Effects of Glucosyl Hesperidin on Serum Lipids in Hyperlipidemic Subjects: Preferential Reduction in Elevated Serum Triglyceride Level

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Summary Although hesperidin lowers serum total cholesterol (TC) or triglyceride (TG) in animal models, its effect in humans remains unclear. Using a soluble hesperidin derivative, glucosyl hesperidin (G-hesperidin), as a hesperidin source, we examined the efficacy on hyperlipidemic subjects. G-Hesperidin was administered to the subjects at 100 or 500 mg/d for 6 wk. The percentage of subjects who had a change in serum cholesterol levels was less than 20%. However, 45–55% of the total subjects showed a reduction in serum TG level. The subjects were classified into normal (TC<230 mg/dL, TG<150 mg/dL), high-TC (TC>230 mg/dL, TG<150 mg/dL) and high-TG (TG>150 mg/dL) types. While serum cholesterol levels scarcely changed in any phenotype, TG level was significantly reduced by administration in the high-TG type. In this phenotype, serum apolipoprotein (apo) C-II and E levels decreased by the administration, but non-apo B. G-Hesperidin also raised low-density lipoprotein (LDL)-cholesterol/apo B in the high-TG type. These results indicate that G-hesperidin preferentially lowers serum TG in hypertriglyceridemic subjects and that this effect is possibly caused by the facilitation of catabolism of TG-rich lipoproteins and may contribute to the reduction of small dense LDL.

Key Words glucosyl hesperidin, triglyceride, apolipoprotein, small dense LDL

Hesperidin is a flavonoid compound that occurs abundantly in citrus fruit peel. This material is well known to decrease capillary fragility and permeability (1). In addition, hesperidin is reported to have various physiological functions (1). Improvement of serum lipid levels is one of these effects, and it is described that hesperidin lowers serum total cholesterol (TC) or triglyceride (TG) in animal models (2–6).

Elevated serum TC level is recognized as a major risk factor for the progression of atherosclerosis (7–9). Furthermore, recent studies have shown that a high level of serum TG is closely associated with the development of coronary artery disease and that this association is attributed to the formation of small dense low-density lipoprotein (LDL), which has smaller particle size than normal LDL (10–16). Although hesperidin is expected to exhibit a preventive effect against atherosclerosis by restoring these serum lipid disorders, its effect in humans is still unclear. Additionally, the use of this flavonoid has been limited because of its low water solubility.

Hijiya et al. have synthesized a soluble form of hespe-

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MATERIALS AND METHODS

Test substances. Tablets consisting of G-hesperidin (100 or 250 mg), maltose (900 or 750 mg) and sucrose stearate (30 mg) were prepared and used for this study. G-Hesperidin was provided by Toyo Sugar Refining Co., Ltd. (Tokyo, Japan). Maltose was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan).
Sucrose stearate was purchased from Mitsubishi-Kagaku Foods Corp. (Tokyo, Japan).

Subjects. Adult male volunteers, who had a serum TC level of at least 200 mg/dL and were not receiving any lipid-lowering drugs, were recruited, and 40 men (27–64 y old) were enrolled in this study. The purpose and nature of the study were fully explained to each volunteer. All subjects gave their informed consent before admission. This study was approved by the Ethics Committee of Hayashibara Biochemical Laboratories, Inc. and carried out in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Experimental protocol. All subjects were randomly divided into two groups of 20: one was the 100 mg/d G-hesperidin administered group (100 mg/d-group) and the other was the 500 mg/d G-hesperidin administered group (500 mg/d-group). The 100 mg/d-group received a 100 mg-tablet and the 500 mg/d-group received two 250 mg-tablets at each bedtime. These tablets were administered for 6 wk, followed by a 4-wk washout period. To examine serum parameters, blood sampling was performed at the beginning of administration (wk 0), at wk 2 and 6 of the administration period, and at the end of the washout period (wk 10). Blood samples were obtained in the early morning after fasting for at least 12 h from 9 p.m. the previous night. The subjects consumed their usual diet and maintained their eating and exercise habits for the period of this study. To assess their dietary habits, the contents of daily meals and snacks were recorded in the diet diary throughout the study.

