Fresh Israeli Jaffa Sweetie Juice Consumption Improves Lipid Metabolism and Increases Antioxidant Capacity in Hypercholesterolemic Patients Suffering from Coronary Artery Disease: Studies in Vitro and in Humans and Positive Changes in Albumin and Fibrinogen Fractions

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The contents of the bioactive compounds in the pummelo−grapefruit hybrid juice named Israeli Jaffa Sweetie and their influence on humans suffering from hypercholesterolemia were studied. It was found that Sweetie juice has a high content of bioactive compounds and a high antioxidant potential. Then 72 hypercholesterolemic patients, ages 43−71 years, after coronary bypass surgery recruited from the Institute pool of volunteers, were randomly divided into two experimental (EG1 and EG2) groups and one control (CG) group, each comprising 24 patients. The diets of EG1 and EG2 patients were daily supplemented with 100 or 200 mL of fresh Sweetie juice, respectively. Before and after diet consumption serum lipid levels, albumin and fibrinogen fractions, and their antioxidant capacities were determined. After 30 consecutive days of Sweetie juice supplemented diets, improvements in serum lipids levels were found in EG1 and EG2 versus CG: (a) total cholesterol, 7.34 versus 8.02 mmol/L, −9.5%, and 6.73 versus 8.02 mmol/L, −16.1%, respectively; (b) low-density lipoprotein cholesterol, 5.63 versus 6.37 mmol/L, −11.6%, and 5.03 versus 6.37 mmol/L, −21.0%, respectively; (c) total glycerides, 2.01 versus 2.27 mmol/L, −11.5%, and 1.71 versus 2.27 mmol/L, −24.7%, respectively. Serum albumin concentration was increased but not significantly in EG1 and EG2 versus CG: 47.5 versus 44.5 g/L, +6.7%, and 47.9 versus 44.5 g/L, +7.6%, respectively. A significant increase in the serum, albumin, and fibrinogen antioxidant capacities in EG2 and to a lesser degree in EG1 was observed. No changes in the CG were found. In conclusion, fresh Sweetie juice contains high quantities of bioactive compounds and has a high antioxidant potential. Diet supplemented with this juice positively influences serum lipid, albumin, and fibrinogen levels and their antioxidant capacities. Addition of fresh Sweetie juice to generally accepted diets may be beneficial for hypercholesterolemic patients.

KEYWORDS: Fresh Sweetie juice; bioactive compounds; hypercholesterolemic patients; serum lipids; albumin; fibrinogen; antioxidant capacities

INTRODUCTION

Vegetables and fruits have many healthful properties (*1*, *2*). Diets rich in these natural products prevent some diseases including coronary artery disease (CAD), one of the most dangerous diseases in the Western industrialized countries. It

was shown that the positive effect of such diets is connected with their high content of dietary fibers and antioxidants (*3−7*). In recent years Israel has produced and exported a new kind of citrus fruit, a pummelo−grapefruit hybrid (*Citrus grandis × Citrus paradisi*), which is named Israeli Jaffa Sweetie. Recently we have investigated this new citrus fruit, which was harvested in the period of December 2001−April 2002. We have found that Sweeties have high contents of dietary fibers and antioxidant compounds (*8*). Diets supplemented with these peeled fruits have improved serum lipid metabolism and increased serum antioxidant potential in rats fed added cholesterol (*8*).

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Consumption of juices has increased in comparison with fruits (*9*−*12*). In our very recent research we have studied the influence of citrus juices on laboratory animals (*13*). We have found that fresh citrus juices positively affect plasma lipid metabolism and plasma antioxidant capacity in rats fed added cholesterol.

It is known that the results of the experiments on laboratory animals cannot be automatically applied to human beings. Therefore, we have decided to investigate the bioactivity of Sweetie juice in an investigation of patients suffering from hypercholesterolemia.

Hypercholesterolemia is the main risk factor for atherosclerosis (*14*). However, only oxidized low-density lipoprotein cholesterol (LDL-C) particles are able to penetrate arterial walls and cause their occlusion and, as a consequence, fatal myocardial infarctions (*15*−*17*). Therefore, the influence of a diet supplemented with Sweetie juice on serum lipids and antioxidant activity in hypercholesterolemic patients suffering from CAD was investigated.

