Bioavailability of Glucosyl Hesperidin in Rats

Mika YAMADA,† Fujimi TANABE, Norie ARAI, Hitoshi MITSUZUMI, Yoshikatsu MIWA, Michio KUBOTA, Hiroto CHAEN, and Masayoshi KIBATA

Hayashibara Biochemical Laboratories, Inc., 1-2-3 Shimoishii, Okayama 700-0907, Japan

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Glucosyl hesperidin (G-hesperidin) is a water-soluble derivative of hesperidin. We compared the absorption and metabolism of G-hesperidin with those of hesperidin in rats. After oral administration of G-hesperidin or hesperidin to rats, hesperetin was detected in sera hydrolyzed with $\beta$-glucuronidase, but it was not detectable in unhydrolyzed sera. Serum hesperetin was found more rapidly in rats administered G-hesperidin than in those administered hesperidin. The area under the concentration-time curve for hesperetin in the sera of rats administered G-hesperidin was approximately 3.7-fold greater than that of rats administered hesperidin. In the urine of both administration groups, hesperetin and its glucuronide were found. Urinary excretion of metabolites was higher in rats administered G-hesperidin than in those administered hesperidin. These results indicate that G-hesperidin presents the same metabolic profile as hesperidin. Moreover, it was concluded that G-hesperidin is absorbed more rapidly and efficiently than hesperidin, because of its high water solubility.

Key words: hesperidin; glucosyl hesperidin; hesperetin conjugates; intestinal bacteria; bioavailability

Chinpi, which is made of Satsuma mandarin peel (Citrus unshiu Marc.), has traditionally been prescribed as a natural medicine for inflammation, allergy, and hepatopathy. It is well-known that hesperidin is one of the primary constituents of Chinpi. Hesperidin is a flavanone glycoside consisting of hesperetin aglycon and disaccharide rutinose. It has multiple biological activities such as reduction of capillary fragility, associated with scurvy, and antilipemic activities.1–4) But the difficulty with hesperidin is low water solubility, less than 0.01%.

Hijiya et al. have synthesized a water-soluble derivative of hesperidin, glucosyl hesperidin (G-hesperidin), by regioselective transglycosylation with cyclodextrin glucanotransferase from Bacillus stearothermophilus.5) G-Hesperidin becomes markedly more soluble. Its water solubility is about 10,000 times greater than that of hesperidin. G-Hesperidin has been reported to possess almost the same antioxidant properties as hesperidin in vitro.6) Moreover, it has been confirmed that the biological activities of G-hesperidin are the same or more than those of hesperidin.7–9) Nishizaki et al. reported that G-hesperidin and hesperidin dose-dependently inhibited passive cutaneous anaphylaxis in rats, and that the effect of G-hesperidin was more continuous than that of hesperidin.10) Furthermore, Chiba et al. showed that G-hesperidin and hesperidin significantly prevented bone loss in the femur in ovariectomized mice.11) G-Hesperidin is susceptible to enzymatic hydrolysis by $\alpha$-glucosidases and this results in release of hesperidin (Fig. 1).10) Hence, it is assumed that this compound could behave as hesperidin in vivo. Several studies have shown that orally administered hesperidin is hydrolyzed by $\beta$-glucosidases to hesperetin aglycon before absorption (Fig. 1).11,12) Then absorbed hesperetin is immediately metabolized to glucuronide and sulfate conjugates in the intestinal epithelium and liver.11,12) Matsumoto et al. have determined that the conjugated metabolites in rat plasma after oral administration of hesperidin are comprised of hesperetin-7-$O$-$\beta$-D-glucuronide and hesperetin-3'$O$-$\beta$-D-glucuronide.12) In addition, homoeriodictyol conjugates as well as hesperetin conjugates have been observed in rat plasma.12)

Flavonoids have been reported to function as their metabolites, including conjugated aglycon, in the body. For instance, it has been shown that quercetin conjugates are detected in rat plasma after oral administration of quercetin and that they exhibit antioxidative activities.13,14) Recently we found that G-hesperidin is effective on hyperlipidemic subjects, suggesting that this derivative functions as hesperetin conjugates in the body.15) Many flavonoids are hardly soluble in water. Therefore in the oral administration test, these flavonoids are usually dissolved in vehicles such as carboxymethyl cellulose sodium and propylene glycol to elevate their

