The Citrus Flavonoids Hesperidin and Naringin
Do Not Affect Serum Cholesterol in Moderately
Hypercholesterolemic Men and Women1–3

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Abstract
The citrus flavonoids hesperidin and naringin have been suggested to lower blood total (TC) and LDL-cholesterol (LDL-C) both in animal models and humans. However, the evidence from previous studies in humans is not convincing. This study evaluated the LDL-C–lowering efficacy of pure hesperidin and naringin in moderately hypercholesterolemic individuals. A total of 204 healthy men and women with a serum TC concentration of 5.0–8.0 mmol/L participated in a randomized, placebo-controlled, parallel trial with 3 groups. A 4-wk preintervention period during which participants refrained from consuming hesperidin and naringin sources preceded the intervention. During the 4-wk intervention, the participants applied the same dietary restrictions and consumed 4 capsules/d providing either placebo (cellulose) or a daily dose of 800 mg hesperidin or 500 mg naringin. Blood samples to measure serum lipids were taken on 2 consecutive days at the beginning and end of the intervention phase. One hundred ninety-four participants completed the study. They maintained their prestudy body weights (mean changes < 0.2 kg in all groups). In all groups, the mean consumption of scheduled capsules was > 99%. Hesperidin and naringin did not affect TC or LDL-C, with endpoint LDL-C concentrations (adjusted for baseline) of 4.00 ± 0.04, 3.99 ± 0.04, and 3.99 ± 0.04 mmol/L for control, hesperidin, and naringin groups, respectively. These citrus flavonoids also did not affect serum HDL-cholesterol and triglyceride concentrations. In conclusion, pure hesperidin and naringin consumed in capsules at mealtime do not lower serum TC and LDL-C concentrations in moderately hypercholesterolemic men and women. J. Nutr. doi: 10.3945/jn.110.124735.

Introduction
Citrus flavonoids such as naringin and hesperidin have been proposed in the literature as potential blood cholesterol-lowering ingredients. These flavonoids exist mainly in their glycoside form in plants. Naringin is mainly present in grapefruits and sour oranges, and hesperidin is present in sweet oranges, mandarins and lemons (1). The colon microflora has the ability to hydrolyze the flavonoid glycosides hesperidin and naringin to generate the corresponding aglycones called naringenin and hesperetin, respectively (2).

Pure naringin or naringenin, naringin-rich grapefruit juices, peels, or peeled grapefruits reduced plasma total cholesterol (TC),4 (−14 to −55%) and hepatic cholesterol concentrations in various animal models (3–14). The evidence for the cholesterol-lowering effect of hesperidin/hesperetin and naringin in animal models is scarcer (9,12). The pathways involved in the potential hypolipidemic properties of hesperidin/hesperetin and naringin/naringenin are not yet fully understood. Studies in animal models showed a depressing effect on 3-hydroxy-3-methylglutaryl-CoA reductase and acyl-CoA: cholesterol acyltransferase activities in the liver (3,5,9,10,12,15), an upregulation of gene expression of key regulators of liver β-oxidation (13,14), and an increase in the excretion of bile acids (3,5–7), whereas effects on neutral sterol excretion were inconsistent (3,4,6,7,9,10,12). In vitro experiments did not confirm a direct effect of hesperidin or naringin on hydroxy-3-methylglutaryl-CoA reductase activity (9,10,16) but did suggest that hesperidin and naringin may reduce acyl-CoA: cholesterol acyltransferase-2 and microsomal triglyceride (TG) transfer protein mRNA (16) as well as the secretion of apolipoprotein B-100 (the apolipoprotein of LDL particles) (14,17), while increasing LDL receptor mRNA (16,18).

The evidence in humans comes mainly from studies in which citrus fruits/juices rich in hesperidin or naringin were tested for their lipid-lowering properties. The consumption of 1–2 peeled Jaffa grapefruits or Sweeties or 100–200 mL fresh Sweetie juice/d reduced plasma TC (−8 to −16%), LDL-cholesterol (LDL-C)