Oxidized LDL is an important marker for the prevention of atherosclerosis (*15*−*17*). Much less is known about the level of serum albumin as such a marker. However, some epidemiological and clinical data consistently show that a reduced level of serum albumin is associated with an increased incident and mortality risk from CAD (*18*, *19*). Some authors indicate that serum albumin is even a significant and independent predictor of the number of atherosclerotic plaques (*20*). There are many explanations of this connection, the most convincing being that the serum albumin level is inversely correlated with fibrinogen and hypoalbuminemia may contribute to atherosclerosis via increased synthesis of fibrinogen (*21*). It was demonstrated that atherosclerosis in addition to LDL-C oxidation is also associated with oxidative modification of proteins. The antiatherosclerotic effects of antioxidants are also due to prevention of protein oxidation (*22*). Therefore, serum albumin and fibrinogen and their antioxidant levels were investigated.

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

MATERIALS AND METHODS

Chemicals. Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid), β-carotene, butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO); 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Fruit Samples. In this investigation Israeli Jaffa Sweetie fruits, which were harvested in the period of December 2002−April 2003, were used. Fruits were cleaned with tap water and dried. Juice was extracted manually. Then a weighed portion (50−100 mL) was lyophilized for 48 h (Virtis model 10-324), and the dry weight was determined. The samples were ground to pass through a 0.5-mm sieve and stored at −20 °C until analysis.

Analyses of Fruits. Dietary fiber in the selected samples was analyzed according to the modified AOAC method. Samples were treated with heat-stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000*g*) to separate the soluble and insoluble fractions and dialysis against water (*23*, *24*).

Total polyphenols and phenolic and ascorbic acids were determined as previously described (*8*). Anthocyanins were determined as follows: 50 mL of Sweetie juice was added to 50 mL of acetonitrile containing 4% acetic acid, mixed, and then centrifuged at 13000*g* for 15 min at 4 °C. The pellet following centrifugation was washed with 50 mL of acetonitrile containing 4% acetic acid and centrifuged. The resulting supernatants were combined with the initial extract. Anthocyanins were estimated according to a pH differential method (*25*).

Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29600. Results were expressed as micrograms of cyanidin 3-glucoside equivalent per milliliter of fresh juice.

Flavonoids were determined as follows: extracted with 5% NaNO2, 10% AlCl3·6H2O, and 1 M NaOH, and absorbance was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as micrograms of catechin equivalents or milligrams per milliliter of fresh juice (*26*, *27*).

Total Antioxidant Potential. There are many methods for total antioxidant determination, and every one has its limitations (*28*). Some antioxidant assay methods give different antioxidant activity trends (*29*). According to our own experience (*8*, *30*) the best combination of the antioxidant tests in experiments in vitro for citrus fruits were the following three assays, which we used in this study.

ABTS Radical Cation Decolorization Assay. The Trolox equivalent antioxidant coefficient (TEAC) value is based on the ability of the antioxidants to scavenge the blue-green 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS•+) radical cation relative to the ABTS•+ scavenging ability of the water-soluble vitamin E analogue Trolox. The 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 different extracts (0.2 mg/mL) or Trolox standards (final concentration = 0−20 μM) in methanol or phosphate-buffered saline (PBS). The absorbance was monitored exactly 1 and 6 min after the initial mixing. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data. To calculate the TEAC, the slope of the plot of the percentage inhibition of absorbance versus concentration for the antioxidant was divided by the slope of the plot of Trolox (*31*).

Antioxidant Assay Using a β-Carotene Linoleate Model System. To an emulsion of β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) was added 40 mL of oxygenated water. Four-milliliter aliquots of this emulsion were added to test samples containing 0.2 mL of fresh juice. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the β-carotene, measuring the absorbance at 470 nm, during $t = 180$ min at an interval of 15 min: $AA = 100[1 - (A_0 - A_t)/(A_0^\circ - A_t^\circ)]$, where A_0 and A_0° are the absorbance values measured at zero time, and A_t and A_t° are the absorbances measured in the test sample and control, respectively, after incubation for 180 min. Trolox and BHA were used as standards in these methods. The kinetics was done during 180 min (*32*).

Radical Scavenging Activity Using DPPH. Five milliliters of a 0.1 mM methanolic solution of DPPH was added to 100 μL of fresh juice and BHA standard. Changes in the absorbance of the samples and standards were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated as % radical scavenging activity = (control OD − sample OD/control OD) × 100 (*32*).

