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Preventive effects of diets supplemented with sweetie fruits in hypercholesterolemic patients suffering from coronary artery disease

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Abstract

Background. Diets rich in vegetables and fruits prevent development of atherosclerosis.

Objective. To investigate the preventive effects of diets supplemented with a new kind of citrus fruit—pummelo–grapefruit hybrid in hypercholesterolemic patients suffering from coronary artery disease (CAD).

Subjects and methods. Sixty-six hypercholesterolemic volunteers after coronary bypass surgery ages 47–68 years were randomly divided into two experimental (EG1 and EG2) groups and one control (CG) group, 22 each. The diets of the patients of the experimental groups (EG1 and EG2) were supplemented with one or two peeled sweeties, respectively. A comprehensive clinical investigation of all 66 patients was done. Blood samples were collected before and after the investigation for a wide range of laboratory tests.

Results. A high content of dietary fibers and antioxidant compounds in peeled sweeties was found. After 30 days of the investigation, peeled sweeties-supplemented diets have decreased plasma lipids levels in EG1 and EG2 vs. CG group: (a) total cholesterol (TC)—7.38 vs. 8.08 mmol/L, − 8.7%, and 6.78 vs. 8.08. mmol/L, −16.1%, respectively; (b) low-density lipoprotein cholesterol (LDL-C)—5.65 vs. 6.39 mmol/L, − 1.6%, and 5.04 vs. 6.39 mmol/L, −21.2%, respectively; (c) triglycerides (TG)—2.01 vs. 2.27 mmol/L, −11.5%, and 1.71 vs. 2.27 mmol/L, −24.7%, respectively. In addition, a significant increase in the plasma antioxidant capacity in EG2, and to a lesser degree in EG1 groups, was observed. No changes in the studied indices in the patients of the CG were detected.

Conclusion. Peeled sweeties have high contents of dietary fibers and antioxidant compounds. Diets supplemented with peeled sweeties positively influence plasma lipid metabolism and plasma antioxidant capacity in patients suffering from hypercholesterolemia. Therefore, the addition of peeled sweeties to a generally accepted antiatherosclerotic diet may be beneficial in prevention of atherosclerosis, mainly in hypercholesterolemic patients.

Keywords: CAD prevention; Citrus fruits; Diets; Hypercholesterolemia; Plasma antioxidant activity

Introduction

Coronary artery disease (CAD) is one of the most dangerous diseases in humans—the principal cause of death in Western civilization [1]. Despite the decline in the incidence of this disease in recent years due to preventive measures, CAD is still the leading cause of morbidity and mortality in the United States [2]. Few relationships in medicine are as well established as that between blood total cholesterol levels and the risk of developing CAD: until now, the high level of plasma total cholesterol is the risk factor number one for atherosclerosis [3]. Mediterranean diets were proposed for prevention of high levels of plasma lipids [4–7]. So, Mattson [6] has shown that Mediterranean alpha-linolic acid-rich diet is effective in controlling blood lipid levels. Spiller [7] has replaced a typical diet high in saturated fat with a Mediterranean-type diet and has found that the plasma cholesterol levels were decreased.

The positive influence of these diets is connected to their low saturated and high monounsaturated fatty acids content [6,7]. However, such diets are rich in dietary fibers and

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antioxidant compounds of vegetables and fruits, which are the main components of these diets, and their role cannot be neglected [8–10].

In the last years, Israel produces and exports a new kind of citrus fruit, a pummelo–grapefruit hybrid (*Citrus grandis* × *C. paradisi*), which is named sweetie in Israel.

Recently, we have investigated in vitro and in vivo these citrus fruits, which were harvested in the period of December 2001–April 2002 [11,12].

We have found that sweeties have high contents of dietary fibers and antioxidant compounds [11]. Diets supplemented with these fruits improved the plasma lipid levels and increased the plasma antioxidant potential in rats fed added cholesterol [12].