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3 Supplemental Figure 1 and Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
4 Abbreviations used: HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; PP, per protocol; TC, total cholesterol; TG, triglyceride.
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Test products. Monteleoder provided the hesperidin and naringin concentrates. Their purity (80% for hesperidin and 93% for naringin) was verified before filling the capsules. For this purpose, hesperidin and naringin concentrates were dissolved in dimethyl sulfoxide at room temperature and diluted with 1% acetic acid in Milli-Q water and acetonitrile before injection. An HPLC system equipped with a gradient HPLC pump (type 480, Separations), a solvent degasser (type GT-103, Separations), an auto-injector (type SIL-10ADyp, Shimadzu), a column thermostat (type Mistral, Separations), and a UV detector (HPLC-UV) (type 484, Waters) was used. The mobile phase consisted of 1% acetic acid in Milli-Q water (eluent A) with acetonitrile as modifier (eluent B). The HPLC column (250 mm-long, 4.6 mm diameter, particle size of 5 μm; type ODS-3 Inertsil, RP-C18, Varian B.V.) was eluted with a gradient flow from 5 to 95% acetonitrile over 40 min at a flow rate of 1 mL/min and was kept at 30°C. The injection volume was 20 μL and the detection was conducted at 330 nm. Concentrations of glycosylflavones were calculated against naringin (93%, Monteleoder) and concentrations of polymethoxylated flavonoids against tangeretin, 95.6%, ChromaDex) using a chromatography data processing system (Perkin Elmer Nelson TotalChrom software).

Because the hesperidin and naringin capsules were intended to contain either 200 mg hesperidin or 125 mg naringin each, microcrystalline cellulose (MC-102, Blanver) was added to both the hesperidin and naringin capsules to ensure a similar weight of all capsules. Two milligrams of silicium dioxide and 4 mg of magnesium stearate were added to each capsule (of all treatments) as antiadherent agents. Metagenics Europe filled the capsules under Hazard Analysis Critical Control Point conditions. Samples from the placebo, hesperidin, and naringin capsules were also analyzed before the start of the intervention with the HPLC-UV method described above to verify their hesperidin and naringin content. Hesperidin capsules provided a mean dose of 198 mg hesperidin each and naringin capsules had a mean content of 117 mg naringin each. Both hesperidin and naringin were undetectable in the placebo capsules. Other flavones such as narirutin, kaempferol neohesperoside, hesperitin, other glycosylated flavonoids, and polymethoxylated flavonoids were present in small amounts (mean total content of 25 mg/capsule) in the hesperidin capsules. In the naringin capsules, other flavonoids detected (naringenin and kaempferol neohesperoside) represented a total of <1 mg/capsule.

The participants received test products in sealed boxes containing 1 daily dose (4 capsules) each at the start of and halfway through the intervention period.

Compliance measurements. The participants registered in a calendar, weekly during the preintervention period and daily during the intervention period, their compliance to the dietary restrictions. They recorded daily their compliance to test product intake (including time of intake of capsules and time of intake of breakfast and dinner) during the intervention period. The study dietician checked the calendar halfway through and at the end of both the preintervention and intervention periods (d = 15, 1, 14, and 28). On these 4 days, the participants also had to complete a health and lifestyle questionnaire in which they recorded all changes to lifestyle and health, including medicine use. On d 14 and 28, the participants returned all used and unused capsule boxes. We counted these boxes and compared with the data in the calendar; in case of inconsistencies, we asked the participants for clarification.

Laboratory analyses. To take into account the day-to-day variability in serum cholesterol concentrations, the participants had to provide 2 overnight (9–14 h) fasting blood samples on consecutive days at the end of the preintervention and intervention periods. We recorded body weight once at the end of the preintervention and intervention phases. TC, LDL-C, HDL-C, and TG were measured with a Hitachi 912 autoanalyzer using the enzymatic colorimetric test CHOD-PAP (Roche, catalog no. 1489232), the homogenous enzymatic colorimetric test LDL-C plus 2nd generation (Roche catalog no. 04714423190), the HDL-C plus 3rd generation test (Roche, catalog no. 04713109190), and the enzymatic colorimetric test GPO-PAP (Roche, catalog no. 1488872), respectively.

Statistical analyses. Serum LDL-C was the key outcome parameter. To detect, with a power of 80% and an α level of 5%, a critical reduction of 0.2 mmol/L (~5%), in LDL-C (SD of the change within an individual of

Participants and Methods

Participants. We recruited 216 men and women via invitation letters and leaflets distributed in Vlaardingen (The Netherlands) and surrounding areas. Participants had to fulfill the following criteria: be an apparently healthy man or postmenopausal woman aged 18–75 y old and have a BMI 20–30 kg/m², serum TC concentration 5.0–8.0 mmol/L, and serum TG concentration < 4.0 mmol/L (see Supplemental Table 1 for detailed inclusion and exclusion criteria). Every volunteer provided written informed consent before any measurement was done. Because of a possible interaction of the citrus flavonoids with Cytochrome P450 3A4 (as reported for grapefruit) (28–30) and a potential impact on the uptake and metabolism of drugs, participants had to refrain from taking any medication, except aspirin, during the intervention period. The Medical Ethical Committee of Wageningen University (Wageningen, The Netherlands) approved the study protocol, information brochure, and recruitment materials. We performed the study from April to June 2008 at the Nutrition and Health department of Unilever R&D Vlaardingen.

