J Nutr Sci Vitaminol, **50**, 211–218, 2004

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

Yoshikatsu MIWA[1], Mika YAMADA[1], Takahiro SUNAYAMA[2], Hitoshi MITSUZUMI[1,*], Yukari TSUZAKI[1], Hiroto CHAEN[1], Yasuo MISHIMA[3] and Masayoshi KIBATA[1,3]

[1]*Research and Development Center, Hayashibara Biochemical Laboratories, Inc., 1–2–3 Shimoishii, Okayama 700–0907, Japan*
[2]*Dai-ichi Central Bldg. Clinic, 6–36–601 Honmachi, Okayama 700–0901, Japan*
[3]*Division of Internal Medicine, National Minami Okayama Hospital, 4066 Hayashima, Okayama 701–0304, Japan*

(Received July 24, 2003)

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 hesperidin, glucosyl hesperidin (G-hesperidin), by regioselective transglycosylation with cyclodextrin glucanotransferase from *Bacillus stearothermophilus* (Fig. 1) (*17*). G-Hesperidin is soluble in water, and its solubility is about 10,000 times greater than that of conventional hesperidin (*17*). Moreover, it has been verified that this derivative releases hesperidin through hydrolysis by brush border α-glucosidases and results in the exhibition of physiological activities (*17*, *18*). We have also found that the supplementation of G-hesperidin significantly lowered serum TG in hyperlipidemic mice and that the lowering effect was probably due to hesperidin released in vivo (*19*). From these findings, G-hesperidin is considered to be available effectively as a hesperidin source.

In this study, we examined the effect of G-hesperidin on serum lipid levels in humans and clarified that G-hesperidin preferentially lowers serum TG.

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).

*To whom correspondence should be addressed.
E-mail: kaihatsu@hayashibara.co.jp

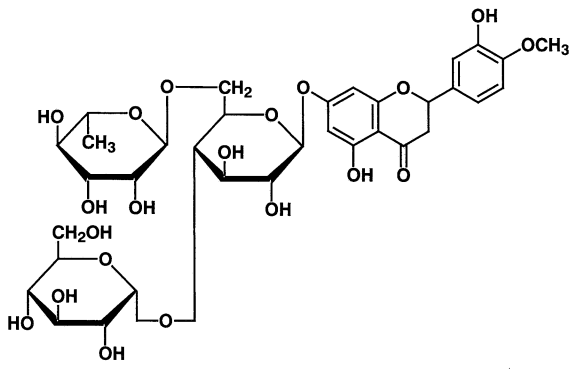

Fig. 1. Chemical structure of glucosyl hesperidin (G-hesperidin).

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 fibrate 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:

$$\text{Efficacy rate (\%)} = \frac{\text{number of subjects in each group who were judged G-hesperidin effective}}{\text{total number of subjects in each group}} \times 100$$

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 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.

	100 mg/d-Group	500 mg/d-Group
Number of subjects	20	20
Age (y)	43.8±10.5	43.6±10.5
Body weight (kg)	67.1±10.7	67.8±9.4
Body mass index	23.1±3.0	23.3±3.0
TC (mg/dL)	227.9±36.0	244.5±26.5
LDL-C (mg/dL)	138.7±31.3	152.7±27.2
HDL-C (mg/dL)	63.1±16.4	65.5±20.8
TG (mg/dL)	120.5±65.9	109.6±59.5
Apo B (mg/dL)	118.6±26.4	133.0±26.1
Apo C-II (mg/dL)	5.2±2.1	5.4±1.7
Apo E (mg/dL)	5.5±1.3	6.0±1.2
GOT (IU/L)	25.9±10.4	27.2±12.0
GPT (IU/L)	36.0±28.4	40.4±34.1
γ-GT (IU/L)	56.7±50.1	72.9±65.5
Creatinine (mg/dL)	1.0±0.1	1.0±0.1

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

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

Serum lipids	Groups	Number of subjects	Number of effective cases[a]	Efficacy rates[b] (%)
TC	100 mg/d	20	2	10
	500 mg/d	20	0	0
LDL-C	100 mg/d	20	2	10
	500 mg/d	20	2	10
HDL-C	100 mg/d	20	4	20
	500 mg/d	20	1	5
TG	100 mg/d	20	11	55
	500 mg/d	20	9	45

[a] 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.
[b] Percentage of effective cases.