Subjects, Clinical Investigation, Laboratory Tests, and Dietary Intervention. One hundred and twenty-two patients between the ages of 43 and 71 years were examined. All of them underwent bypass surgery due to two- or three-vessel CAD. The clinical manifestations of CAD in these patients had appeared at least 2 years before the coronary bypass surgery, but following surgery they were free of anginal syndrome. No lipid-lowering and/or antioxidant increasing drugs were used during the 30 days of the investigation. All patients were at least 12 months after the surgery. From these 122, 72 hypercholesterolemic patients were chosen and randomly divided into three groups: two experimental (EG1 and EG2) and one control (CG), each of 24. All patients had consumed a generally accepted diet for coronary atherosclerosis patients (vegetables, fruits, and limited quantities of fats). This diet contained ~1700 kcal, and the percentage of energy was 66% of carbohydrates, 25% of protein, and 9% of fat.

For 30 consecutive days this diet was supplemented once a day by 100 and 200 mL of fresh Sweetie juice for the EG1 and EG2 groups, respectively; 100 and 200 mL of fresh Sweetie juice were equal to one or two peeled Sweetie fruits, respectively. The patients of the CG group
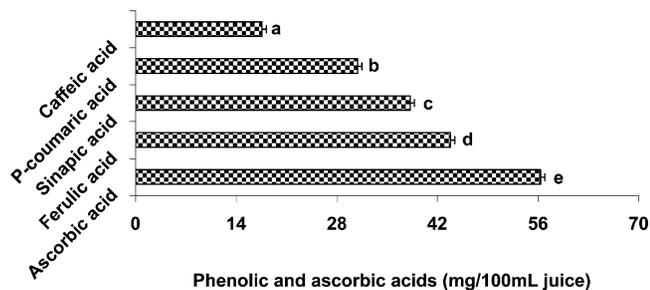
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Figure 1. Phenolic and ascorbic acids in fresh Sweetie juice: mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

instead of juice consumed mineral water. An assigned member of the investigation team checked the consumption of diets, lifestyle, and physical activity of all 72 patients.

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 and instrumental tests were performed. During the trial period there were no complications.

After an overnight fast, blood samples were collected a day before and a day after investigation. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), plasma circulation fibrinogen (PCF), prothrombin time (PT), factor VIIag, factor VIIc, and plasminogen activator inhibitor (PAI) tests were determined as previously described (*33*). Albumin from human serum was precipitated with 2 M ammonium sulfate, and fibrinogen was precipitated by methanol, purified by sequential DEAE anion-exchange chromatography, and then dialyzed against water for 72 h and lyophilized (*34, 35*).

Protein assays were performed according to the Lowry method (*36*). Human serum albumin (HSA) and fibrinogen (HSF) were used as standards, respectively.

In the past we have used the total radical-trapping antioxidative potential test (TRAP) and the lipid peroxidation assay (MDA) for determination of the plasma antioxidant activity (*37*). According to our experience, the above-mentioned tests are not specific for determination of the antioxidant potential of both serum and serum proteins in laboratory animals and humans alike. Therefore, the serum and serum albumins and fibrinogen antioxidant activities were determined by Trolox equivalent antioxidant capacity test (*38*). The results were expressed as millimoles per liter.

Statistical Analysis. Values are given as means ± standard deviation (SD) of five times analyzed samples. Where appropriate, the data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA) following by Duncan's new multiple-range test to assess differences between groups means. Differences of $P < 0.05$ were considered to be significant.

RESULTS

In Vitro Studies. The contents of total, soluble, and insoluble dietary fibers were 2.81 ± 0.19, 2.1 ± 0.19, and 0.71 ± 0.7% of fresh juice, respectively.

The content of total polyphenols was 885.1 ± 27.1 μg/mL of fresh Sweetie juice. The contents of phenolic and ascorbic acids are shown in **Figure 1**. As can be seen, the highest concentrations were of ferulic, sinapic, and *p*-coumaric acids and the lowest was of caffeic acid. The differences in the contents of phenolic acids were significant ($P < 0.05$).

The content of ascorbic acid was significantly higher than that of each of the phenolic acids ($P < 0.05$).

The content of anthocyanins was 1.3 ± 0.1 μg of cyanidin 3-glucoside equivalent/mL of fresh juice.

