

Changes in Plasma Lipid and Antioxidant Activity in Rats as a Result of Naringin and Red Grapefruit Supplementation

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The aim of this investigation was to compare the influence of naringin versus red grapefruit juice on plasma lipid levels and plasma antioxidant activity in rats fed cholesterol-containing and cholesterol-free diets. The antioxidant activity of a correlated quantity of red grapefruit juice was higher than that of naringin. Forty-two male Wistar rats were randomly divided into six groups of 7 named control, naringin, grapefruit, Chol, Chol/naringin, and Chol/grapefruit. The rats of the control group were fed basal diet (BD) and 1–2 mL of distilled water. To the BD of the other five groups were added 0.46–0.92 mg of naringin dissolved in 1–2 mL of distilled water (naringin), 1–2 mL of red grapefruit juice (grapefruit), 1% of nonoxidized cholesterol (NOC) and 1–2 mL of distilled water (Chol), 1% of NOC and 0.46–0.92 mg of naringin in 1–2 mL of water (Chol/naringin), and 1% of NOC and 1–2 mL of red grapefruit juice (Chol/grapefruit). After 30 days of different feeding, it was found that diets supplemented with red grapefruit juice and to a lesser degree with naringin improved the plasma lipid levels mainly in rats fed cholesterol and increased the plasma antioxidant activity. In conclusion, naringin is a powerful plasma lipid lowering and plasma antioxidant activity increasing flavonone. However, fresh red grapefruit is preferable than naringin: it more effectively influences plasma lipid levels and plasma antioxidant activity and, therefore, could be used as a valuable supplement for disease-preventing diets.

KEYWORDS: Naringin; red grapefruit; plasma lipids; antioxidant activity; rats

INTRODUCTION

The beneficial health effects of diets supplemented with different fruits and vegetables have enhanced the interest in their bioactive compounds (1–3). It was shown that the positive influence of these natural products is mostly connected with their antioxidants and certain parts of dietary fiber (4). Citrus fruits have a high content of these substances and, as a consequence, a high antioxidant capacity (5). Naringin is one of the flavonones and is a permanent component of grapefruit juice (6). Ross et al. (6) have analyzed nine commercial brands

of grapefruit juice for their flavonoid content using HPLC methods. Naringin has been identified in all grapefruit juices. Others received similar results (7). Some authors claim that naringin can alter cholesterol metabolism and antioxidant status when rats are fed a diet high in cholesterol (8). To assess these properties of naringin, it was decided to compare the well-known plasma lipid-lowering and antioxidant effects of red grapefruit juice with correspondent quantities of this flavonone in rats fed cholesterol-containing and cholesterol-free diets (9, 10).

As far as we know, there are not such comparative investigations that also include experiments on laboratory animals.

MATERIALS AND METHODS

Chemicals. All reagents were of analytical grade. Deionized and distilled water was used throughout. All used chemicals, naringin, Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid), and

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2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co., St. Louis, MO.

Samples and Their Preparation. Israeli Jaffa red Star Ruby (Sunrise) grapefruits (*Citrus paradisi*) of the same maturity degree harvested in 2003 were purchased in the Sharon region from a farmer. The grapefruit juice (GJ) was prepared manually and prevented from oxidizing (11).

Determination of Bioactive Compounds. Dietary fiber, minerals, some essential trace elements, total polyphenols, flavonoids, anthocyanins, and naringin in GJ were determined as previously described (11–13).

Determination of Total Antioxidant Potential. There are many methods for total antioxidant determination, and each has its limitation (14). Some of the antioxidant assays give different antioxidant activity trends (15). Therefore, in this experiment the antioxidant potential of GJ and naringin was determined according to two methods using ABTS^{•+} with K₂S₂O₈ and with MnO₂.

(A) ABTS^{•+} radical cation was generated by the interaction of ABTS (250 μM) and K₂S₂O₈ (40 μM). After the addition of 990 μL of ABTS^{•+} solution to 10 μL of Trolox standards (final concentration = 0–20 μM) in phosphate-buffered saline (PBS), the absorbance was monitored at exactly 1 and 6 min after the initial mixing (16).

(B) ABTS^{•+} was prepared as well by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide on a Whatman no. 5 filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μM Whatman PVDF syringe filter. This solution was then diluted in a 5 mM PBS, pH 7.4, to an absorbance of 0.70. The percentage decrease of the absorbance at 734 nm in (A) and (B) was calculated and plotted as a function of the concentration of the samples and of Trolox for the standard reference data (16).