Experimental design. The study had a double-blind, placebo-controlled, randomized parallel design with 3 treatments: 4 capsules providing 800 mg/d of hesperidin, 4 capsules providing 500 mg/d of naringin, or 4 placebo capsules containing microcrystalline cellulose. The daily doses of hesperidin (800 mg) and naringin (400 mg) used in this study were the 95th percentile of intake based on national dietary intake surveys. The daily doses of hesperidin (800 mg) and naringin (400 mg) used in this study were the estimated 95th percentile of intake based on national dietary intake surveys. In 2 other studies, 100–300 mg/d glucosyl-hesperidin in tablets had either no effect on LDL-C (26) or lowered it significantly only in hypertriglyceridemic individuals and after 8 wk of consumption (27). However, all these human studies were either not placebo controlled (22,24–27), not blinded (19–21), not randomized (23,24), or used a “before and after” design (22). Consequently, it was not possible to firmly conclude that hesperidin or naringin do lower plasma cholesterol concentrations in humans.

Therefore, the present study aimed at testing, in a randomized, placebo-controlled, adequately powered, proof-of-principle study whether hesperidin and naringin exert a cholesterol-lowering effect in moderately hypercholesterolemic individuals. We used high doses of hesperidin and naringin to optimize the chances of detecting a significant cholesterol-lowering effect. For standardizing the daily intake and avoiding possible interactions with a food matrix, the participants consumed pure hesperidin, naringin, or cellulose (as placebo) in a capsule format.
0.4 mmol/L for duplicate measurements based on previous clinical trials from our group) with each of the active treatments (hesperidin and naringin) compared with the placebo using a 1-sided test, 61 participants/group were needed to complete the study. Taking a 13% dropout rate into account, 210 participants were recruited to take part in the study as well as 6 reserve volunteers to replace possible dropouts before the start of the intervention period.

Results are expressed as means and SD or SEM where appropriate and P < 0.05 was considered significant. The statistical analysis was performed for both the per protocol (PP) population (excluding the noncompliant participants) (n = 188) and the intention to treat population (including all randomized participants for whom the end of intervention data for the primary outcome variable were available, n = 194). Because this study was a proof-of-principle study, results obtained from the PP population are considered as the main outcome and are presented here. Overall, the results of the intention to treat analysis were similar to those of the PP analysis.

Differences in group means of continuous baseline participant characteristics (age, weight, BMI, and serum lipids) were tested using an ANOVA and a χ² analysis. Differences in group means of serum lipid concentrations were determined by an ANCOVA using a model including cohort, gender, treatment, respective baseline lipid variable, age, and BMI change as covariables. TG concentrations were log transformed, because their distribution was slightly skewed. All statistical analyses were performed using the SAS software (SAS Institute, version 9.1).

Results

Participants. Based on the screening results, 249 volunteers fulfilled all inclusion and exclusion criteria. Following exclusions by lot, the exclusion of 2 wrongly included participants as well as dropouts, a total of 204 volunteers and 1 reserve volunteer (107 men, 98 women) started the study. During the study, 8 participants dropped out because of medical reasons and 2 participants dropped out because of personal reasons (holiday plans, n = 1, and work appointments interfering with study visit days, n = 1) (Supplemental Fig. 1). A total of 194 participants (102 men and 92 women) completed the study. We excluded 5 participants from the PP analysis following the blind review of the data: 3 participants gained >2 kg body weight during the intervention period and 2 participants reported insufficient compliance to background diet (n = 1) or test product intake (n = 1) (Supplemental Fig. 1). In addition, we excluded 1 participant from the PP analysis, because he was an outlier for baseline TG concentrations, resulting in an interaction on serum TG between treatment and baseline TG, which disappeared after exclusion of this subject.