Abbreviations: G-hesperidin, glucosyl hesperidin; AUC, area under the concentration-time curve; SGLT1, sodium-dependent glucose transporter 1

† To whom correspondence should be addressed. Fax: +81-86-221-6405; E-mail: kaihatsu@hayashibara.co.jp
Materials and Methods

Chemicals. Hesperetin, β-glucuronidase type VII-A (EC 3.2.1.31, from Escherichia coli), castanospermine, p-nitrophenyl-α-glucoside, and p-nitrophenyl-β-glucoside were purchased from Sigma (St. Louis, MO). Hesperidin was purchased from Wako Pure Chemical (Osaka, Japan). G-Hesperidin was obtained from Hayashibara Biochemical Laboratories (Okayama, Japan). Other chemicals were of analytical or HPLC grade.

Animals and diets. Male Sprague-Dawley rats (6 weeks old, Charles River Japan, Atsugi, Japan) were housed in an air-conditioned room (23 ± 2 °C) under a 12 h light-dark cycle, with free access to tap water and MF diet (Oriental Yeast, Tokyo). The present study was approved by the Animal Use Committee of Hayashibara Biochemical Laboratories, Inc., and the animals were handled according to the Guidelines for the Care and Use of Laboratory Animals. Rats were fasted overnight, and 1 mmol/kg of G-hesperidin or hesperidin in 2 ml of distilled water was administered orally by gastric intubation. We have found that G-hesperidin is effective on hyperlipidemic mice at this dose level per day.6)

Before and at 0.25, 0.5, 1, 3, 6, 9, 12, and 27 h after administration, blood was withdrawn from the tail vein into a MICROTAiNER® (Becton Dickinson, Franklin Lakes, NJ). Serum was prepared by centrifugation at 10,000 × g for 2 min. Urine was collected in metabolic cages the day before, and for 27 h after administration of G-hesperidin or hesperidin, in five different fractions: 0–2, 2–4, 4–8, 8–11, and 11–27 h. These serum and urine samples were stored at −20 °C. The rats were not allowed to feed until the last blood sampling.

HPLC analysis. Individual serum and urine samples were divided into two aliquots. One aliquot of serum and urine samples was used for the quantification of metabolites released by hydrolysis with β-glucuronidase, a deconjugation enzyme. For hydrolysis by β-glucuronidase, serum and urine samples (50 μl) were incubated with 500 units/ml β-glucuronidase, which was prepared in 0.1 mM sodium acetate buffer (pH 5.0) at 37 °C for 2 h in the presence of 0.5 mM castanospermine, an α-glucosidase inhibitor. Then these mixtures were acidified with 0.1 ml of 0.01 M oxalic acid. The other portions of serum and urine samples remained without hydrolysis by β-glucuronidase and were used for direct quantification of non-conjugated metabolites. These samples were also acidified with 0.1 ml of 0.01 M oxalic acid. After acidification, both hydrolyzed and unhydrolyzed samples were applied to a Sep-Pak C18 cartridge (Waters, Milford, MA). The cartridge was washed with 0.01 M oxalic acid, distilled water, and 25% methanol, and then 1 ml of methanol eluate was obtained. The eluate was evaporated to dryness, and the residue was dissolved in 100 μl of methanol/water/acetic acid (60:39:1, v/v/v). After filtration (0.45 μm filter unit), the filtrate (10 μl) was injected onto an Inertsil ODS-3 column (250 × 4.6 mm i.d., GL Sciences, Tokyo) and analyzed by HPLC. The mobile phase contained the following: solvent A, methanol/acetic acid (100:1, v/v); solvent B, water/acetic acid (100:1, v/v). The gradient program was as follows: the initial composition was A/B = 35/65 for 0–5 min, followed
by a linear gradient of 35/65–80/20 for 5–55 min. The column temperature was kept at 40 °C, and the flow rate was 0.5 ml/min. The elution was monitored with a photodiode array detector (Waters) at 285 nm. The compounds existing in the samples were identified by comparing their HPLC retention times (t_R) with those of standard G-hesperidin, hesperidin, and hesperetin, and quantified by measuring the peak areas based on calibration plots of the peak area of the standards. Recoveries of these compounds from serum and urine by this method were >95%. The detection limits for these compounds were 0.05 μM.