The contents of flavonoids were 56.9 ± 2.4 μg of catechin equivalent/mL of fresh juice.
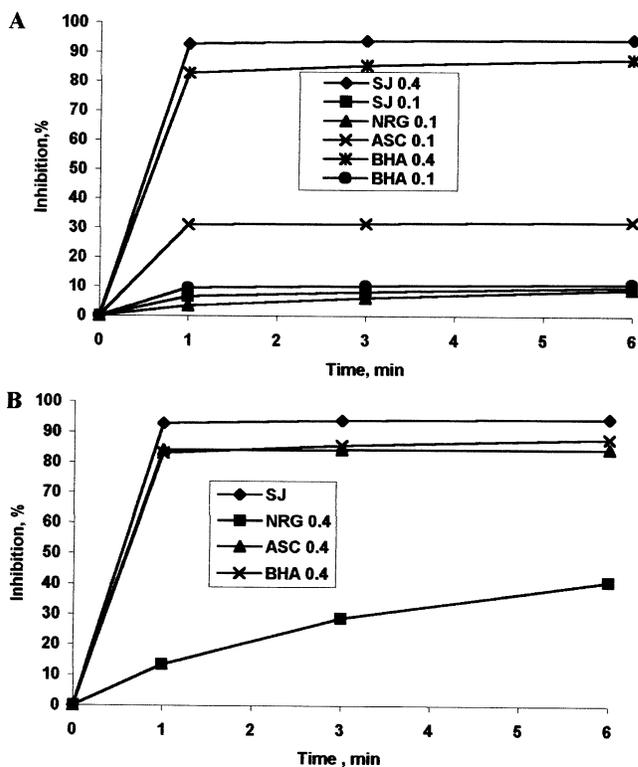

Figure 2. Kinetics of ABTS scavenging effect: (**A**) Sweetie juice (SJ 0.4) at 0.4 mg/mL; (SJ 0.1) at 0.1 mg/mL; ascorbic acid (ASC 0.1) at 0.1 mg/mL; butylated hydroxyanisole (BHA 0.4) at 0.4 mg/mL; (BHA 0.04) at 0.04 mg/mL; naringin (NRG 0.1) at 0.1 mg/mL; (**B**) (SJ 0.4) at 0.4 mg/mL; (NRG 0.4) at 0.4 mg/mL; ascorbic acid (ASC 0.4) at 0.4 mg/mL; (BHA 0.4) at 0.4 mg/mL.
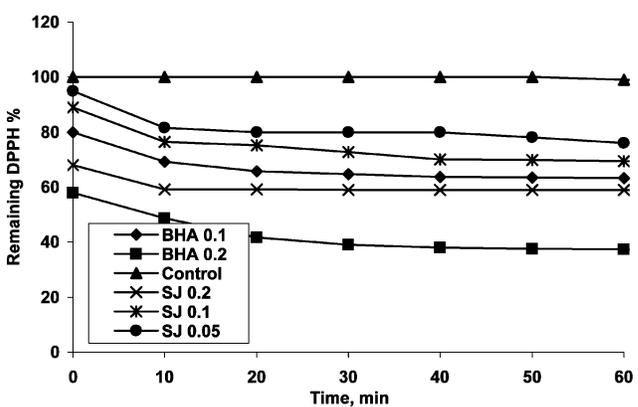

Figure 3. Kinetics of DPPH scavenging effects: BHA at concentrations of 0.1 and 0.2 mg/mL; Sweetie juice at concentrations of 0.2 mg/mL (SJ 0.2), 0.1 mg/mL (SJ 0.1), and 0.05 mg/mL (SJ 0.05); C, control.

The antioxidant potential of the methanol extract of 0.4 mg/mL of Sweetie juice (SJ) using the β-carotene linoleate model system showed 89% antioxidant activity. Similarly, the methanol extract of 0.4 mg/mL of SJ using the DPPH radical scavenging activity method showed 87% antioxidant activity. As can be seen, the used methods have proven that the antioxidant potential of fresh Sweetie juice is very high.