Table 3. Classification of subjects according to hyperlipidemia phenotypes.

	100 mg/d-Group (n=20)			500 mg/d-Group (n=20)		
	Normal type	High-TC type	High-TG type	Normal type	High-TC type	High-TG type
Number of subjects	5	8	7	3	13	4
TC (mg/dL)	201.0±10.5	247.9±36.3	224.1±36.5	207.0±20.3	250.2±19.5	253.8±31.3
LDL-C (mg/dL)	119.2±13.2	157.4±27.6	131.1±35.5	121.0±9.2	160.0±26.4	152.5±24.6
HDL-C (mg/dL)	65.4±15.2	67.4±15.1	56.6±18.8	68.0±11.1	68.2±24.3	54.8±9.3
TG (mg/dL)	77.0±26.2	88.5±19.7	188.1±67.5	85.0±18.0	85.4±28.8	206.8±55.9
Apo B (mg/dL)	95.6±7.5	125.0±25.6	127.7±28.4	103.3±12.5	137.6±24.8	142.9±25.9
Apo C-II (mg/dL)	3.2±0.5	4.8±2.0	7.0±1.3	4.8±1.2	5.2±1.8	6.7±1.0
Apo E (mg/dL)	4.6±1.0	5.7±1.6	5.9±1.1	4.7±0.8	6.0±1.1	7.0±0.5

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-TC type (TC>230 mg/dL, TG<150 mg/dL) and high-TG type (TG>150 mg/dL).

high efficacy rate for serum TG was obtained for both groups. The rate was 55% in the 100 mg/d-group and 45% in the 500 mg/d-group.

Effects 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-TC 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-TC type and 7 high-TG type; and the 500 mg/d-group consisted of 3 normal type, 13 high-TC 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-TC 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-TC 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

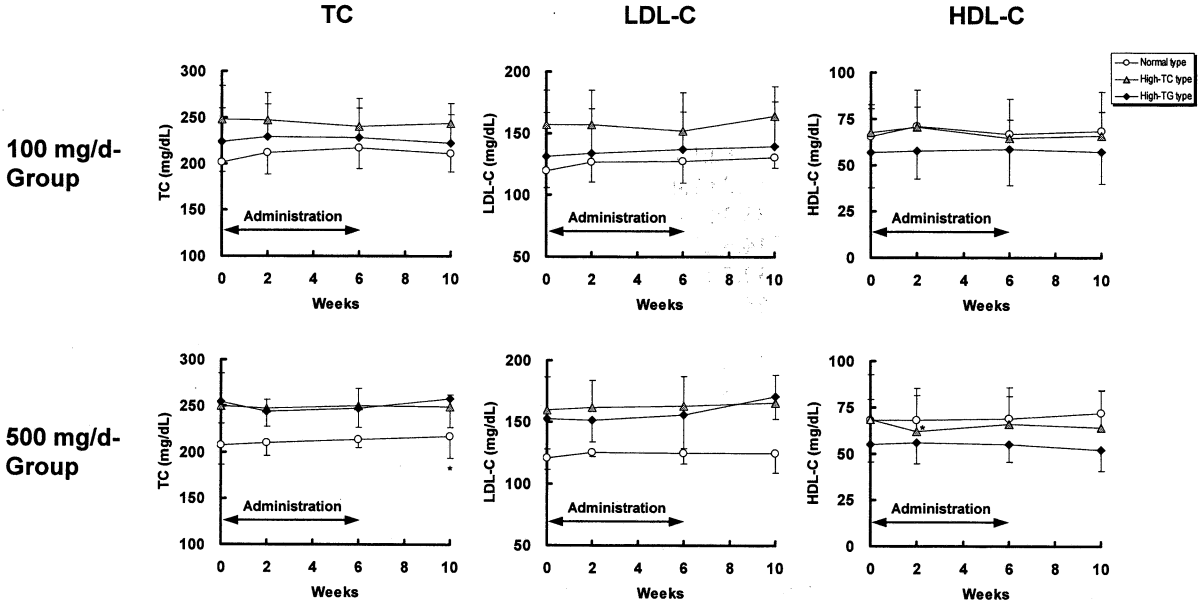

Fig. 2. Changes in serum cholesterol levels of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum cholesterol levels were measured at each time point. Values are means±SD. * $p<0.05$ vs.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