Preparation of small intestine and cecal content homogenates. Rats were sacrificed by diethyl ether anesthesia. The small intestine was excised, rinsed with cold Dulbecco’s phosphate-buffered saline (Ca^2+ and Mg^2+ free), and homogenized in 4 volumes of 100 mM potassium phosphate buffer (pH 7.0) with a glass-Teflon homogenizer. The homogenate was centrifuged at 3,000 × g for 15 min, and the supernatant was used as an enzyme source. The cecum was removed, and its contents were drained by finger pressure into a tube. The cecal contents thus obtained were suspended in 4 volumes of 100 mM potassium phosphate buffer (pH 7.0) and sonicated for 5 min. After centrifugation at 400 × g for 5 min, the supernatant was used as an enzyme source.

Assay for glucosidase activities. The α-glucosidase activity of small intestine and cecal content homogenates was estimated by measuring the rate of formation of p-nitrophenol from p-nitrophenyl-α-glucoside. 17) Reaction mixtures (2 ml) consisted of 50 mM potassium phosphate buffer (pH 7.0), 5 mM p-nitrophenyl-α-glucoside, and a sample of enzyme solution, and were incubated at 37 °C for 15 min. The p-nitrophenol liberated was determined by measuring the absorbance at 400 nm in the presence of 100 mM Na_2CO_3. One unit of α-glucosidase activity was defined as the amount of enzyme that generates 1 μmol of p-nitrophenol per min under the conditions mentioned above.

The β-glucosidase activity of small intestine and cecal content homogenates was determined using p-nitrophenyl-β-glucoside as a substrate. 18) Reaction mixtures (2 ml) consisted of 50 mM sodium acetate buffer (pH 5.0), 5 mM p-nitrophenyl-β-glucoside, and a sample of enzyme solution, and were incubated at 37 °C for 15 min. The p-nitrophenol liberated was determined as described above. One unit of β-glucosidase activity was defined as the amount of enzyme that produces 1 μmol of p-nitrophenol per min under the assay conditions.

Hydrolysis of G-hesperidin by small intestine and cecal content homogenates. The hydrolytic ability of small intestine and cecal content homogenates toward G-hesperidin was examined by measuring the amounts of hesperidin and hesperetin released. Reaction mixtures were composed of 1 ml of 2 mg/ml G-hesperidin and 1 ml of enzyme solution. After incubation for the indicated times at 37 °C, the hesperidin and hesperetin released from G-hesperidin were determined by HPLC analysis, as described above.

Data analysis. Values are given as the means ± SD. The significant difference between the means of two groups was statistically analyzed by Mann-Whitney’s U test. The significance level was set at p < 0.05 for all tests. Statistical analysis was performed with Stat View version 5.0 for Windows (SAS Institute, Cary, NC).

Results

Quantification of the metabolites derived from G-hesperidin and hesperidin in rat serum

The metabolites of flavonoids have frequently been reported to be present in blood circulation in the form of glucuronide, sulfate, and methylate conjugates. 12,19) To examine the absorption and metabolism of G-hesperidin and hesperidin in vivo, each of these compounds was orally administered to rats. In the case of hesperidin administration, the conjugates circulating in the blood were reported to be mainly glucuronides, and, although minor in quantity, sulfate/glucuronide conjugates were also detected. 12) Therefore in this study, the sera of rats were subjected to hydrolysis with β-glucuronidase only, and this method did not allow us to distinguish the number and position of conjugated moieties.

Figure 2 shows representative chromatograms of standards and β-glucuronidase hydrolysate of rat serum taken after oral administration of G-hesperidin or hesperidin. In both administration groups, hesperetin (t_R = 48.4 min) was detected in the hydrolysate (Fig. 2B and C). A peak (t_R = 46.7 min) was assumed to be homoeriodictyol based on the study of Matsumoto et al. 12) A peak corresponding to hesperetin was not obtained in the hydrolyte of rat serum before oral administration of G-hesperidin or hesperidin (data not shown).