Serum parameter analyses. Serum TC, LDL-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and TG were measured using an automated analyzer. Serum apolipoprotein (apo) B, C-II and E were measured by immunoturbidimetry. Moreover, serum glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl-transferase (γ-GT) and creatinine were determined in accordance with standard methods. All the analyses were performed by FALCO Biosystems Ltd. (Kyoto, Japan).

Evaluation of LDL particle size. LDL particle size was evaluated based on LDL-C/apo B, which was calculated from measurement values of serum LDL-C and apo B. Since one molecule of apo B is bound to a LDL particle, LDL-C/apo B represents cholesterol content per one LDL particle (20). This value is correlated with LDL particle size, and when it is less than 1.2, the presence of small dense LDL is speculated (20).

Evaluation of efficacy for serum lipids. For serum TC, LDL-C or TG, G-hesperidin was judged effective when each lipid level at wk 6 was at least 10% lower than the initial value (wk 0). For serum HDL-C, G-hesperidin was judged effective when it led to at least 10% increase relative to the initial value at wk 6. These judgement criteria were established by reference to the potency of statin or fibrates trials, which are known to improve serum lipid levels (21). The efficacy rate of G-hesperidin for each serum lipid was calculated by the following formula:

Efficacy rate (%) = (number of subjects in each group who were judged G-hesperidin effective)/(total number of subjects in each group)×100

Classification of subjects according to hyperlipidemia phenotypes and efficacy analyses in each phenotype. Based on initial serum TC and TG values, all subjects were classified into the following 3 phenotypes: normal type (TC<230 mg/dL, TG<150 mg/dL), high-TC type (TC>230 mg/dL, TG<150 mg/dL) and high-TG type (TG>150 mg/dL). The criteria for the phenotype classification were determined according to the description of Ikeda et al. (22). In each phenotype, the efficacy of G-hesperidin was assessed by analyzing the changes of initial values in serum lipids, apolipoproteins and LDL particle size (LDL-C/apo B) at wk 2, 6 and 10.

Statistical analyses. Data were expressed as means± SD. Week 0 values were considered as baselines, and the changes from the baselines in each group were examined by paired t-test. The significance level was set at p<0.05 for all tests. Statistical analyses were performed with Stat View version 5.0 for Windows (SAS Institute, Cary, NC).

RESULTS

Characteristics of the subjects

Baseline characteristics of the subjects are shown in Table 1. The ages of the 100 mg/d-group and the 500 mg/d-group were 43.8±10.5 years and 43.6±10.5 years, respectively. Serum TC level was 227.9±36.0 mg/dL in the 100 mg/d-group and 244.5±26.5 mg/dL in the 500 mg/d-group. No change was recognized in dietary habits with all subjects throughout the study. There was no change in body weight or body mass index in either group during the study. In addition, there were no changes in serum GOT, GPT, γ-GT and creatinine levels in either group during the administration period. No clinical signs related to the treatment were observed in the groups.

Effects of G-hesperidin administration on serum lipids

We examined what percentage of the subjects showed an effect of G-hesperidin on serum lipids. The results are shown in Table 2 as the efficacy rates of G-hesperidin. Low efficacy rates, which were less than 20%, for serum TC, LDL-C and HDL-C were obtained in the 100 mg/d-group and the 500 mg/d-group. On the other hand, a
Table 1. Baseline characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>100 mg/d-Group</th>
<th>500 mg/d-Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43.8±10.5</td>
<td>43.6±10.5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.1±10.7</td>
<td>67.8±9.4</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.3±1.0</td>
<td>23.2±3.0</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>227.9±36.0</td>
<td>244.5±26.5</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>138.7±31.3</td>
<td>152.7±27.2</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>63.1±16.4</td>
<td>65.5±20.8</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>120.5±65.9</td>
<td>109.6±59.5</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>118.6±26.4</td>
<td>133.0±26.1</td>
</tr>
<tr>
<td>Apo C-II (mg/dL)</td>
<td>5.2±2.1</td>
<td>5.4±1.7</td>
</tr>
<tr>
<td>Apo E (mg/dL)</td>
<td>5.5±1.3</td>
<td>6.0±1.2</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>25.9±10.4</td>
<td>27.2±12.0</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>36.0±28.4</td>
<td>40.4±34.1</td>
</tr>
<tr>
<td>γ-GT (IU/L)</td>
<td>56.7±50.1</td>
<td>72.9±65.5</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD for data measured at wk 0.