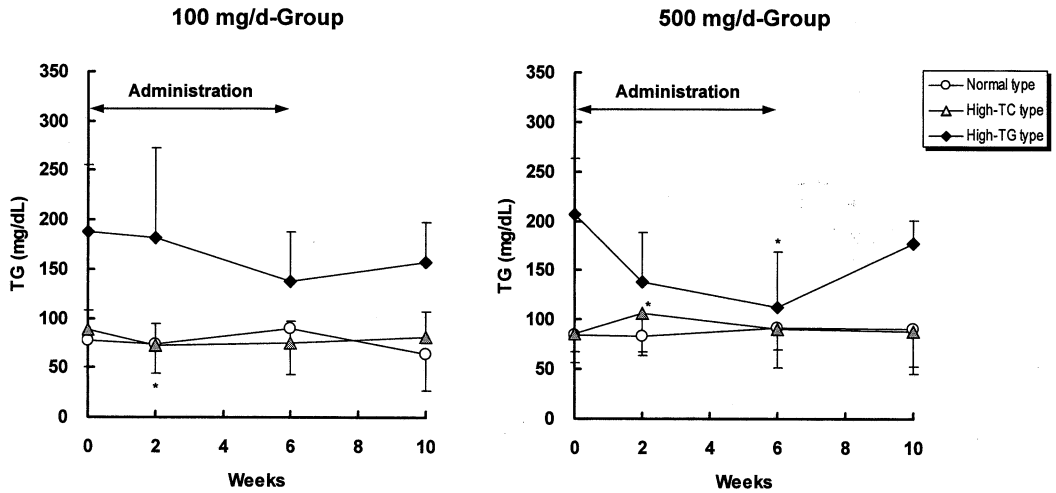

Fig. 3. Changes in serum TG level of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum TG level was measured at each time point. Values are means±SD. * $p<0.05$ vs. wk 0 value.

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 de-crease 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