The serum kinetics of hesperetin–glucuronide is shown in Fig. 3. After dosing with hesperidin, hesperetin–glucuronide was detected at 6 h, and reached a maximum (about 1.3 μM) at 9–12 h. On the other hand, in the administration of G-hesperidin, hesperetin–glucuronide was detected as early as 15 min after administration, and reached a maximum (6.3 μM) at 6 h. The serum concentration of hesperetin conjugate observed after administration of G-hesperidin was higher than that of hesperidin throughout the experimental period, except at 27 h. In particular, the differences in the serum concentrations of the conjugate between the administration of G-hesperidin and hesperidin were significant until 9 h. The area under the concentration-time curve (AUC) for hesperetin–glucuronide in the sera of rats administered G-hesperidin was approximately 3.7-fold higher than that of rats administered hesperidin (Table 1). After hydrolysis with β-glucuronidase, a high
Fig. 2. Representative HPLC Chromatograms of Standard G-Hesperidin, Hesperidin, and Hesperetin (A), and \(\beta\)-Glucuronidase Hydrolysate of Rat Serum Taken at 6 h after Oral Administration of 1 mmol/kg Hesperidin (B) or G-Hesperidin (C).

Fig. 3. Changes in the Serum Concentration of Hesperetin-Glucuronide in Rats Given G-Hesperidin or Hesperidin.
G-Hesperidin (●) or hesperidin (○) was given to rats by gastric intubation (1 mmol/kg). Serum samples were treated with \(\beta\)-glucuronidase. Values are the means ± SD (n = 5). ** Significantly different from the value of hesperidin administration at \(p < 0.01\).
level of hesperidin was detected in the serum of one out of five rats administered G-hesperidin (data not shown). In contrast, no free hesperetin, hesperidin, or G-hesperidin was detected in the unhydrolyzed sera of rats administered G-hesperidin or hesperidin.

**Quantification of the metabolites derived from G-hesperidin and hesperidin in rat urine**

In order to determine further the metabolic fate and bioavailability of G-hesperidin and hesperidin, we examined the excretion of metabolites in rat urine after administration of both compounds. Figure 4 shows representative chromatograms of β-glucuronidase hydrolysate of rat urine taken after oral administration of G-hesperidin or hesperidin. Hesperetin ($t_R = 48.4$ min) and homoeriodictyol ($t_R = 46.7$ min) were detected in the hydrolyzed urine (Fig. 4A and B). The peak corresponding to hesperetin was not detected in the hydrolysate of rat urine before oral administration of G-hesperidin or hesperidin (data not shown).

The changes in cumulative urinary excretion of hesperetin–glucuronide are shown in Fig. 5A. After dosing with hesperidin, hesperetin–glucuronide was detected between 0 and 2 h, and reached a maximum (about 586 μM) between 8 and 11 h. A similar tendency was observed in the administration of G-hesperidin: hesperetin–glucuronide was detected between 0 and 2 h, and reached a maximum (about 829 μM) between 8 and 11 h. The urine concentration of hesperetin conjugate was higher in rats administered G-hesperidin than in those administered hesperidin throughout the experimental period. The differences in urinary excretion of the conjugate between the administration of G-hesperidin and hesperidin were especially significant until 8 h. In addition, hesperetin was detected in the unhydrolyzed urine of rats administered G-hesperidin or hesperidin.

The changes in cumulative urinary excretion of non-conjugated hesperetin are shown in Fig. 5B. After supplementation with hesperidin, free hesperetin was detected between 0 and 2 h, and culminated (at about 115 μM) between 8 and 11 h. Similarly, G-hesperidin administration also led to detection of free hesperetin between 0 and 2 h, and its urine level culminated (at about 661 μM) between 8 and 11 h. The urine concentration of non-conjugated hesperetin was higher in rats administered G-hesperidin than in those administered hesperidin throughout the experimental period ($p < 0.05$ in urine collected between 2 and 4 h, and $p < 0.01$ in urine collected between 8 and 11 h).

Moreover, glucuronized hesperidin and non-conjugated hesperidin were found in the urine until 4 h after administration of G-hesperidin, but they were not found in the case of hesperidin administration (Fig. 5C and D). In the urine of rats administered G-hesperidin, the excretion of hesperidin conjugate and free hesperidin was earlier than that of hesperetin conjugate and free hesperetin respectively. Furthermore, the levels of hesperidin conjugate and free hesperidin were in lower

**Table 1.** Total Area under the Serum Concentration-Time Curve of Hesperetin–Glucuronide in Rats Administered G-Hesperidin or Hesperidin

<table>
<thead>
<tr>
<th>Supplement</th>
<th>AUC $0 \rightarrow 27$ h (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Hesperidin</td>
<td>$67.6 \pm 12.0^{**}$</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>$18.2 \pm 7.3$</td>
</tr>
</tbody>
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Values are the means ± SD ($n = 5$). ** Significantly different from the value of hesperidin administration at $p < 0.01$.