Table 3. Classification of subjects according to hyperlipidemia phenotypes.

<table>
<thead>
<tr>
<th></th>
<th>100 mg/d-Group (n=20)</th>
<th>500 mg/d-Group (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal type</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>High-TG type</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>High-TG type</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>201.0±10.5</td>
<td>207.0±20.3</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>119.2±13.2</td>
<td>121.0±9.2</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>65.4±15.2</td>
<td>68.0±11.1</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>77.0±26.2</td>
<td>85.0±18.0</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>95.6±7.5</td>
<td>103.3±12.5</td>
</tr>
<tr>
<td>Apo C-II (mg/dL)</td>
<td>3.2±0.5</td>
<td>4.8±1.2</td>
</tr>
<tr>
<td>Apo E (mg/dL)</td>
<td>4.6±1.0</td>
<td>4.7±0.8</td>
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</tbody>
</table>

Each value represents the mean±SD for data measured at wk 0. The subjects were classified into 3 phenotypes on the basis of their initial serum TC and TG values: normal type (TC<230 mg/dL, TG<150 mg/dL), high-TG type (TC>230 mg/dL, TG<150 mg/dL) and high-TG type (TG>150 mg/dL).

Triglyceride-Lowering Effect of Glucosyl Hesperidin

Table 2. Efficacy rates of G-hesperidin for serum lipids.

<table>
<thead>
<tr>
<th>Serum lipids</th>
<th>Groups</th>
<th>Number of subjects</th>
<th>Number of effective casesa</th>
<th>Efficacy ratesb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>100 mg/d</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>500 mg/d</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LDL-C</td>
<td>100 mg/d</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>500 mg/d</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>HDL-C</td>
<td>100 mg/d</td>
<td>20</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>500 mg/d</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>TG</td>
<td>100 mg/d</td>
<td>20</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>500 mg/d</td>
<td>20</td>
<td>9</td>
<td>45</td>
</tr>
</tbody>
</table>

For each serum lipid, G-hesperidin was judged effective when it led to at least 10% improvement relative to the initial value at wk 6 (TC, LDL-C and TG: ≤ -10%, HDL-C: ≥ +10%). Values represent the number of subjects who were judged G-hesperidin effective.

a Percentage of effective cases.

Effect of G-hesperidin administration in each hyperlipidemia phenotype

To further investigate the efficacy of G-hesperidin, we classified the subjects into 3 phenotypes (normal type, high-TG type and high-TG type) based on their initial serum TC and TG values. The 100 mg/d-group consisted of 5 normal type, 8 high-TG type and 7 high-TG type; and the 500 mg/d-group consisted of 3 normal type, 13 high-TG type and 4 high-TG type (Table 3).

Changes in serum cholesterol levels. In the individual phenotypes mentioned above, we analyzed the changes in serum cholesterol levels after G-hesperidin administration. As a result, no obvious changes in serum TC, LDL-C and HDL-C levels were observed in any phenotype of the 100 mg/d-group and the 500 mg/d-group during the administration period (Fig. 2).

Change in serum TG level. The changes in serum TG level after G-hesperidin administration examined in each phenotype are shown in Fig. 3. Normal and high-TG types of both groups showed no obvious changes in serum TG level during the administration period.

In contrast, the high-TG type subjects of both groups tended to have decreased in serum TG levels during the administration period. This decrease was more pronounced in the 500 mg/d-group and about 50% reduction in serum TG level was observed at wk 6. This effect was dependent on the dose level and the period of G-hesperidin administration and had a tendency to disappear 4 wk after the cessation of treatment.

Changes in serum apolipoprotein levels. In each phenotype, we also investigated whether the serum levels of 3 apolipoproteins (apo B, C-II and E), which are correlated with serum lipids, are altered by G-hesperidin administration. The result is shown in Fig. 4.

Initial serum apo B, C-II and E levels of high-TG and high-TG type subjects tended to be high in both groups, compared to those of normal type. In particular, serum apo C-II was remarkably elevated in the high-TG type.

No obvious changes in serum apo B level were
detected in any phenotype of either group during the administration period, however a slight reduction (5%) was observed in the high-TC type of the 500 mg/d-group.