Kinetics of ABTS scavenging effect is shown in **Figure 2**. As can be seen, three curves of standards such as naringin (NRG) and BHA at 0.1 mg/mL and Sweetie juice of 0.1 mg/mL (**Figure 2A**) were close at the end point of 6 min and differ from each other only ~2% inhibition. BHA in a concentration of 0.4 mg/mL was close in this value to juice (0.4 mg/mL) and
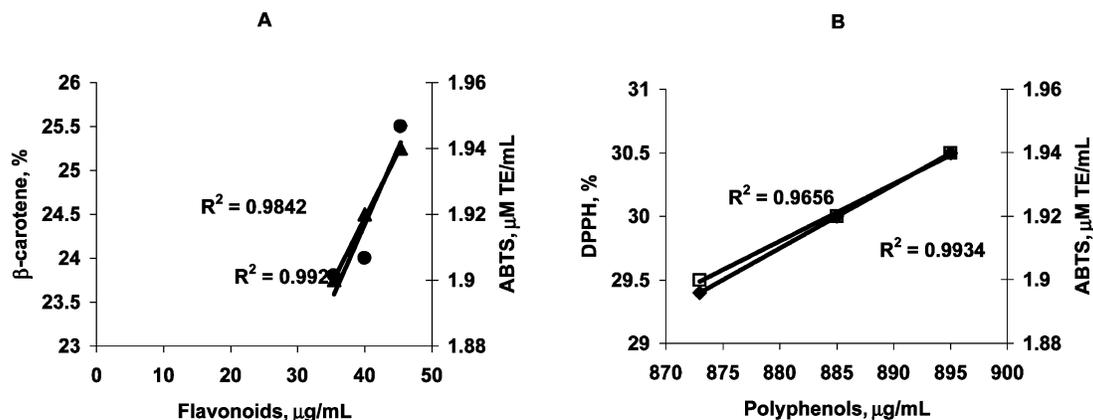
A

B

Figure 4. Relationship, calculated by a linear regression analysis for Sweetie juice: (**A**) ●, flavonoids (μg/mL, *X*) to β-carotene (% inhibition, Y_1), and ▲, flavonoids (μg/mL, *X*) to ABTS (μM TE/mL; Y_2); (**B**) ◆, polyphenols (μg/mL, *X*) to DPPH scavenging effect (% inhibition, Y_1), and □, polyphenols (μg/mL, *X*) to ABTS (μM TE/mL, Y_2).
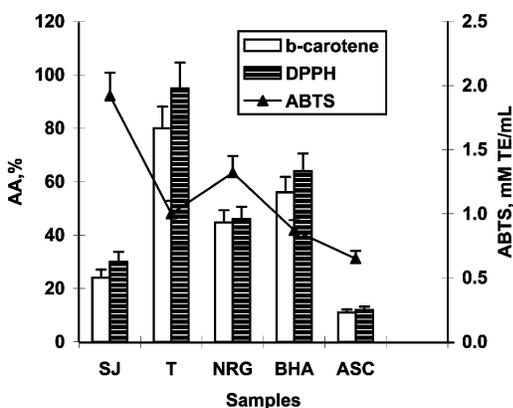

Figure 5. Relationship between three scavenging methods (β-carotene, DPPH, and ABTS radicals) and various standard substances.

differs only by ~10%. Juice (0.4 mg/mL) was ~3 times higher in percentage of inhibition than ascorbic acid (ASC) at 0.4 mg/mL. The inhibition percentage increased when the concentration of ASC and NRG increased as well (**Figure 2B**). It was interesting to match the concentrations of the standards to the concentrations of the juices [curves SJ, BHA, ASC, and NRG at the same concentration of 0.4 mg/mL (**Figure 2B**) and at a concentration of 0.1 mg/mL (curves SJ, BHA, ASC, and NRG (**Figure 2A**)].

Kinetics of DPPH scavenging effects is shown in **Figure 3**. Sweetie juice (0.1 and 0.05 mg/mL) about twice as close to the standard of 0.1 mg/mL BHA and differs only by 4% of remaining DPPH. The scavenging activity of BHA at a concentration of 0.2 mg/mL showed higher activity than Sweetie juice at 0.1 and 0.05 mg/mL. Kinetics of DPPH (**Figure 3**) is calculated on the remaining activity of DPPH; therefore, if the curve on the figure is lower, then the antioxidant activity is higher.

Correlations of the antioxidant values and the antioxidant compounds are shown in **Figure 4**. As can be seen, the correlation coefficients between flavonoids and β-carotene and ABTS were 0.9842 and 0.9928, respectively (**Figure 4A**), and between polyphenols and ABTS and DPPH were 0.9934 and 0.9656, respectively (**Figure 4B**). Correlation coefficients between soluble dietary fibers (SDF) and β-carotene and ABTS were lower than those of polyphenols and flavonoids—between 0.5103 and 0.4749, respectively.