![Fig. 4. Representative HPLC Chromatograms of β-Glucuronidase Hydrolysate of Rat Urine Taken between 4 and 8 h after Oral Administration of 1 mmol/Kg Hesperidin (A) or G-Hesperidin (B).]
concentrations than the respective forms of hesperetin in the urine of rats administered G-hesperidin.

Hydrolysis of G-hesperidin by small intestine and cecal content homogenates

It has been reported that G-hesperidin is hydrolyzed to hesperidin by \( \alpha \)-glucosidases (Fig. 1).\(^{10}\) Furthermore, it has been explained that orally administered hesperidin is hydrolyzed to hesperetin by \( \beta \)-glucosidases from intestinal bacteria and then released hesperetin is absorbed into blood (Fig. 1).\(^{1,11,12,20}\) We observed that G-hesperidin administration as well as hesperidin administration led to detection of hesperetin conjugate in rat serum (Fig. 3). Therefore, G-hesperidin was considered to be finally hydrolyzed to hesperetin via hesperidin in the intestinal tract. In order to clarify this further, small intestine and cecal content homogenates from rats were examined for their hydrolytic ability toward G-hesperidin.

As shown in Table 2, high activity of \( \alpha \)-glucosidase was detected in small intestine homogenate, but \( \beta \)-glucosidase activity was extremely low in this homogenate. On the other hand, cecal content homogenate showed high activities of \( \alpha \)- and \( \beta \)-glucosidases, which probably derived from intestinal bacteria. These homogenates were used as enzyme sources and incubated with G-hesperidin at 37°C. As a result, the small intestine homogenate completely hydrolyzed G-hesperidin to hesperidin at 1 h (Fig. 6). Moreover, the cecal content homogenate generated not only hesperidin but also hesperetin for 4 h (Fig. 6).

Discussion

G-Hesperidin, a water-soluble derivative of hesperidin, has been shown to exhibit the same biological activities as hesperidin \textit{in vivo},\(^{7-10}\) but the behavior of this derivative in the body remains to be fully clarified. In this study, we compared the metabolic fate and the bioavailability of G-hesperidin with those of hesperidin in rats.
After dosing with G-hesperidin or hesperidin, hesperetin–glucuronide was found in the sera of both administration groups (Fig. 3). Moreover, hesperetin–glucuronide (Fig. 5A) and non-conjugated hesperetin (Fig. 5B) were excreted in the urine of both administration groups. These results indicate that G-hesperidin presents the same metabolic profile as hesperidin. It has been reported that orally administered hesperidin is absorbed as hesperetin after hydrolysis by /C12-glucosidases from intestinal microflora, and that absorbed hesperetin is mainly metabolized to glucuronide conjugate in the intestinal tissue and liver.12) It appears likely that G-Hesperidin is converted to hesperidin in the intestinal tract, thereby causing the metabolism mentioned above.

Serum hesperetin–glucuronide was found sooner in rats administered G-hesperidin than in those administered hesperidin (Fig. 3), and furthermore, AUC for this conjugate was more pronounced in the administration of G-hesperidin (Table 1). We also observed that the urinary excretion of metabolites was higher in rats administered G-hesperidin than in those administered hesperidin (Fig. 5). These observations indicate that G-hesperidin is absorbed more efficiently than hesperidin. It has been reported that the extent of flavonoid absorption is affected by its solubility in the digestion process.16,21) According to the report, quercetin, which is hardly soluble in water, is elevated in absorption after administration to rats when it is dissolved in propylene glycol.16) Therefore, it is thought that the elevated absorption efficiency of G-hesperidin is due to its high water solubility.

Additionally, the profile of G-hesperidin hydrolysis in the intestinal tract was investigated using small intestine and cecal content homogenates from rats. It was observed that G-hesperidin was hydrolyzed to hesperidin by small intestine homogenate containing abundant /α-glucosidases (Table 2 and Fig. 6). On the other hand, this derivative was hydrolyzed to hesperidin and hesperetin in an ordered way by cecal content homogenate containing both /α- and /β-glucosidases (Table 2 and Fig. 6). /α-Glucosidases have been demonstrated to exist in the brush border of the small intestine.22) Moreover, it has been shown that cecal contents have an abundance of intestinal bacteria and that this microflora produces /β-glucosidases as well as /α-glucosidases.23,24) Furthermore, Kometani et al. have reported that G-hesperidin is hydrolyzed to hesperidin by /α-glucosidases, but that it can not undergo the action of /β-glucosidases.10) On the basis of these findings, our observations suggest that orally administered G-hesperidin is initially hydrolyzed to hesperidin by small intestinal /α-glucosidases, and that released hesperidin is subsequently hydrolyzed to hesperetin by /β-glucosidases from intestinal bacteria.