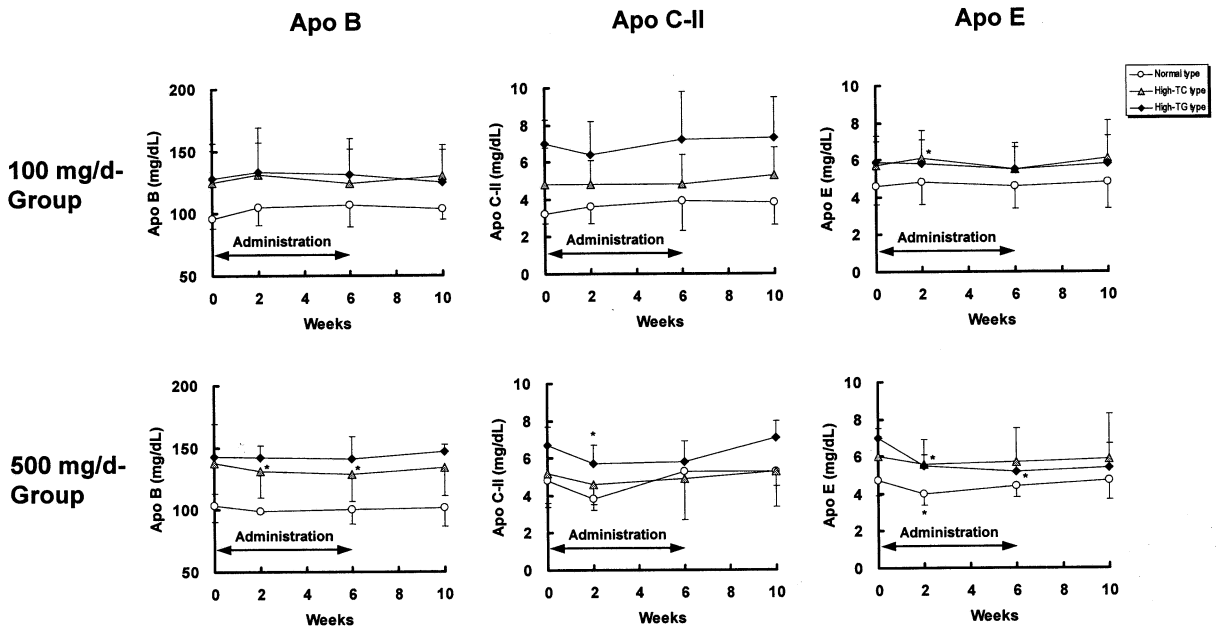

Fig. 4. Changes in serum apolipoprotein levels of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10, and serum apolipoprotein levels were measured at each time point. Values are means±SD. * $p<0.05$ vs. wk 0 value.

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-TC 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

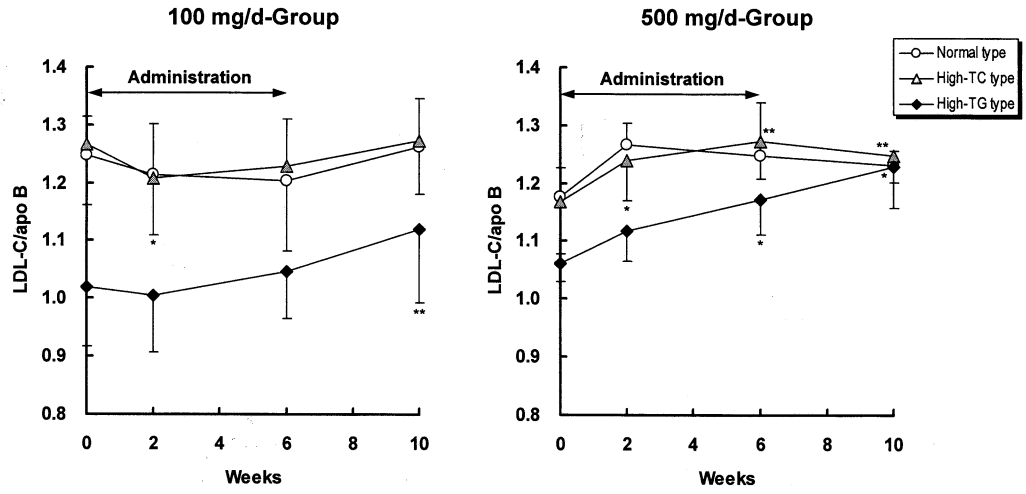

Fig. 5. Changes in LDL-C/apo B of respective phenotypes. The tablets containing G-hesperidin were administered at prescribed doses for 6 wk. Blood sampling was performed at wk 0, 2, 6 and 10. LDL-C/apo B was calculated from the measurement values of serum LDL-C and apo B at each time point. Values are means±SD. * $p<0.05$ vs. wk 0 value, ** $p<0.005$ 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-II/apo C-III ratio (*31*, *32*). As with fibrates, we recently found that 24-wk administration of G-hesperidin raised the serum apo C-II/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 to 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.

Acknowledgments

We would like to thank Ms. Michiyo Tsujita for her excellent technical and secretarial assistance in this study.