Table 1. Plasma Lipids (Millimoles per Liter) in the Control, EG1, and EG2 Groups after Completion of the Investigation (Means, SD, and 95% CI, *n* = 12)[a]

diet	TC	LDL-C	HDL-C	TG
control	8.02 ± 0.4a [7.15–8.97]	6.37 ± 0.2a [5.95–6.81]	1.22 ± 0.1a [1.00–1.44]	2.27 ± 0.1a [2.05–2.51]
EG1	7.34 ± 0.3a [6.69–7.99]	5.63 ± 0.2b [5.19–6.07]	1.31 ± 0.1a [1.09–1.53]	2.01 ± 0.1a [1.79–2.23]
EG2	6.73 ± 0.3b [6.08–7.38]	5.03 ± 0.2c [4.59–5.67]	1.38 ± 0.1a [1.16–1.60]	1.71 ± 0.1b [1.49–1.93]
two-way ANOVA *P* value				
EG1	NS	<0.01	NS	NS
EG2	<0.0125	<0.0005	NS	<0.0005

[a] Means in columns without letters in common differ significantly ($P < 0.05$). Abbreviations: CI, confidence intervals, EG1, experimental group 1, the diet of which was supplemented with one peeled Sweetie fruit; EG2, experiment group 2, the diet of which was supplemented with two peeled Sweetie fruits; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. TC, total cholesterol; TG, triglycerides.
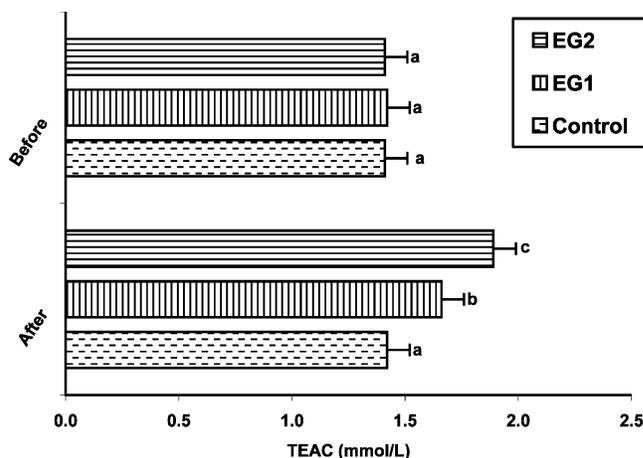

Figure 6. Changes of plasma antioxidant activity after completion of the investigation: mean $\pm$ SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

The relationship among the three scavenging methods is shown in **Figure 5**. As can be seen, the SJ scavenging effect in β-carotene and DPPH methods was higher than that of ASC and lower than that of BHA. Oppositely, in the ABTS method SJ showed a much higher antioxidant capacity than BHA.
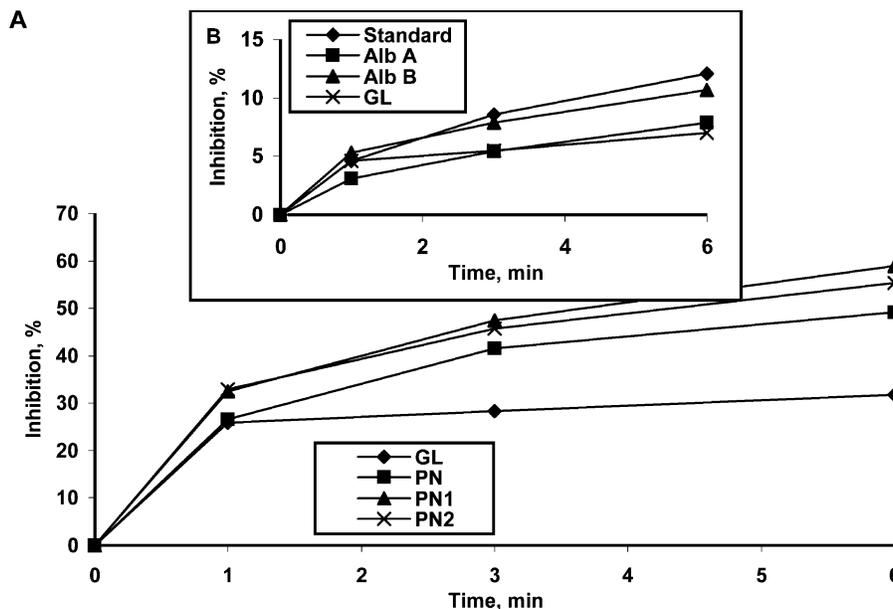
Figure 7. Kinetics of ABTS scavenging effect. Abbreviations: Alb A, human serum albumin after SJC; Alb B, human serum albumin before SJC; GL, glutathione; HSA; glutathione; 0.025 mg/mL; PN, patient; SJC, Sweetie juice consumption.