These methods were applied for the determination of naringin and grapefruit juice and also for assessment of the plasma antioxidant activities.

Rats and Diets. The Animal Care Committee of the Warsaw Agricultural University approved this study. Wistar male rats (*n* = 42) with a mean weight of 115 g at the beginning of the study were provided by the Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland).

In the present as in our previous experiments the same successful proven protocol was used (11, 13).

The rats were housed in plastic metabolic cages and were randomly divided into six groups of seven. These groups were named control, naringin, grapefruit, Chol, Chol/naringin, and Chol/grapefruit. During the 30 days of the experiment, the rats of all six groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, and vitamin and mineral mixtures (11). The rats of the control group were fed basal diet (BD) and 1–2 mL of distilled water. To the BD of the five other groups were added 0.46–0.92 mg of naringin dissolved in 1–2 mL of distilled water (naringin); 1–2 mL of red grapefruit juice (grapefruit), 1% of nonoxidized cholesterol (NOC), and 1–2 mL of distilled water (Chol); 1% of NOC, 0.46–0.92 mg of naringin dissolved in 1–2 mL of distilled water (Chol/naringin); and 1% of NOC and 1–2 mL of red grapefruit juice (Chol/grapefruit). The dietary cholesterol was determined by high-performance liquid chromatography (HPLC) and was found not to contain cholesterol oxides. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to rats. These diets contained as percentages of energy 68% carbohydrates, 23% protein, and 9% fat. The calculated energy of the used diets was from 395.3 to 400.3 kcal/100 g, and the difference was not significant.

All rats were fed once a day at 10:00 a.m. ad libitum. They had unrestricted access to drinking water. Naringin dissolved in a corresponding amount of distilled water and GJ were induced by intubation into the stomach. To get the rats used to the maximal quantity of juice or water, in the first 2 weeks every animal received naringin in only 1 mL of distilled water or 1 mL of GJ per day; in the third week, 1.5 mL; and in the last week of the trial, 2 mL per day (13). The food intake and body weight gains were monitored daily. The amount of naringin used was increased, and in the last week of the experiment 0.92 mg of naringin was dissolved in 2 mL of distilled water.

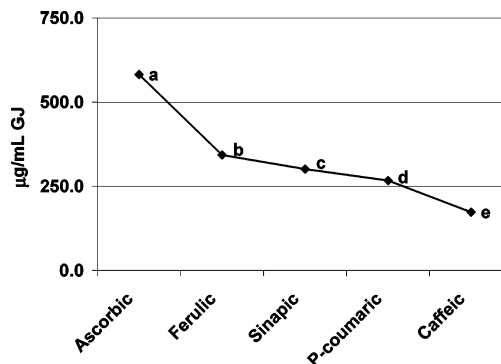


Figure 1. Contents of phenolic and ascorbic acids in red grapefruit juice (means of five measurements). Values with different letters are significantly different ($P < 0.05$).

It is generally accepted that the most reliable data of the blood lipid metabolism can be obtained from fasting animals, 14–16 h after the last feeding. Therefore, the food was removed from the cages at 6 p.m. the day before, and the samples were collected at 9:00 a.m. the next day. The plasma was prepared and used for laboratory tests. Under general urethane narcosis (1.8 g of urethane/1 kg of animal body weight), the abdomen was opened to take samples of bile-pancreatic juice (17).

Two time points were used in this experiment: before and after 30 days of different feeding. At these time points, a wide range of laboratory tests was performed. The plasma TC, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined as previously described in detail without using coefficients of correlation (17).

The collection and determination of bile flow, bile cholesterol, and bile acids were done as previously described (17).

Statistical Analysis. To verify the statistical significance of the studied parameters, means \pm SD of five times analyzed samples were defined. When appropriate, differences between groups were tested by two-way ANOVA. The P values of <0.05 were considered to be significant.

RESULTS

In Vitro. The contents of total, insoluble, and soluble dietary fibers in GJ were 2.87, 2.11, and 0.76%, respectively.

The contents of total polyphenols, anthocyanins, and flavonoids were 967.2 ± 71.1 , 70.4 ± 5.2 , and 50.9 ± 4.3 g/mL of fresh GJ, respectively.