In Fig. 6, G-hesperidin released hesperidin just after the addition of homogenates (0 h). As Kometani et al. have explained, G-hesperidin has a high susceptibility to /α-glucosidases.10) Hence it was assumed that this derivative experienced rapid hydrolysis in the presence of the enzymes. Moreover, the initial concentration of G-hesperidin in the reaction with cecal content homogenate was slightly lower than that in the reaction with small intestine homogenate (Fig. 6). This difference suggests that G-hesperidin partially binds to cecal contents.

The reason G-hesperidin is efficiently absorbed remains unclear, but there is a possibility that an active transport system of glucose by sodium-dependent absorption.
glucose transporter 1 (SGLT1) is involved in the high absorption efficiency of G-hesperidin. Quercetin glycosides, which have been well investigated as to their absorption and metabolism, have been verified to be absorbed rapidly in preference to quercetin aglycon.25–27 As for the mechanism of this phenomenon, Hollman et al. have suggested that the aglycon moiety of quercetin glycosides, namely the quercetin group, might be drawn into the enterocytes by its glucose moiety through SGLT1.25 In addition, Day et al. have explained that lactase phlorizin hydrolase, a β-glucosidase existing on the brush border of the small intestine, might contribute to the rapid absorption of quercetin glycosides in association with SGLT1.27 Similar mechanisms might lie in the absorption process of G-hesperidin, because this derivative possesses one glucose molecule introduced by transglycosylation and efficiently releases hesperidin, a substrate for β-glucosidases, by hydrolysis in the small intestine.

Recently, Kim et al. reported that G-hesperidin permeated across the Caco-2 cell monolayer, a model of the small intestinal epithelium, via the paracellular pathway, but hesperidin did not.28 In this case, the difference in permeability between G-hesperidin and hesperidin was considered to be due to the water solubility of these compounds.28 Such a passive transport system might explain the high absorbability of G-hesperidin after administration to rats. Nevertheless, the administration of G-hesperidin did not lead to the detection of its intact form in rat serum (Fig. 3). Permeated G-hesperidin might be hydrolyzed to hesperetin by α- and β-glucosidases derived from enterocytes during its transepithelial transport in vivo.

In the present study, not only hesperetin and its conjugate but also hesperidin and its conjugate were detected in the urine of rats administered G-hesperidin (Fig. 5). This result suggests the possibility that hesperidin released from G-hesperidin is directly absorbed across the brush border. As mentioned above, it is thought that orally administered G-hesperidin is hydrolyzed to hesperidin and glucose by α-glucosidases on the brush border of the small intestine. It appears likely that a portion of released hesperidin is deglycosylated to hesperetin by β-glucosidases from intestinal bacteria or epithelialocytes and is subsequently absorbed across the intestinal epithelium. In contrast, another part of released hesperidin is probably transported into the enterocytes without deglycosylation. Furthermore, it is probable that the transported hesperidin, which is partially conjugated, is immediately removed from blood circulation and excreted into the urine without renal tubular reabsorption. We also observed that hesperidin conjugate was present in the serum of one out of five rats administered G-hesperidin, supporting the conclusion that released hesperidin is partially absorbed in the intact form across the brush border.

It has been reported that hesperidin is not toxic in high-dose and long-term administration studies.1,29,30 On the basis of these findings, hesperidin has been regarded as an extremely safe compound. The safety of G-hesperidin has also been confirmed in long-term administration studies using mice and rats.6,31 In a human study, no clinical changes related to 24-week administration of G-hesperidin were observed in subjects.32 Hence the safety of G-hesperidin is considered to be comparable to that of hesperidin. Additionally, we observed that the metabolites, such as hesperetin, hesperidin, and their conjugates, were rapidly excreted in rat urine after administration of G-hesperidin (Fig. 5). This observation confirms the safety of G-hesperidin.

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References

9) Ohtsuki, K., Abe, A., Mitsuzumi, H., Kondo, M.,