The proportion of males and females was fairly similar and did not significantly differ among the 3 groups (Table 1). The groups did not differ in baseline age, weight, BMI, blood lipid concentration, or the TC:HDL-C ratio. Body weight ranged from normal to overweight in all groups. Mean baseline TC and LDL-C concentrations in the 3 groups were in the high range of borderline high concentrations (ranging from optimal to very high) and TG were normal (with a range from normal to high) according to the National Cholesterol Education Program classification (33). Mean changes in body weight (0.09 ± 0.09 kg, 0.19 ± 0.10 kg, and 0.20 ± 0.09 kg for the control, hesperidin, and naringin groups, respectively) and BMI (0.02 ± 0.03 kg/m², 0.07 ± 0.03 kg/m², and 0.07 ± 0.03 kg/m² for the control, hesperidin, and naringin groups, respectively) over the intervention period were small and did not differ among the groups (P = 0.629 and 0.554, respectively).

Adverse events. Eight participants dropped out for medical reasons: antibiotic use (n = 1, during preintervention), myocardial infarction (n = 1, during preintervention), eye surgery (n = 1), medicine use (n = 2; 1 participant started taking antibiotics for a urinary tract infection during preintervention and 1 because of previously undisclosed use of medication during preintervention), dermatitis (n = 1, d 2, placebo group), unspecified gastroduodenitis (n = 1, d 3 after start of hesperidin treatment), or unspecified psychosis (n = 1, preexisting, not reported at screening).

Other spontaneous reports of adverse events were nontreatment-related complaints of mild background diseases (e.g. headache, common colds, and dental problems). Following the report of an unspecified gastroenteritis after 3 d of treatment, we included elicited reporting of gastrointestinal complaints in the health and lifestyle questionnaire. For none of the adverse events was there a trend toward differences among treatment groups.

Compliance. Compliance with the treatment was high. In all groups, the mean percentage of test product intake was >99% (99.8 ± 1.1%, 99.6 ± 0.9%, and 99.4 ± 1.7%, for placebo, hesperidin, and naringin, respectively). We excluded from the PP analysis 1 participant who did not consume any test product during the last 4 d of the intervention period. Except for 1 participant who reported major changes in diet and physical activities during the last 2 wk of the preintervention period, which we excluded from the PP analysis, there were no major deviations in compliance with background diet and physical activity.

Serum lipids. Hesperidin and naringin did not affect baseline-adjusted, end-of-intervention serum concentrations of TC, LDL-C, HDL-C, and TG compared with the placebo treatment (Table 2). They also did not affect the TC:HDL-C ratio. In all groups, the nonadjusted end-of-intervention serum TC, LDL-C, HDL-C, and TG concentrations remained close to the baseline concentrations (Supplemental Table 2).

Discussion

Previous human studies had design limitations and controversial outcomes concerning the potential blood lipid-lowering effect of hesperidin and naringin (19–26). The present randomized placebo-controlled study showed that neither hesperidin nor

### TABLE 1
Baseline anthropometric characteristics and serum lipid concentrations of the participants included in the PP analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Hesperidin</th>
<th>Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>65</td>
<td>59</td>
<td>64</td>
</tr>
<tr>
<td>Age, y</td>
<td>60.1 ± 8.2</td>
<td>61.0 ± 8.6</td>
<td>59.8 ± 8.8</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>53.8</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.0 ± 12.3</td>
<td>74.4 ± 9.5</td>
<td>75.5 ± 9.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 2.3</td>
<td>25.1 ± 2.1</td>
<td>25.6 ± 2.0</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>6.18 ± 0.85</td>
<td>6.18 ± 0.83</td>
<td>6.11 ± 0.88</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.97 ± 0.71</td>
<td>3.98 ± 0.77</td>
<td>3.98 ± 0.78</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.51 ± 0.45</td>
<td>1.52 ± 0.40</td>
<td>1.50 ± 0.39</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.38 ± 1.20</td>
<td>4.34 ± 1.33</td>
<td>4.31 ± 1.13</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.44 ± 0.60</td>
<td>1.31 ± 0.54</td>
<td>1.27 ± 0.50</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 P-values obtained from an ANOVA for continuous variables (age, weight, BMI, serum lipids) and a χ² analysis for categorical variables (gender).
3 One outlier that was responsible for a baseline × treatment interaction was excluded from analysis.
TABLE 2  Baseline-adjusted serum lipid concentrations in moderately hypercholesterolemic men and women after consuming capsules containing placebo, hesperidin (800 mg/d), or naringin (500 mg/d) for 4 wk.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hesperidin</th>
<th>Naringin</th>
<th>P-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>65</td>
<td>59</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>6.22 ± 0.05</td>
<td>6.19 ± 0.05</td>
<td>6.19 ± 0.05</td>
<td>0.864</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.00 ± 0.04</td>
<td>3.99 ± 0.04</td>
<td>3.99 ± 0.04</td>
<td>0.987</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.54 ± 0.02</td>
<td>1.53 ± 0.02</td>
<td>1.51 ± 0.02</td>
<td>0.375</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.31 ± 0.05</td>
<td>4.29 ± 0.05</td>
<td>4.37 ± 0.05</td>
<td>0.516</td>
</tr>
<tr>
<td>TG(^3), mmol/L</td>
<td>1.26 ± 0.03</td>
<td>1.24 ± 0.04</td>
<td>1.28 ± 0.04</td>
<td>0.717</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (PP population).
2 In addition with the baseline value, cohort, gender, BMI change, and age were included in the model as covariates.
3 The statistical analysis was performed on log-transformed TG values; the TG concentrations presented here are back-transformed from the log values.

