Ingesting 0 to 500 mg of Ascorbic Acid

Symbols represent ingestion of ascorbic acid amount as follows: 0 mg, \( \bullet \); 50 mg, \( \blacksquare \); 100 mg, \( \square \); 200 mg, \( \triangle \); 500 mg, \( \triangle \).

(B) The areas under the plasma ascorbic acid time–concentration curves after oral ingestion. The values are means \( \pm \) S.E.M., \( n=5 \).
100 mg of ascorbic acid and then continued to increase but at a lesser rate.

**Excretion of Ascorbic Acid from Urine** The time to excretion of urinary ascorbic acid after oral ingestion is graphed in Fig. 2A. The y-axis indicates fractional excretion with creatinine collection. The peak times of ascorbic acid excretion were 3 to 4 h after oral ingestion of 50, 100, 200 and 500 mg. The total amount excreted for 6 h after oral ingestion increased dose-dependently (Fig. 2B).

**Absorption and Excretion of Ascorbic Acid in Acerola Juice** Considering the above results, we determined the most effective ascorbic acid dose for this comparative study was 50 mg. Accordingly, the subjects drank acerola juice containing 50 mg of ascorbic acid with the results shown in Fig. 3. The y-axis of Fig. 3A indicates \( \Delta C \) calculated by the plasma ascorbic acid concentration, as follows: \( \Delta C = \text{concentration at each time} - \text{initial concentration} \). The \( \Delta C_{\text{max}} \) of acerola juice exceeded that of ascorbic acid alone. In contrast, the urinary excretion of ascorbic acid at 1, 2 and 5 h after ingestion of acerola juice were significantly less than that of ascorbic acid (Fig. 3B). The \( \Delta AUC \) value of ascorbic acid in plasma after acerola juice ingestion was a little greater than that of ascorbic acid alone (Table 2). Moreover, the net 6-h urinary excretion of ascorbic acid after acerola juice ingestion was less than that of ascorbic acid alone.

**DISCUSSION**

In the present study, we measured the time-dependent changes of the plasma and urinary ascorbic acid levels after a single oral ingestion by healthy Japanese males, because little information about ascorbic acid bioavailability is available in this population. Levine *et al.* measured the AUC value in plasma after oral or intravenous doses of 200, 500 and 1250 mg ascorbic acid. Bioavailability was complete for the 200 mg dose, but much lower for the higher doses. Our data were almost similar to theirs suggesting that there are no differences between the population groups in this aspect of ascorbic acid bioavailability.

### Table 2. Average AUC of Plasma and Urinary of Ascorbic Acid

<table>
<thead>
<tr>
<th>Source</th>
<th>( \Delta AUC ) of plasma ascorbic acid (h·μM)</th>
<th>Net 6-h urinary excretion of ascorbic acid (μg/mg creatinin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>40.8±8.9</td>
<td>105.3±16.4</td>
</tr>
<tr>
<td>Acerola juice</td>
<td>43.4±3.6</td>
<td>54.0±16.4</td>
</tr>
</tbody>
</table>

Values are means±S.E.M.
To investigate the possibility that some components of food affect the absorption and excretion of ascorbic acid differently from that in ascorbic acid alone, we compared the bioavailability of ascorbic acid alone and that naturally present in acerola juice. As seen in Fig. 3 and Table 2, the acerola juice tended to promote the absorption of ascorbic acid in plasma and suppressed its excretion in urine better than the ascorbic acid alone.

Similarly, Vinson and Bose\textsuperscript{19)\textsuperscript{20)} reported that the ascorbic acid in citrus extract was more bioavailable than ascorbic acid alone in human subjects. They studied under two conditions. Their subjects were either saturated with or deprived of ascorbic acid in the body, then tested for the absorption and excretion of ascorbic acid in citrus extract versus ascorbic acid alone. In the subjects who were deprived with ascorbic acid in the body, the AUC value for ascorbic acid containing 500 mg citrus extract was greater than for ascorbic acid alone. In addition, significantly less ascorbic acid containing citrus extract was excreted. In contrast to the subjects who were saturated with ascorbic acid had a completely opposite outcome. These results suggest that the effect of some food components depends on ascorbic acid fractional saturation of subjects.

Bates et al.\textsuperscript{19)\textsuperscript{20)} also investigated this issue by using stable isotope-labeled ascorbic acid. There, grape juice containing anthocyanins, flavanols and flavonols tended to reduce absorption compared with ascorbic acid alone. We presume that their negative results were caused by using subjects who were saturated with ascorbic acid in the body. They concluded that the flavonoid in grape juice inhibited the ascorbic acid transporter, sodium-dependent vitamin C transporter 1 (SVCT1) in their report. Song et al.\textsuperscript{20)} reported that the most potent inhibitor class of flavonoids, for example, myricetin, quercetin and anthocyanin, also had inhibitory capacity for ascorbic acid transport in a cell culture model. However, they noted that inhibition was eliminated if glycosylated residues were present at the C3 position of benzopyran. Acerola juice, which was used in the present study, contains cyanidin-3-α-O-rhamnoside and pelargonidin-3-α-O-rhamnoside. The C3 positions of these two polyphenols are glycosylated by rhamnose. Apparently, then, these two polyphenols in acerola juice are not inhibitors of SVCT1. In addition, the content of these polyphenols in diluted acerola juice was 6.5 μM and 1.0 μM, respectively. According to the inhibition study, the concentration giving 50% inhibition (IC\textsubscript{50}) of cyanindin was 84 μM. Therefore, even if the anthocyanin in acerola juice is hydrolyzed in the ileum and intestines, it is considered that its presence does not affect SVCT1, because the content is less than the IC\textsubscript{50} value of cyanindin.

In conclusion, the data in this study indicate that some component(s) of acerola juice affects the absorption of ascorbic acid into plasma and minimizes its excretion via urine. Clarification of not only those effects but also their mechanisms will assure the Japanese population’s most effective intake of vitamin C.

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