Although the serum apo C-II level was unchanged in the normal and high-TC types of both groups and the high-TG type of the 100 mg/d-group, it significantly decreased by about 15% in the high-TG type subjects of the 500 mg/d-group during the administration period. This decrease had a tendency to be abrogated during the washout period.

The change in serum apo E level was similar to that in the apo C-II level: high-TG type subjects of the 500 mg/d-group had a significant reduction of 20% apo E level after the G-hesperidin administration. This effect also had a tendency to disappear 4 wk after the cessation of treatment.

Change in LDL particle size. In respective phenotypes, we further examined the change in LDL-C/apo B as an index of LDL particle size. The result is shown in Fig. 5.

The initial LDL-C/apo B of high-TG type subjects was lower than that of the normal and high-TC types in both groups, and the value was less than 1.2. From this observation, the LDL particle size of high-TG type subjects was suggested to be smaller than that of the normal and high-TC types. Additionally, it was speculated that the high-TG type possesses small dense LDL.

In both groups, the LDL-C/apo B of normal and high-TC types remained within the normal range (>1.2) throughout the administration period. A slight elevation of the value was recognized in the 500 mg/d-group, but this was a change caused within the normal

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**Fig. 2.** Changes in serum cholesterol levels of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum cholesterol levels were measured at each time point. Values are means±SD. *p<0.05 vs. wk 0 value.

**Fig. 3.** Changes in serum TG level of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum TG level was measured at each time point. Values are means±SD. *p<0.05 vs. wk 0 value.
range.

On the other hand, the LDL-C/apo B of high-TG type was elevated in both groups during the administration period, indicating an expansion of LDL particle size. This effect had a tendency to depend on the dose level and the period of G-hesperidin administration.

DISCUSSION

Hesperidin is shown to lower the serum TC level in rats by the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (4, 5). In addition, this flavonoid is reported to exhibit a serum TG-lowering effect in rats through the suppression of pancreatic lipase (6). However, the effects in humans remain to be fully clarified.

In this study, we examined whether 6-wk administration of G-hesperidin, as a hesperidin source, leads to the efficacy for serum lipids in subjects whose serum TC level is more than 200 mg/dL. As a consequence, the case of subjects who had a change in serum cholesterol (TC, LDL-C or HDL-C) was less than 20% in each the 100 mg/d-group and the 500 mg/d-group (Table 2). On the other hand, many effective cases for serum TG were observed, and 45–55% of all subjects showed a reduction in this serum lipid level (Table 2). These findings suggest that G-hesperidin has a favorable effect on serum TG, but not on serum cholesterol, in humans.

The effect of G-hesperidin was further clarified by analyzing the changes in serum lipid levels of respective phenotypes. As shown in Table 3, the study subjects were classified into normal type (TC<230 mg/dL, TG<150 mg/dL), high-TG type (TC>230 mg/dL, TG<150 mg/dL) and high-TG type (TG>150 mg/dL). While serum cholesterol levels showed no change in any phenotype (Fig. 2), the serum TG level was significantly reduced in high-TG type subjects during the administration period (Fig. 3). From these results, we assume that G-hesperidin is effective in cases of elevated serum TG level.

Under present experimental conditions, G-hesperidin failed to exert the serum TC-lowering effect that had been reported in rats. The reason may be ascribed to the difference of species. It is known that the effect of HMG-CoA reductase inhibitors is diverse in different animal species (23, 24). For instance, compactin or pravastatin, an HMG-CoA reductase inhibitor, effectively suppresses the enzyme in humans and rabbits, and results in decreasing their serum TC levels, but it fails to exhibit the action in rats (25–27). Such species specificity is likely to be the cause of the lack of G-hesperidin action in humans. Thus, there is a possibility that humans and rats differ in susceptibility to G-hesperidin. However, further investigation of dosage is also required to determine the modification of serum TC level by G-hesperidin.