Hesperidin consumed in capsule format exerted a TC- or LDL-C-lowering effect in moderately hypercholesterolemic individuals. The doses of 800 mg of hesperidin and 500 mg of naringin used in the present study corresponded to the 95th percentile of daily consumption in Western populations (31,32). The rationale for using such high doses was to minimize the chance of not detecting an LDL-C-lowering effect. Doses comparable (25) or lower (19–21,27) than used in the present study were reported to significantly lower LDL-C by ~7 to ~21%. Therefore, the absence of an LDL-C-lowering effect of hesperidin or naringin cannot be attributed to the dose used in this study.

Hesperidin and naringin/naringenin significantly reduced LDL-C (3,5–7) and affected key steps of cholesterol metabolism (5–7,12) already after 3–4 wk in previous studies in animal models. A duration of 3–4 wk is usually needed to establish a new steady state in cholesterol metabolism and stabilization of plasma cholesterol concentrations after dietary interventions such as changes in dietary fats (34) or plant sterol-enriched food consumption (35) in humans. Moreover, some of the studies in which significant LDL-C reductions were observed had treatment durations of 4 wk (19–21). Therefore, the 4-wk duration of the present study is judged as sufficient to observe, if any, a full LDL-C-lowering effect.

Jung et al. (25) reported a significant LDL-C-lowering effect with the consumption of 400 mg/d naringin in volunteers with a mean baseline LDL-C concentration of 3.45 mmol/L but a nonsignificant decrease in volunteers with a lower mean baseline LDL-C concentration (4.09 mmol/L). It could thus be hypothesized that the mean baseline LDL-C concentration of ~4 mmol/L in the present study may have contributed to the absence of effect on LDL-C. However, 500 mg of glucosyl-hesperidin also had no LDL-C-lowering effect in another study where participants had a higher mean baseline LDL-C concentration (5.6 mmol/L) (26). In addition, to take into account the large range of individual baseline LDL-C concentrations (from optimal to very high) in this study, we included baseline LDL-C concentrations as covariates in the statistical analysis, which results in a lower variability in the estimate of the treatment effect. Therefore, the absence of a significant cholesterol-lowering effect of hesperidin and naringin observed in the present study cannot be ascribed to particularly low baseline LDL-C concentrations. Furthermore, it should be noted that both nonadjusted and adjusted end-of-intervention serum lipids remained close to baseline concentrations, indicating that there was not even a tendency for a cholesterol-lowering effect.

Given the reported high compliance in this study and the fact that compliance with the food and/or capsule consumption requirements was high in previous nutrition intervention trials performed by our group, it is unlikely that the lack of efficacy in the present study was related to a lack of compliance with the prescribed dose requirement. In addition, although detailed dietary intake data were not recorded, the fairly good compliance with the dietary and lifestyle instructions is reflected by the fact that body weights were stable (±0.2 kg change) in each of the groups and that serum lipid concentrations in the control group remained unchanged during the intervention period (see values for the control group in Table 1 and Supplemental Table 2). Moreover, to ensure that the (absence of) impact of hesperidin or naringin on TC and LDL-C concentrations was not confounded by a potential impact of the test products on body weight itself (36), we included changes in BMI in the statistical analysis as covariates.