Similar results were obtained with the comparison with Trolox, *p*-coumaric acid, and other standards (*13*). In general, SJ was a stronger scavenger of ABTS radicals. Employment of different scavengers in the determination of antioxidant methods has shown as well a different reaction of SJ.

Studies in Humans. The heart rate, systolic and diastolic blood pressure, and the weight of the patients after completion of the investigation were without significant changes (data not shown).

The changes in the lipid levels are summarized in **Table 1**. As can be seen, the fresh Sweetie juice supplemented diets have decreased serum lipids levels in the EG1 and EG2 groups versus the CG group: TC, 7.34 versus 8.02 mmol/L, −9.5%, and 6.73 versus 8.02 mmol/L, −16.1%, respectively; LDL-C, 5.63 versus 6.37 mmol/L, −11.6%, and 5.03 versus 6.37 mmol/L, −21.0%, respectively; TG, 2.01 versus 2.27 mmol/L, −11.5%, and 1.71 versus 2.27 mmol/L, −24.7%, respectively. The increases in HDL-C in the EG1 and EG2 groups versus the CG group were minimal (+2.1 and +2.3%, respectively).

After completion of the trial, the level of the serum albumin was increased in patients of the EG1 and EG2 groups versus the CG group but not significantly: 45.9 versus 44.5 g/L, +3.1%, and 46.1 versus 44.5 g/L, +3.6%, respectively.

Also, the serum albumin antioxidant activity in patients of the EG1 and EG2 groups versus the CG group was increased: 1.51 versus 1.22 mmol/L, +23.8%, and 1.74 versus 1.22 mmol/L, +42.6%, for the EG1 and EG2 groups, respectively.

The serum antioxidant activity in patients of the EG1 and EG2 groups versus the CG group was increased (**Figure 6**): 1.66 versus 1.42 mmol/L, +16.9%, and 1.89 versus 1.42 mmol/L, +33.1%, respectively.

The kinetics of ABTS scavenging effect of human serum is shown in **Figure 7**. Human serum has different antioxidant scavenging capacities (**Figure 7A**). The antioxidant capacities of patients N, N1, and N3 varied from 0.97 to 1.13 to 1.2 μM. These numbers can be compared with glutathione of 0.2 mg/ mL. The antioxidant activity of human serum as well as in the albumin fraction was increased after juice consumption and was 0.22 in comparison with 0.16 μM before juice consumption.
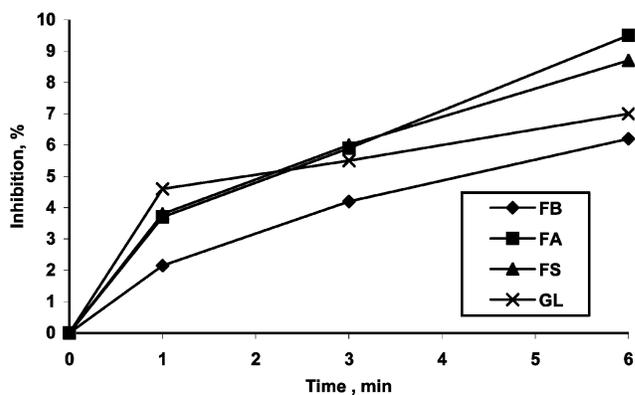

Figure 8. Kinetics of ABTS scavenging effect. Abbreviations: FA, fibrinogen after SJC; FB, before SJC; FS, standard; GL, glutathione (0.025 mg/mL); SJC, Sweetie juice consumption.

The antioxidant activity of 0.14 μM is comparable with glutathione of 0.025 mg/mL.

No significant changes in PCF concentration, prothrombin time (PT), factor VIIag, factor VIIc, and plasminogen activator inhibitor test (PAI) were observed (data not shown). However, an increase in fibrinogen antioxidant capacity of ~1.5 times was registered after Sweetie juice consumption (SJC) (**Figure 8**). This increase was slightly higher than that for albumin fraction.

No significant changes in the TEAC values of serum, serum albumin, and fibrinogen in the CG group of patients were registered.