The contents of dietary fibers, total polyphenols, anthocyanins, and flavonoids in the present used GJ were comparable with the contents of these bioactive compounds in the GJ of the 2002 harvest (11).

The results of the determination of the contents of phenolic and ascorbic acids are summarized in **Figure 1**. As can be seen, the content of ferulic acid was significantly higher than those of sinapic, *p*-coumaric, and caffeic acids. The content of ascorbic acid was significantly higher than that of every phenolic acid ($P < 0.05$).

The results of the determination of the contents of the studied trace elements are summarized in **Figure 2**. As can be seen, the content of Fe was the highest and of Mn, the lowest ($P < 0.05$).

The content of naringin was 0.46 mg/mL of fresh GJ. The antioxidant activity of naringin and GJ was estimated by ABTS scavenging radical using MnO₂ and K₂S₂O₈ (18).

The antioxidant activity of naringin (mM TE/L) was 0.21 ± 0.02 and 0.26 ± 0.02 with K₂S₂O₈ and MnO₂, respectively. The antioxidant activity of GJ (mM TE/L) was 4.75 ± 0.4 and 5.17 ± 0.5 with K₂S₂O₈ and MnO₂, respectively.

Table 1. Weight Gains, Food Consumption, and Feed Efficiency Ratio in All Six Groups^a

| group | wt gain (g/day) | food consumption (g/day) | consumption by intubation (mL/day) | feed efficiency ratio |
|-----------------|-----------------|--------------------------|------------------------------------|-----------------------|
| control | 4.38 ± 0.65a | 15.92 ± 1.84a | water, 1–2 mL | 0.274 ± 0.014a |
| Chol | 3.88 ± 0.66a | 15.38 ± 1.67a | water, 1–2 mL | 0.251 ± 0.024a |
| naringin | 3.82 ± 1.02a | 15.97 ± 1.19a | naringin in water, 1–2 mL | 0.236 ± 0.046a |
| grapefruit | 3.75 ± 0.53a | 15.03 ± 1.34a | grapefruit juice, 1–2 mL | 0.248 ± 0.017a |
| Chol/naringin | 3.91 ± 1.04a | 15.81 ± 1.12a | naringin in water, 1–2 mL | 0.244 ± 0.056a |
| Chol/grapefruit | 4.71 ± 0.86b | 16.58 ± 1.28b | grapefruit juice, 1–2 mL | 0.282 ± 0.033b |

^a Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($P < 0.05$).

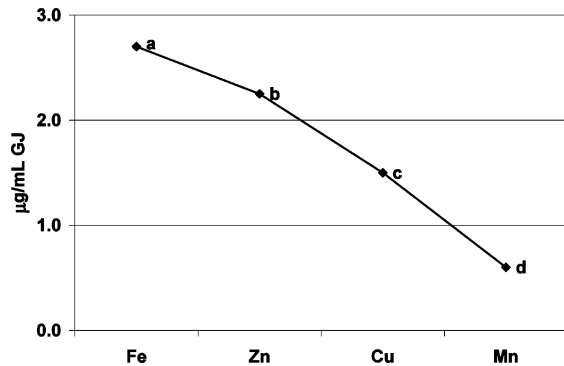


Figure 2. Contents of essential trace elements in red grapefruit juice (means of five measurements). Values with different letters are significantly different ($P < 0.05$).

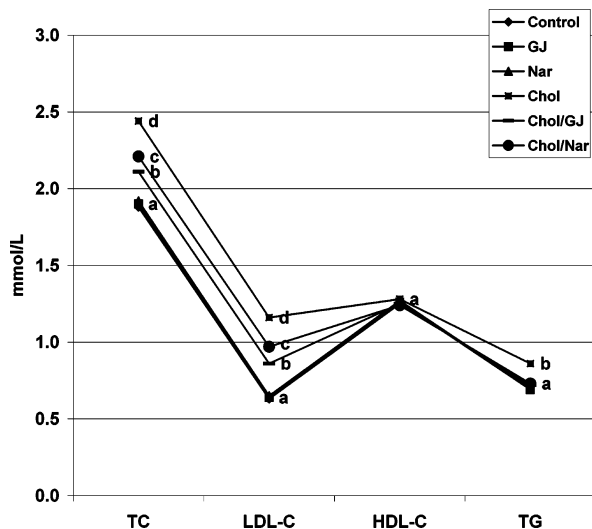


Figure 3. Changes in the plasma lipid levels after completion of the experiment ($n = 7$). Values with different letters are significantly different ($P < 0.05$).