As mentioned above, Kawaguchi et al. have described that hesperidin may suppress the intestinal uptake of dietary lipids by inhibiting pancreatic lipase (6). This is considered as a mechanism of lowering serum TG by G-hesperidin and if it might be true, cholesterol levels must be down, too. Nevertheless, the decrease in serum TG level could not be explained only by the suppression of lipid absorption, because G-hesperidin was administered at bedtime 2–3 h after food intake. We observed that elevated values of serum apo C-II and apo E occurred in high-TG type subjects and that these apolipoprotein levels were significantly reduced by G-hesperidin administration (Fig. 4). Apo C-II and apo E are bound to very-low-density lipoprotein (VLDL), which contains abundant TG, or TG-rich lipoproteins that are

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**Fig. 4.** Changes in serum apolipoprotein levels of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum apolipoprotein levels were measured at each time point. Values are means±SD. *p<0.05 vs. wk 0 value.
generated in the catabolic process of VLDL (28, 29); and furthermore, their high values reflect a deficiency of VLDL catabolism. Then, our results may be possibly considered that G-hesperidin could enhance VLDL catabolism in high-TG type subjects. Lipoprotein lipase (LPL), a TG-hydrolyzing enzyme, could play a critical role in the catabolic process of VLDL. On this account, the authors speculate that the present results could be associated with the activation of LPL, so that G-hesperidin accelerates VLDL catabolism through the activation of LPL, and this effect might contribute to the augmentation of removing TG from VLDL. Fibrate drugs, which are widely prescribed to lower serum TG, have been demonstrated to augment LPL activity at the level of transcription by activating a nuclear receptor, peroxisome proliferator-activated receptor (PPAR-α) (30). In addition, it has also been reported that these drugs suppress the expression of apo C-III, a LPL antagonist, via the activation of PPAR-α, resulting in an elevated serum apo C-III/apo C-III ratio (31, 32). As with fibrates, we recently found that 24-wk administration of G-hesperidin raised the serum apo C-III/apo C-III ratio in 8 high-TG type subjects (0.45±0.08 at baseline and 0.50±0.12 at wk 24; p<0.05) (unpublished data). This finding suggests a possibility that G-hesperidin affects PPAR-α in a similar manner as fibrates. Therefore, we speculate that G-hesperidin may augment LPL activity through this nuclear receptor. Further investigations focussed on PPAR-α are required to clarify the mechanisms of the TG-lowering effect by G-hesperidin. On the other hand, it is well known that cholesteryl ester transfer protein (CETP) as well as LPL contributes to the removal of the TG from VLDL (33, 34). However, it seems likely that CETP is not affected by G-hesperidin because the serum HDL-C level was unchanged during the administration period (Fig. 2).

Moreover, an elevated serum apo B level was also observed in high-TG type at wk 0, but these was no significant change during the administration period (Fig. 4). Nevertheless, hesperetin, which is released from hesperidin through deglycosylation by bacteria in the gastrointestinal tract, has been reported to suppress apo B secretion in cultured human hepatocytes (35, 36). Since many investigators have observed that hesperidin is deglycosylated to its aglycon (hesperetin) before absorption (1, 37, 38), there is a possibility that this flavonoid administration causes the reduction of serum apo B level. Recently, we have confirmed that hesperetin conjugated with glucuronic acid is detected in the serum of rats after oral administration of G-hesperidin (unpublished data). From this result, it can be presumed that G-hesperidin as well as hesperidin is absorbed in the form of aglycon. Hence, further analyses such as investigation of the administration period and in vitro experiments are in progress in our laboratory to elucidate the influence of G-hesperidin on apo B level.

It has been demonstrated that patients with a high serum TG level experience an increase in TG-rich lipoproteins by the deficiency of the ability to catabolize VLDL (39–41). These hypertriglyceridemic patients possess small dense LDL with high frequency (13, 14), and this abnormal LDL has been verified to be closely involved in coronary artery disease (15, 16). As expected, the initial LDL-C/apo B of high-TG type subjects was less than 1.2, suggesting the presence of small dense LDL (Fig. 5). In these subjects, the G-hesperidin administration led to an increase in LDL particle size as judged by a raise in LDL-C/apo B (Fig. 5). This finding implies that G-hesperidin facilitates VLDL catabolism and results in the reduction of small dense LDL formation.

In conclusion, the present study has indicated that a 6-wk administration of G-hesperidin preferentially lowers the serum TG level in hypertriglyceridemic subjects and that this effect is possibly due to the facilitation of removing TG from TG-rich lipoproteins. This action also participates in the suppression of small dense LDL for-
mation, suggesting a potential for preventing atherosclerosis progression.

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REFERENCES