REFERENCES

1) Garg A, Garg S, Zaneveld LJD, Singla AK. 2001. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother Res* **15**: 655–669.
2) Rathi AB, Nath N, Chari SN. 1983. Action of vitamin P like compounds on lysosomal status in hypercholesterolemic rats. *Acta Vitaminol Enzymol* **5**: 255–261.
3) Monforte MT, Trovato A, Kirjavainen S, Forestieri AM, Galati EM, Lo Curto RB. 1995. Biological effects of hesperidin, a citrus flavonoid. (note II): hypolipidemic activity on experimental hypercholesterolemia in rat. *IL Farmaco* **50**: 595–599.
4) Bok SH, Lee SH, Park YB, Bae KH, Son KH, Jeong TS, Choi MS. 1999. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *J Nutr* **129**: 1182–1185.
5) Park YB, Do KM, Bok SH, Lee MK, Jeong TS, Choi MS. 2001. Interactive effect of hesperidin and vitamin E supplements on cholesterol metabolism in high cholesterol-fed rats. *Int J Vitam Nutr Res* **71**: 36–44.
6) Kawaguchi K, Mizuno T, Aida K, Uchino K. 1997. Hesperidin as an inhibitor of lipases from porcine pancreas and *Pseudomonas*. *Biosci Biotechnol Biochem* **61**: 102–104.
7) Demchuk AM, Hess DC, Brass LM, Yatsu FM. 1999. Is cholesterol a risk factor for stroke? Yes. *Arch Neurol* **56**: 1518–1520.
8) Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* **333**: 1301–1307.
9) Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JMO, Wun CC, Davis BR, Braunwald E. 1996. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* **335**: 1001–1009.
10) Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Mänttäri M, Heinonen OP, Frick MH. 1992. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation* **85**: 37–45.
11) Grundy SM, Vega GL. 1992. Two different views of the relationship of hypertriglyceridemia to coronary heart disease. Implications for treatment. *Arch Intern Med* **152**: 28–34.
12) Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Kramsch DM, Blankenhorn DH. 1994. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation* **90**: 42–49.
13) Feingold KR, Grunfeld C, Pang M, Doerrler W, Krauss RM. 1992. LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes. *Arterioscler Thromb* **12**: 1496–1502.
14) Kazumi T, Kawaguchi A, Hozumi T, Nagao M, Iwahashi M, Hayakawa M, Ishihara K, Yoshino G. 1999. Low density lipoprotein particle diameter in young, non-obese, normolipidemic Japanese men. *Atherosclerosis* **142**: 113–119.
15) McNamara JR, Jenner JL, Li Z, Wilson PWF, Schaefer EJ. 1992. Change in LDL particle size is associated with change in plasma triglyceride concentration. *Arterioscler Thromb* **12**: 1284–1290.
16) Austin MA, Mykkänen L, Kuusisto J, Edwards KL, Nelson C, Haffner SM, Pyörälä K, Laakso M. 1995. Prospective study of small LDLs as a risk factor for non-insulin dependent diabetes mellitus in elderly men and women. *Circulation* **92**: 1770–1778.
17) Hijiya H, Miyake T. 1991. Alpha-glycosyl hesperidin, and its preparation and uses. European Patent Publication No. 0402049.
18) Ohtsuki K, Abe A, Mitsuzumi H, Kondo M, Uemura K, Iwasaki Y, Kondo Y. 2002. Effects of long-term administration of hesperidin and glucosyl hesperidin to spontaneously hypertensive rats. *J Nutr Sci Vitaminol* **48**: 420–422.
19) Yamada M, Mitsuzumi H, Tsuzaki Y, Miwa Y, Chaen H, Yamamoto I. 2003. Antioxidant activity of glucosylated vitamin P and its suppressive effect on oxidative stress in hyperlipidemic mice. *J Jpn Soc Nutr Food Sci* **56**: 355–363.
20) McNamara JR, Small DM, Li Z, Schaefer EJ. 1996. Differences in LDL subspecies involve alterations in lipid composition and conformational changes in apolipoprotein B. *J Lipid Res* **37**: 1924–1935.
21) Bruckert E, De Gennes JL, Malbecq W, Baigts F. 1995. Comparison of the efficacy of simvastatin and standard fibrate therapy in the treatment of primary hypercholesterolemia and combined hyperlipidemia. *Clin Cardiol* **18**: 621–629.
22) Ikeda Y, Takagi A. 2001. Involvement of environmental factors on development of hyperlipidemia. *Nippon Rinsho* **59** (suppl 2): 670–676.
23) Shiomi M, Ito T. 2001. Animal models for spontaneous hyperlipidemia. *Nippon Rinsho* **59** (suppl 2): 617–620.
24) Tsujita Y, Kuroda M, Shimada Y, Tanzawa K, Arai M, Kaneko I, Tanaka M, Masuda H, Tarumi C, Watanabe Y, Fujii S. 1986. CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. *Biochim Biophys Acta* **877**: 50–60.
25) Endo A, Tsujita Y, Kuroda M, Tanzawa K. 1979. Effects of ML-236B on cholesterol metabolism in mice and rats: lack of hypocholesterolemic activity in normal animals. *Biochim Biophys Acta* **575**: 266–276.
26) Endo A. 1988. Chemistry, biochemistry, and pharmacology of HMG-CoA reductase inhibitors. *Klin Wochenschr* **66**: 421–427.
27) Fujioka T, Nara F, Tsujita Y, Fukushige J, Fukami M, Kuroda M. 1995. The mechanism of lack of hypocholesterolemic effects of pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, in rats. *Biochim Biophys Acta* **1254**: 7–12.