To recommend the use of sweeties for prevention of atherosclerosis, it must be shown that diets supplemented with these fruits improve the plasma lipid levels and increase the plasma antioxidant activity also in humans. Therefore, it was decided to investigate the influence of diet supplemented with peeled sweeties on hypercholesterolemic patients suffering from CAD.

As far as we know, there are no such investigations.

Materials and methods

Fruit samples

A new kind of citrus fruit, a pummelo–grapefruit hybrid (*C. grandis* × *C. paradisi*), which is named sweetie, was investigated. In this study, sweeties (cultivar Jaffa) harvested in the period of December 2002–April 2003 were used.

These fruits were cleaned with tap water and dried. The peeled fruits were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. Then a weighed portion (50–100 g) was lyophilized for 48 h (Virtis model 10-324) and the dry weight was determined. The sample was ground to pass through a 0.5-mm sieve and stored at −20 °C until analyzed.

Analyses of fruits

Total, soluble and insoluble dietary fibers were determined according to Prosky et al. [13] and the results were expressed in grams per 100 fresh weight (FW).

Total and free phenols were measured at 765 nm using Folin–Ciocalteu reagent with gallic acid as the standard and the results were expressed in μmol/g FW [14].

Phenolic acids were determined by fluorometry and HPLC [15] with a 25-cm 10 μm C_{18} column (Supelco, Inc. Bellefonte, PA) using a solvent of 86% water/4% acetic acid/10% methanol with a flow rate of 2 ml/min at 320 nm. In addition, ascorbic acid was determined by HPLC at 254 nm [16]. The results were expressed in mg/100 g FW.

The total anthocyanins were estimated by a pH differential method [17]. Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5 using $A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as micrograms of cyanidin-3-glucoside equivalent per gram of FW.

Flavonoids were extracted with 5% $NaNO_2$,10% $AlCl_3 \cdot 6H_2O$ and were measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed in mg/g FW [18,19].

There are many methods for total antioxidant determination and every one has its limitations [20]. Some antioxidant assay methods give different antioxidant activity trends [21]. Therefore, the total antioxidant potential of peeled sweeties was determined by the two following tests:

1. Total antioxidant status (TAA test). The TAA was estimated using the ferrylmyoglobin/ABTS method [22]. This technique measures the relative ability of antioxidant substances to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$), compared with standard amounts of the synthetic antioxidant Trolox, the water-soluble vitamin E analogue. The radical cation $ABTS^{\cdot+}$, generated in the aqueous phase from ABTS through the peroxidation action of metmyoglobin, is a blue/green chromogen with characteristic absorption at 734 nm. Results were expressed as millimoles of Trolox equivalents (TE) per gram of FW.
2. Scavenging activity against nitric oxide (NO). Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution was assayed spectrophotometrically by using the Greiss reagent and absorbance was measured at 542 nm [23,24]. Results were expressed as percentage of inhibition (AE) × 10^3.

Subjects, clinical investigation and laboratory tests

The study population was recruited from patients-volunteers who have previously undergone coronary bypass surgery due to CAD in the Institute of Cardiology of the University Medical Center, Rehovot, Israel.

The subjects gave written informed consent to a protocol approved by the responsible Institutional Committee on human experimentations, based on the Helsinki Declaration of 1975 as revised in 1983.

One hundred sixteen male patients between the ages of 47 and 68 years were examined. All of them underwent bypass surgery due to three-vessel CAD. In all of them, the clinical manifestation of CAD have appeared at least 2 years before the coronary bypass surgery, but following the surgery, these patients were free of anginal syndrome without additional medication. No lipid-lowering medicine was used during the 30 days of the investigation.