In Vivo. The addition of GJ, naringin, and/or cholesterol to the diets did not lead to significant differences in food consumption, body weight gains, and feed efficiencies ratio in all five experimental groups. However, all three variables in rats of the Chol/GJ group were significantly higher ($P < 0.05$) than in the other five groups (Table 1).

At baseline, the six diet groups did not differ from one another in plasma lipid concentrations (data not shown). The results of the changes after the experiment are summarized in Figure 3. As can be seen, the GJ- and, to a lesser degree, naringin-supplemented diets in groups fed cholesterol significantly hindered the rise of TC, LDL-C, and TG. The supplementation of GJ and naringin did not influence the level of HDL-C.

Changes in the pancreatic bile flow are shown in Figure 4. It was found that the addition of grapefruit juice and naringin

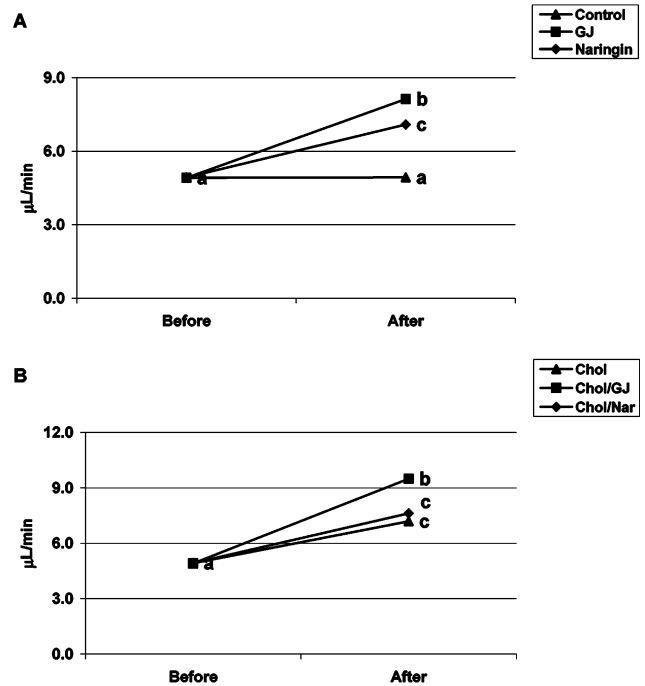


Figure 4. Changes in the pancreatic bile flow ($n = 7$). Addition of GJ and naringin increased significantly the flow (A). The same index was increased significantly ($P < 0.05$) in all three groups fed cholesterol-containing diets (B). Values with different letters are significantly different ($P < 0.05$).

to diets of rats fed cholesterol-free diets (Figure 4A) increases significantly the pancreatic bile flow in both GJ and naringin groups ($P < 0.05$).

The same index was increased significantly ($P < 0.05$) in all three groups fed cholesterol-containing diets (Figure 4B).

Changes in the bile cholesterol concentration are shown in Figure 5. As can be seen, that addition of grapefruit juice and naringin to diets of rats fed cholesterol-containing diets increases significantly the bile cholesterol concentration ($P < 0.05$). Also, the increase in the bile cholesterol concentration in the rats of the Chol group was found, but in the Chol/GJ group it was significantly higher ($P < 0.05$).

Changes in the bile acids concentration are shown in Figure 6. As can be seen, a significant increase was registered only in the Chol/GJ and Chol/Nar groups ($P < 0.05$).

At the end of the trial, an increase in the plasma antioxidant capacity in both GJ and naringin dietary groups was found (Figure 7) as shown by a significant increase in the TEAC values. However, the increase was significant ($P < 0.05$) only in the rats fed a diet supplemented with grapefruit juice (GJ group).