28) Andersson Y, Majd Z, Lefebvre AM, Martin G, Sechkin AV, Kosykh V, Fruchart JC, Najib J, Staels B. 1999. Developmental and pharmacological regulation of apolipoprotein C-II gene expression. Comparison with apo C-I and apo C-III gene regulation. *Arterioscler Thromb Vasc Biol* **19**: 115–121.

29) Weisgraber KH. 1994. Apolipoprotein E: structure-function relationships. *Adv Protein Chem* **45**: 249–302.

30) Schoonjans K, Staels B, Auwerx J. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* **37**: 907–925.

31) Staels B, Vu-Dac N, Kosykh VA, Saladin R, Fruchart JC, Dallongeville J, Auwerx J. 1995. Fibrates down-regulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. *J Clin Invest* **95**: 705–712.

32) Haubenwallner S, Essenburg AD, Barnett BC, Pape ME, DeMattos RB, Krause BR, Minton LL, Auerbach BJ, Newton RS, Leff T, Bisgaier CL. 1995. Hypolipidemic activity of select fibrates correlates to changes in hepatic apolipoprotein C-III expression: a potential physiologic basis for their mode of action. *J Lipid Res* **36**: 2541–2551.

33) Nozue T, Inazu A, Mabuchi H. 2001. High density lipoprotein. *Nippon Rinsho* **59** (suppl 2): 75–79.

34) Okada M. 2001. Functions of cholesteryl ester transfer protein (CETP). *Nippon Rinsho* **59** (suppl 2): 236–239.

35) Borradaile NM, Carroll KK, Kurowska EM. 1999. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids* **34**: 591–598.

36) Wilcox LJ, Borradaile NM, de Dreu LE, Huff MW. 2001. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J Lipid Res* **42**: 725–734.

37) Ameer B, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. 1996. Flavanone absorption after naringin, hesperidin, and citrus administration. *Clin Pharmacol Ther* **60**: 34–40.

38) Manach C, Morand C, Gil-lzquierdo A, Bouteloup-Demange C, Rémésy C. 2003. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *Eur J Clin Nutr* **57**: 235–242.

39) Tan CE, Forster L, Caslake MJ, Bedford D, Watson TDG, McConnell M, Packard CJ, Shepherd J. 1995. Relations between plasma lipids and postheparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women. *Arterioscler Thromb Vasc Biol* **15**: 1839–1848.

40) Sone H, Takahashi A, Shimano H, Ishibashi S, Yoshino G, Morisaki N, Saito Y, Kawazu S, Teramoto T, Fujita T, Shiba T, Iwamoto Y, Kuzuya N, Akanuma Y, Yamada N. 2002. HMG-CoA reductase inhibitor decreases small dense low-density lipoprotein and remnant-like particle cholesterol in patients with type-2 diabetes. *Life Sci* **71**: 2403–2412.

41) Guerin M, Egger P, Soudant C, Le Goff W, van Tol A, Dupuis R, Chapman MJ. 2002. Dose-dependent action of atorvastatin in type IIB hyperlipidemia: preferential and progressive reduction of atherogenic apo B-containing lipoprotein subclasses (VLDL-2, IDL, small dense LDL) and stimulation of cellular cholesterol efflux. *Atherosclerosis* **163**: 287–296.