All patients were recruited at least 12 months after the surgery and the results of the laboratory tests were identical to the results of laboratory tests usually performed before coronary bypasses surgery. From these 116, 66 hypercholesterolemic patients between the ages of 48 and 66 years were chosen and randomly divided into three groups: two experimental (EG1 and EG2) and one control (CG), 22 each. All patients have consumed the usual Israeli diet recommended for patients with coronary atherosclerosis: rich in vegetables and fruits and limited quantities of fats [25,26]. For 30 consecutive days, the diet for the patients of the EG1 and EG2 groups was supplemented once a day by one or two peeled sweetie fruits, respectively. The patients of the CG consumed only the usual diet (not supplemented with sweeties).

Assigned member of the investigation team checked the consumption of the diets, the lifestyle and physical activity of the patients of all three groups.

Before and after completion of the study, every patient was examined. Systolic and diastolic blood pressure, heart rate and weight were registered. A wide range of laboratory tests was performed. During the trial period, there were no treatment complications.

Blood samples a day before and a day after 30 days of this investigation were collected after an overnight fast. Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fibrinogen, prothrombin time, Factor VII ag, Factor VII c and plasminogen activator inhibitor (PAI) were determined as previously described [25,26].

In the past, we have used in our experiments total radical-trapping antioxidative potential test (TRAP) and lipid peroxidation assay (MDA) for determination of the plasma antioxidant activity [27]. According to our experience, the above mentioned tests are not specific for determination of the plasma antioxidant potential in laboratory animals and humans alike. Therefore, the plasma total antioxidant potential in our patients was determined by Trolox equivalent antioxidant capacity test (TEAC) [28]. TEAC was done using the relative ability of antioxidant substances to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$). The relative ability was measured at 734 nm and compared with Trolox. Results were expressed as mmol/L Trolox equivalent.

Statistical methods

Values are given as means ± SD of five-times-analyzed in vitro samples. Where appropriate, the data were tested by two-way ANOVA. In the assessment of the antioxidant capacity, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The P values of <0.05 were considered significant.

In vitro

The contents of total, soluble and insoluble dietary fiber in peeled sweeties were 1.31 ± 0.07, 0.43 ± 0.03 and 0.86 ± 0.05 g/100 FW, respectively.

The antioxidant profile of fresh peeled sweeties and fresh peeled white grapefruits is reflected in the Table 1.

As can be seen, the contents of total and free phenols, total flavonoids and anthocyanins in peeled sweeties are higher than in peeled grapefruits but not significantly.

Both methods used (TAA and NO) have proven that the antioxidant potential of the peeled sweeties is very high and higher than in peeled grapefruits but not significantly.

The contents of phenolic and ascorbic acids are shown in Fig. 1. As can be seen, the highest concentration was of ferulic (29.7 ± 0.9), sinapic (26.1 ± 0.8) and *p*-coumaric (21.3 ± 0.7) acids, and the lowest of caffeic acid (12.2 ± 0.6) mg/100g FW. The differences in the contents of phenolic acids were significant ($P < 0.05$). The total concentration of the four hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic) was 89.3 mg/100 g FW. The content of ascorbic acid (38.1 mg/100 g FW) was significantly higher than of every one of the phenolic acids ($P < 0.05$).

The best correlation (R^2 = 0.9446 and 0.9466) was between total phenols and the total antioxidant activity as determined by TAA and NO test, respectively (Figs. 2A and B). In addition, the correlation between free phenols and the total antioxidant activity (R^2 = 0.8212 and 0.8746, Figs. 2A

Table 1
Antioxidant profile of fresh peeled sweeties and white grapefruits[a]

	Total phenols[b] (μmol/g)	Free phenols[b] (μmol/g)	Total anthocyanins[c] (μg/g)	Flavonoids (mg/g)	TAA[d] (mmol TE/g)	NO test (AE) × 10^3
Peeled sweeties	9.2 ± 0.9	1.2 ± 0.1	1.1 ± 0.1	0.54 ± 0.07	6.95 ± 0.5	15.4
Peeled grapefruits	8.9 ± 0.9	1.0 ± 0.1	1.0 ± 0.1	0.52 ± 0.07	6.89 ± 0.5	15.1

[a] Data are means ± SD of five measurements.
[b] Data expressed as μmol/g of gallic acid equivalents.
[c] Data expressed as μg/g of cyanidin-3 glucoside per gram of fresh fruit.
[d] Data expressed as millimoles of Trolox equivalents per gram of fresh fruit.