A decrease in the plasma antioxidant capacity after completion of the trial was registered in all groups of rats fed cholesterol

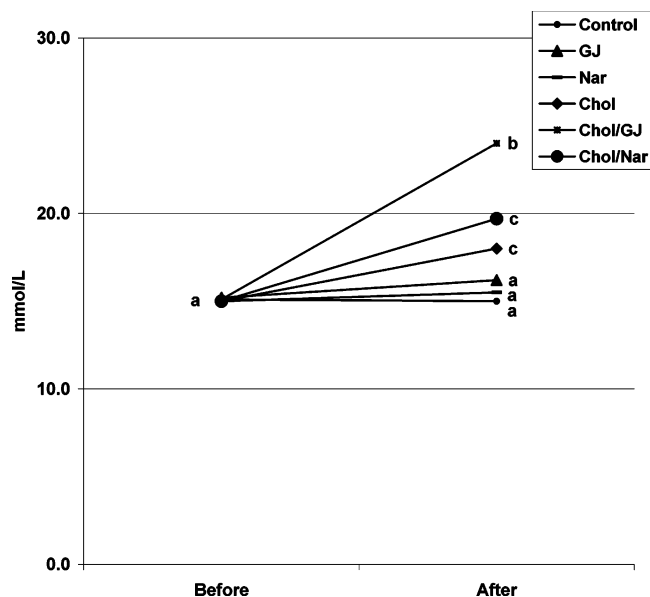


Figure 5. Changes in the bile cholesterol concentration ($n = 7$). Addition of grapefruit juice and naringin to diets of rats fed cholesterol-containing diets increased significantly ($P < 0.05$) the bile cholesterol concentration in the rats of the Chol group. However, the increase in the Chol/GJ group was significantly higher ($P < 0.05$). Values with different letters are significantly different ($P < 0.05$).

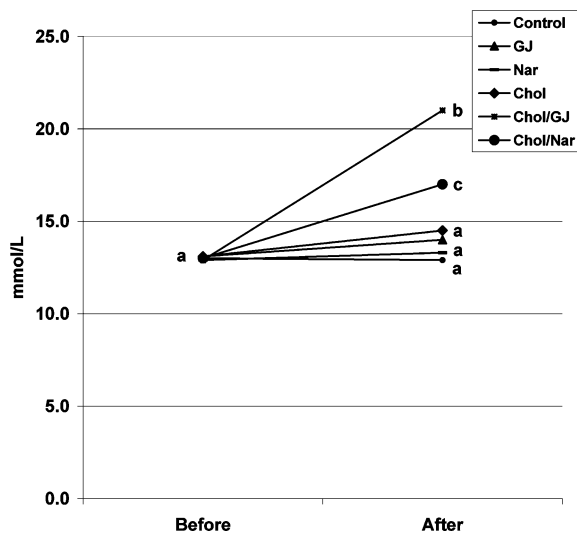


Figure 6. Changes in the bile acids concentration ($n = 7$). A significant increase ($P < 0.05$) was found only in the Chol/GJ and Chol/Nar groups. Values with different letters are significantly different ($P < 0.05$).

(**Figure 8**). However, this decrease was significant ($P < 0.05$) only in the rats of the Chol diet group (**Figure 8**).

No significant changes were observed in all studied parameters in the rats of the control group.

DISCUSSION

Naringin is one of the most powerful flavonones, which is a permanent component of grapefruit juices (6, 7). According to these authors, naringin can be identified in all grapefruit juices (6, 7). It was shown that naringin and aglycon naringenin (19) are responsible for citrus fruits' antioxidative activity (20). Some authors claim that naringin supplementation can alter cholesterol metabolism and antioxidant status when rats are fed added cholesterol (8). To assess the possible bioactivity of naringin, it was decided to compare the well-known plasma lipid-lowering

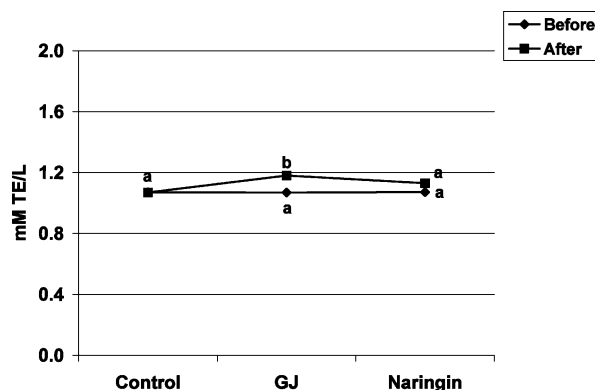


Figure 7. Increase in plasma antioxidant activity in GJ and naringin diet groups ($n = 7$). However, the increase was significant only in rats of the GJ diet group. Values with different letters are significantly different ($P < 0.05$).