We used pure hesperidin and naringin in a capsule format to standardize the dose and avoid interactions with food matrices. The participants consumed the capsules at mealtime, because capsules are likely to be consumed at this occasion in a real-life setting. Studies in humans have shown that hesperidin (37) and naringin (38) are poorly absorbed as intact molecules. Colonic bacteria presumably cleave the sugar moiety of hesperidin and naringin in the lower intestinal tract, generating the absorbable aglycone forms, i.e., hesperetin and naringenin (2). We chose glycosides for this study, because this is the main form in which hesperidin and naringin are present in citrus fruits, and history of consumption was available from published studies to determine the doses that could be used without safety concerns. The measurement of hesperidin and naringin content in the ingredients used to prepare the capsules as well as in the capsules themselves confirmed that they were present in the expected dosage and in the same chemical form as found in citrus fruits and their juices. The possibility of a denaturation of hesperidin and naringin during extraction resulting in the formation of other compounds that would not exert the same biological activity as hesperidin and naringin can therefore be excluded. Moreover, it is unlikely that the meal components, which are digested and absorbed mostly in the upper gastrointestinal tract, may affect the absorption of hesperidin and naringin in the colon. A recent study showed that the concomitant ingestion of food did not affect the absorption of citrus flavonoids from orange juice (39). These data further support that consumption with a meal should not explain the lack of a cholesterol-lowering effect of hesperidin or naringin capsules in the present study.

A limitation of this study is that we did not assess the bioavailability of hesperidin and naringin. Although urine flavonoid concentrations have been suggested to be unsuitable biomarkers of dietary intake (40), repeated postconception measurements of hesperidin/naringin and their metabolites in plasma would have allowed confirming the high compliance reported by the participants and helped in estimating the concentrations achieved in the blood. Nevertheless, data from previous bioavailability studies provide insight into the kinetics of absorption of these citrus flavonoids that can be used to analyze the present outcome. Peak plasma concentrations of hesperetin and naringenin, the metabolites of hesperidin and naringin, respectively, are observed soon after ingestion (4–7 h) (37,40,41). Because the putative mechanisms of action of hesperidin and naringin for a cholesterol-lowering effect were suggested to take place in the liver, the capsules were consumed 2 times/d in the present study to ensure a more regular presence of these flavonoids in the blood circulation. Thus, frequency of
intake should have maximized the chances of observing a potential cholesterol-lowering effect of hesperidin and naringin. On the other hand, the bioavailability of hesperidin and naringin is limited, likely due to their conversion in large proportions into insoluble compounds (chalcones) by the colon flora (42). Peak plasma concentrations of hesperetin after the consumption of orange juice or hesperetin capsules providing 125–135 mg hesperetin equivalent were in the range of 2.2–2.7 μmol/L (40,41), and the ingestion of naringenin from capsules or naringin from grapefruit juice (135–199 mg naringenin equivalent) lead to peak plasma concentrations of 6.0–7.3 μmol/L (40,41). It is possible that such circulating concentrations may not be sufficient to affect liver enzyme activities and gene expression/transcription. Indeed, after consumption of ~440 mg of hesperidin (37), a dose comparable to that used in this study, peak plasma concentrations of the aglycone hesperetin (1.28 μmol/L) were markedly lower than the concentrations of hesperetin (200 μmol/L) shown to affect LDL-receptor transcription in HepG2 liver cells (18). Another hypothesis for explaining the lack of cholesterol-lowering effect of hesperidin and naringin in the present study could be that homeostatic responses would partially or completely compensate the cholesterol-lowering effects of hesperidin and naringin. However, the effects reported previously on key steps of cholesterol metabolism (3–7,9,10,12–18) as well as on weight balance (36) are either in line with reductions in blood lipids or at least do not provide evidence for compensation processes.

Nonplacebo controlled studies in hypercholesterolemic healthy volunteers (25) and coronary by-pass patients (19–21), as well as in hypertriglyceridermic participants (26,27) showed significant reductions in plasma TG with the consumption of hesperidin/naringin in capsules/tablets (25,19–21) or Sweetie fruits/juice (26,27). These data suggested that hesperidin and naringin could also have plasma TG-lowering properties. Nonplacebo controlled studies (26,27) also suggested that hesperidin could exert some lipid-lowering effects in volunteers with higher baseline TG concentrations. However, the present placebo-controlled study did not show any TG-lowering effect of hesperidin or naringin in participants with a mean baseline TG concentration ≤ 1.4 mmol/L. Similarly, the present study found no effect on HDL-C concentrations, whereas increases in this variable were reported previously with orange juice (22) and Sweetie fruit/ juice (20,21) in nonplacebo-controlled studies.

In conclusion, the present study showed that an intake of 500 mg/d naringin or 800 mg/d hesperidin administered twice daily with main meals did not lower serum TC and LDL-C in moderately hypercholesterolemic individuals. This outcome suggests that the citrus flavonoids hesperidin and naringin exert no cholesterol-lowering effect in humans, at least not when consumed in capsule format.

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