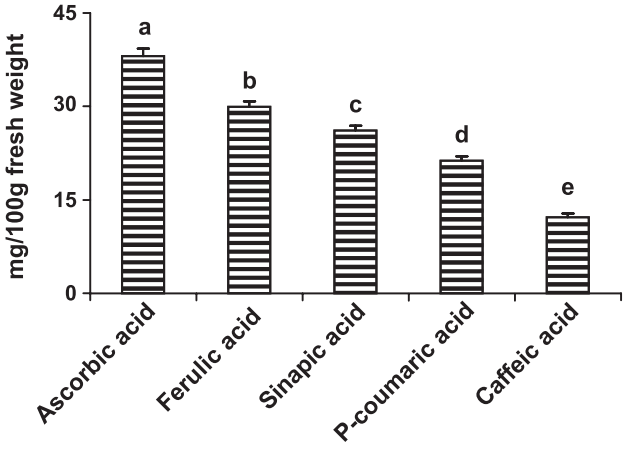

Fig. 1. Phenolic and ascorbic acids in fresh peeled sweeties. (Horizontal lines) mean ± SD. Bars with different letters are significantly different ($P < 0.05$).

and B), between anthocyanins and the total antioxidant activity (R^2 = 0.8068 and 0.8612, Fig. 3A), and between flavonoids and the total antioxidant activity (R^2 = 0.932 and

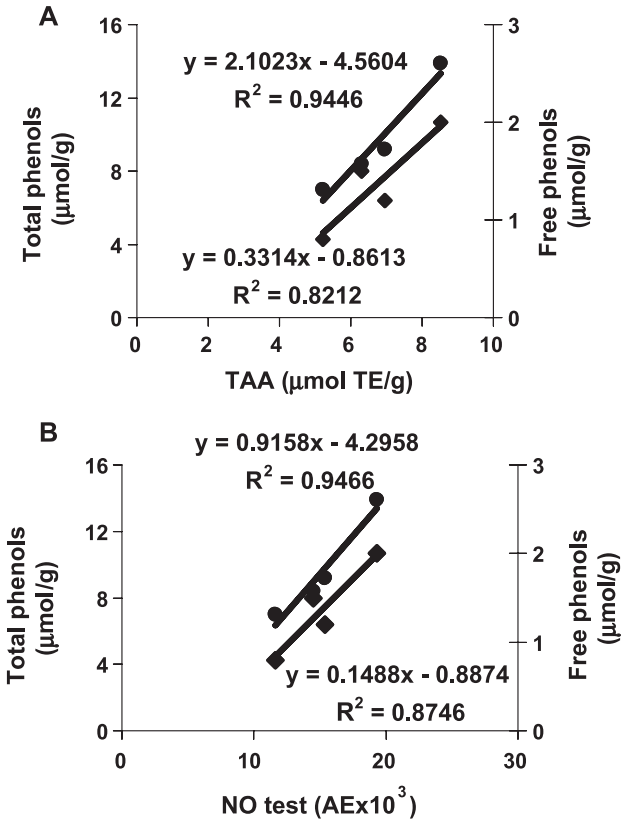

Fig. 2. (A and B) Relationship, calculated by linear regression analysis for the studied peeled sweeties between total and free phenols and TAA and NO tests. (A) ●, concentration of total phenols (µmol/g; Y_1) to TAA test (µmol TE/g; X); ♦, concentration of free phenols (µmol/g; Y_2) to TAA test (µmol TE/g; X). (B) ♦, concentration of total phenols (µmol/g; Y_1) to NO test [(AE ($\times 10^3$), X]; and ♦, concentration of free phenols (µmol/g; Y_2) to NO test [(AE ($\times 10^3$), X]. TAA, total antioxidant activity; TE, Trolox equivalent; NO, scavenging activity against nitric oxide; AE, antioxidant efficiency.