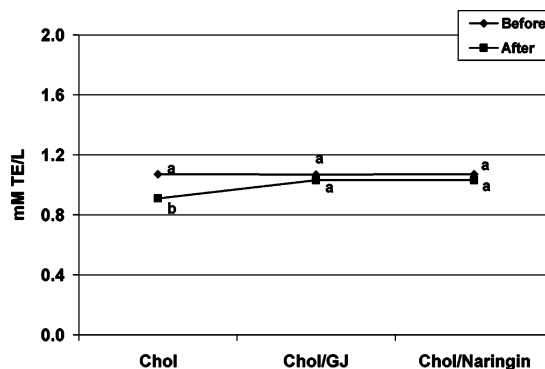


Figure 8. Decrease in plasma antioxidant activity in all groups of rats fed cholesterol after completion of the feeding period ($n = 7$). However, the decrease was significant only in rats of the Chol diet group. Values with different letters are significantly different ($P < 0.05$).

and antioxidant activity of red grapefruit juice with correspondent quantities of this flavonone.

It was found that the contents of the main bioactive compounds in the used fresh red grapefruit juice (diet fibers, trace elements, total polyphenols, phenolic and ascorbic acids, and others) were comparable with the data of others and the results of our previous investigations (9–11).

As was expected, the antioxidant potential of the used fresh red grapefruit juice was higher than that of the corresponding quantity of naringin. This is connected to the additional bioactive compounds in the fresh red grapefruit juice and to the biochemical links between them (12).

There were no significant differences in the food intake, weight gain, and feed efficiency ratio among the five diet groups. Only in the Chol/GJ group were these indices higher. These results correspond with others (8).

After completion of the feeding period, a plasma lipid-lowering effect only in groups of rats fed cholesterol-added diets supplemented with either grapefruit juice or naringin was registered. Also, these data are in accordance with the data of others (10, 21).

The liver plays a major role in the synthesis and net excretion of cholesterol, either directly as free cholesterol in the bile or after conversion into bile acid (21). It was reported that supplementation of citrus bioflavonoid mixture or naringin lowers plasma cholesterol concentration and fecal neutral sterol in high-cholesterol-fed rats (22). Therefore, it was interesting to know how both fresh grapefruit juices and the corresponding quantity of naringin dissolved in distilled water affect the bile

pancreatic flow and the biliary bile cholesterol and biliary bile acids concentrations. It was found that fresh grapefruit juice and to a lesser degree naringin dissolved in distilled water increased all three of the above-mentioned indices, mainly in groups of rats fed cholesterol. These data indicate that the plasma lipid-lowering effect of grapefruit juice and naringin is genuine, and the mechanism is through the increase in the bile flow and the biliary bile cholesterol and biliary bile acids concentrations.

As was mentioned, grapefruit juice and naringin did not affect the lipid levels in rats fed diets without cholesterol. These results were expected: it was already demonstrated by other authors and in our previous experiments on laboratory animals and in investigations of patients that the hypolipidemic effect of fruits and vegetables is evident only in animals fed cholesterol and in hypercholesterolemic patients (11, 23).

A significant increase in the plasma antioxidant capacity was found in the grapefruit dietary group and to a lesser degree in the naringin group. However, in groups fed added cholesterol, a decrease in the plasma antioxidant capacity was registered. The decrease in groups whose diets were enriched with either grapefruit juice or naringin (Chol/GJ and Chol/naringin) was significantly less than in the Chol group. Therefore, this investigation demonstrates that the addition of grapefruit juice or naringin hinders the decrease in the plasma antioxidant capacity. Also, other authors have shown that a cholesterol-supplemented diet decreases the plasma antioxidant capacity (24, 25).

In conclusion, (i) naringin is a powerful plasma lipid lowering and plasma antioxidant capacity increasing flavonone and (ii) fresh red grapefruit juice is preferable to naringin: it positively influences plasma lipid levels and plasma antioxidant capacity and could be used as a valuable supplement for disease-preventing diets.

ABBREVIATIONS USED

ABTS, 2, 2-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation; Chol, nonoxidized cholesterol; GJ, grapefruit juice; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Nar, naringin; NOC, nonoxidized cholesterol; TC, total cholesterol; TE, Trolox equivalent; TEAC, Trolox equivalent antioxidant coefficient; TG, triglycerides; TPH, total phospholipids.

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