DISCUSSION

It is an established fact that an elevated level of total cholesterol is the major risk factor for atheroclerosis and that hypercholesterolemia is the anatomic foundation of this disease (*14*). However, only oxidized LDL-C particles are able to penetrate arterial walls and cause occlusions of arteries (*15*– *17*). It was shown that supplementation of diets with a combination of vitamins, which possess antioxidant properties,

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slows atherosclerotic progression in hypercholesterolemic persons (*39*). Therefore, only a remedy that exercises hypocholesterolemic and antioxidant effects could be successful in the prevention of atherosclerosis in general and coronary atherosclerosis in particular (*15−17*).

For many years our team of dieticians, biochemists, and cardiologists has been studying various kinds of nutritional products (*40−42*) including citrus fruits (*30*) in order to improve diets for patients suffering from hypercholesterolemia. Recently we have investigated in vitro and in experiments on laboratory animals a new kind of citrus fruit—a pummelo—grapefruit hybrid named Sweetie (*8*). We used peeled fruits. As was mentioned, in recent years more and more consumers prefer fruit juices (*10−12*). Therefore, in the present investigation we have decided to study Sweetie juice in vitro. We tried to find out if addition of fresh Sweetie juice to the usual antiatherosclerosis diet could lead to the above-mentioned objectives: hypocholesterolemic and antioxidant effects.

The results of the investigation in vitro have shown that fresh Sweetie juice contains high concentrations of bioactive substances (mainly antioxidant compounds) and possesses high antioxidant ability. These results are in accordance with our previous data, when Sweeties harvested in 2001−2002 were investigated (*8*). Despite different climatic conditions in these two seasons, the results of the investigations in vitro were without significant differences. Therefore, it can be suggested that the main reason for the different contents of the bioactive compounds in the same fruits is not the climatic but the soil conditions in various geographical areas (*43*).

The results of the investigation of humans have shown that the generally accepted antiatherosclerosis diet supplemented with 200 mL and, to a lesser degree, with 100 mL of fresh Sweetie juice led to a significant improvement in the serum lipid levels and in the serum antioxidant activity in hypercholesterolemic CAD patients.

These results could be predicted: fruits containing high quantities of dietary fibers and antioxidant compounds have positively influenced the serum lipid levels and the serum antioxidant activity in experiments on laboratory animals (*40−42*).

Some epidemiological investigations reported that a reduced level of serum albumin is associated with an increased morbidity and mortality from CAD (*19, 20, 44*). A recent study proved that a low level of serum albumin is associated with an increased risk of all-cause and cardiovascular mortality as well as with coronary heart disease and stroke incidence (*45*). It was shown that low serum albumin is a powerful predictor of cardiovascular adverse events in healthy subjects and patients with subclinical atherosclerosis (*46*). The same authors claim that low serum albumin may be particularly useful for risk prediction in patients with few traditional risk factors of atherosclerosis.

In this prospective clinical study we have found that diets supplemented with fresh Sweetie juice led to an increase in the concentration of serum albumin and in its antioxidant activity.

Some investigators found that plasma fibrinogen is an efficient antioxidant (*47, 48*). Kaplan et al. (*47*) have shown that removal of fibrinogen from plasma enhanced oxidation, whereas addition of fibrinogen restored inhibition. Also, Olinescu and Kummerow (*48*) claim that fibrinogen, like albumin, acts as a supplementary antioxidant defense mechanism against oxidative stress. We found that consumption of Sweetie juice increases the antioxidant activity of fibrinogen.

It can be suggested that the increase in antioxidant activities of albumin and fibrinogen may be due to interaction of these plasma proteins with antioxidants from the used fresh juice. This suggestion could be supported by the fact that in patients of the CG group, who did not consume Sweetie juice, the antioxidant activity of serum albumin and fibrinogen remains unchanged.

Therefore, the results of this in vitro and in human investigation show that Sweetie juice possesses cholesterol-lowering and antioxidant properties.

In conclusion, fresh Israeli Jaffa Sweetie juice contains high concentrations of antioxidant compounds and as a consequence possesses a high antioxidant potential. Diet supplemented with fresh Sweetie juice positively influences serum lipid levels and serum, albumin, and fibrinogen antioxidant activities. Therefore, addition of fresh Sweetie juice to a generally accepted diet may be beneficial in the prevention of atherosclerosis, mainly in hypercholesterolemic patients.

ABBREVIATIONS USED

AA, antioxidant activity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ASC, ascorbic acid; β-carotene, β-carotene bleaching; BHA, butylated hydroxyanisole; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging test; SJ, Sweetie juice; GL, glutathione; NRG, naringin; PN, patient; T, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); TE, Trolox equivalent; TEAC, Trolox equivalent antioxidant capacity.

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