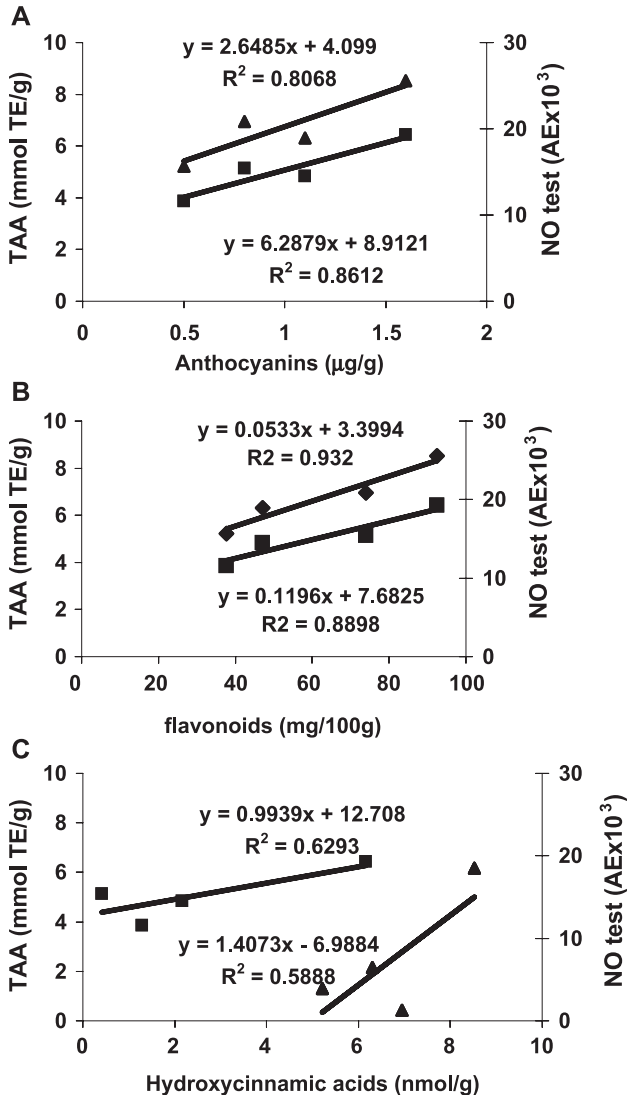

Fig. 3. (A–C) Relationship, calculated by linear regression analysis for the studied peeled sweeties between anthocyanins and TAA and NO tests (Fig. 3A) and between flavonoids and TAA and NO tests (Fig. 3B). (A) ▲, concentration of anthocyanins (µg/g; X) to TAA test (mmol TE/g; Y_1); and ■, concentration of anthocyanins (µg/g; X) to NO test [(AE) $\times 10^3$), Y_2]. (B) ♦, concentration of flavonoids (mg/100g, X) to TAA test (mmol TE/g; Y_1); ■, concentration of flavonoids (mg/100g, X) to NO test [(AE) $\times 10^3$), Y_2]. (C) ■, concentration of hydroxycinnamic acids (nmol/g, X) to TAA test (mmol TE/g; Y_1). ▲, concentration of hydroxycinnamic acids (nmol/g, X) to NO test [(AE) $\times 10^3$), Y_2]. TAA, total antioxidant activity; TE, Trolox equivalent, NO, scavenging activity against nitric oxide.

0.8898, Fig. 3B) as determined by TAA and NO test, respectively, was high. The correlation between hydroxycinnamic acids and total antioxidant activity (R^2 = 0.6293 and 0.5888 for TAA and NO test, respectively) was relatively low (Fig. 3C).

Clinical data

The heart rate, the systolic and diastolic blood pressure, and the weight of the patients after completion of the

Table 2
Plasma lipids[1] (mmol/L) in the control, EG1 and EG2[2] groups after completion of the investigation

Diets	TC	LDL-C	HDL-C	TG
Control	8.08 ± 0.4[a]	6.39 ± 0.2[a]	1.29 ± 0.1[a]	2.27 ± 0.1[a]
EG1	7.38 ± 0.3[a]	5.65 ± 0.2[b]	1.33 ± 0.1[a]	2.01 ± 0.1[a]
EG2	6.78 ± 0.3[b]	5.04 ± 0.2[c]	1.39 ± 0.1[a]	1.71 ± 0.1[b]
Two-way ANOVA (P value)				
EG1	NS	<0.01	NS	NS
EG2	<0.0125	<0.0005	NS	<0.0005

[1]Values are means ± SD, $n = 22$.
[2]Means in columns with different letters differ significantly ($P < 0.05$).
EG1, experimental group, which diet was supplemented with one peeled sweetie fruit; EG2, experimental group, which diet was supplemented with two peeled sweetie fruits; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

investigation were without significant changes (data not shown).

Laboratory data

The changes in the lipid levels are summarized in Table 2. As can be seen, peeled sweeties-supplemented diets after completion of the investigation have decreased plasma lipid levels in EG1 and EG2 vs. CG group: (a) TC, −8.7% and −16.1% for EG1 and EG2, respectively; (b) LDL-C, −11.6% and −21.2% for EG1 and EG2, respectively; (c) TG, −11.5% and −24.7% for EG1 and EG2, respectively. The increase in the HDL-C in the EG1 and EG2 vs. control group was minimal (+3.1% and +7.7% for EG1 and EG2, respectively).

After the trial, an increase in the plasma antioxidant activity in patients of both experimental groups was found (Fig. 4): an increase in TEAC (1.64 vs. 1.42, +15.5% and 1.87 vs. 1.41 mmol/L, +32.6%) for the EG1 and EG2 groups, respectively. No significant changes in the TEAC values in patients of the CG group were registered.

Discussion

Atherosclerosis is a multifactorial process based on the action of various risk factors [29]. According to Ross [29], the form and content of the advanced lesions of atherosclerosis demonstrate the results of three fundamental biological processes: (a) accumulation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T-lymphocytes; (b) formation by the proliferated smooth muscles cells of large amounts of connective tissue matrix, including collagen, elastic fibers and proteoglycans; and (c) accumulation of lipids, principally in the form of cholesteryl esters and free cholesterol within the cells as well as in the surrounding connective tissues.

Some authors have found signs of inflammatory and immunological nature of atherosclerosis [30–34] and one of them even claims that atherosclerosis is a true inflammatory disease [33]. However, after the paper "Beyond cholesterol: modifications of low density lipoprotein that increases its atherogenicity" was published [35], more and more authors support the theory that atherosclerosis is mainly an oxidative disease [36–38]. The oxidative theory is widely used as a basis for prevention and treatment of this disease [35–38]. According to this theory, only rich in cholesterol-oxidized LDL-C particles are able to penetrate arterial walls causing their occlusions [35]. The most important damages are in the coronary arteries. CAD is a consequence of atherosclerotic process in these arteries. Recently Perez et al. [39] have proven that oxidative stress is a central mechanism for the pathogenesis of CAD and atherogenesis. It was shown in experiment on laboratory animals that antioxidant vitamins supplementation reduces progression of established atherosclerosis by suppressing oxidative and inflammatory reac-

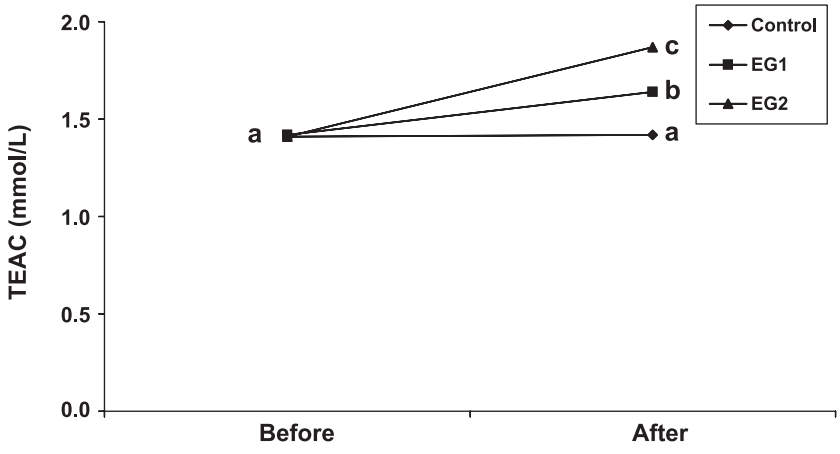

Fig. 4. The changes of the plasma antioxidant activity after completion of the investigation. (Horizontal lines) Antioxidant activity values. Symbols with different letters are significantly different ($P < 0.05$).

tions and increasing nitric oxide levels [40]. In addition, the data of 6-year-long investigation in the framework of "The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study" show that supplementation with combination of vitamin E and slow-release vitamin C slows down atherosclerotic progression in hypercholesterolemic persons [41].

Intensive multiple risk factor reduction has a beneficial effect on coronary atherosclerosis in men and women: the disease regression is twice as frequent in the risk reduction group as in control group [42]. An integral part of these preventive measures are proper diets. The general principles of such low calories diets are limited quantities of fats and lot of vegetables and fruits [43,44]. Therefore, more and more scientists are studying these natural products, which could be effective in prevention of atherosclerosis [44,45]. In the last 15 years, our international team of experts in preventive cardiology and dietetics are studying vegetables, fruits, oils and alcoholic beverages to enrich diets intended for prevention of coronary atherosclerosis [24,25,44–46].

Recently, a new kind of citrus fruits (pummelo–grapefruit hybrid named sweetie in this country) draw our attention. We have studied this fruit in vitro [11,12,46] and in experiments on laboratory animals [12] and have compared with other citrus fruits [11,12]. We have found in our studies in vitro [11,12] that sweeties possess higher concentrations of bioactive compounds and that diets supplemented with this fruit more positively affect plasma lipid levels and plasma antioxidant activity in laboratory animals fed added cholesterol than white grapefruits [12]. In this investigation, we tried to find out if addition of peeled sweeties to a generally accepted antiatherosclerotic diet could lead to the desired changes in plasma lipid levels and plasma antioxidant activity also in hypercholesterolemic patients suffering from CAD.

The results of the investigation in vitro have shown that peeled sweeties contain a high concentration of biologically active compounds: dietary fibers, phenolic and ascorbic acids, anthocyanins and flavonoids. The concentrations of these compounds are comparable with their concentrations in grapefruits [12]. A high antioxidant potential of peeled sweeties was found as determined by total antioxidant tests used.

As was stated, the aim of present investigation was to find out if the new kind of citrus fruits could be recommended for use in diets for patients suffering from hypercholesterolemia.

The results of this investigation have shown that supplementation to the usual antiatherosclerotic diet of two and to less degree with one peeled sweetie fruits during 30 consecutive days led to hypocholesterolemic effect and to increased plasma antioxidant activity in hypercholesterolemic CAD patients.

These results could be predicted: natural products containing high quantities of dietary fibers and antioxidant compounds positively influence plasma lipid levels and plasma antioxidant status in experiments on laboratory animals [12,27,31,40,44].

In conclusion, (a) diets supplemented with peeled sweeties positively influence plasma lipid levels and plasma antioxidant activity in hypercholesterolemic patients, (b) the optimal quantity is two peeled sweetie fruits per day, and (c) addition of peeled sweeties to a generally accepted antiatherosclerotic diet may be beneficial in prevention of atherosclerosis, mainly in hypercholesterolemic patients.

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