© Med Sci Monit, 2005; 11(12): BR465-472
PMID: 16319784

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Received: 2004.08.30
Accepted: 2005.01.04
Published: 2005.12.01

Effect of acute and chronic grapefruit, orange, and pineapple juice intake on blood lipid profile in normolipidemic rat

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: Departmental sources

Summary

Background: High fruit intake is known to be associated with reduced risk of coronary heart disease. Our objective was to determine the effects of acute and chronic juice [grapefruit, orange, and pineapple] intake on plasma lipid profile and lipoprotein metabolism in normolipidemic rats.

Material/Methods: The effects of acute juice intake were studied after three hours of a single juice-lipid load instilled intragastrically. In the chronic study, blood samples from fasted animals were subjected to analyses after six months of either water [control] or water-juice [1:1] intake.

Results: In the acute study, pineapple and grapefruit significantly decreased plasma triacylglycerol [TAG], and chylomicron [CM] TAG and cholesterol concentrations concomitantly with delayed gastric emptying. Plasma cholesterol levels and very-low-density lipoprotein [VLDL] secretion and metabolism were not affected. In the chronic study, only grapefruit significantly decreased plasma and VLDL TAG concentrations and relative VLDL particle size with respect to other groups. All juices significantly increased VLDL apolipoprotein B [apoB] secretion, but plasma total apoB concentrations were highest in the grapefruit group and lowest in the orange and pineapple groups. No effect on blood cholesterol levels was observed.

Conclusions: The cardioprotective benefit of chronic juice intake in normolipidemic rat may be chiefly through mechanisms independent of a direct effect on blood lipid profile, although orange and pineapple, but not grapefruit, relatively improved the metabolism and clearance of blood lipoprotein particles. As a result of delayed gastric emptying, grapefruit and pineapple juices may moderate sharp increases in postprandial plasma TAG concentrations accompanying peak digestion and absorption.

key words: **apolipoprotein B • grapefruit • lipoprotein metabolism • orange • pineapple**

Abbreviations: **Apo B48** – apolipoprotein B48; **ApoB100** – apolipoprotein B100; **CM** – chylomicron; **HDL** – high-density lipoprotein; **IDL** – intermediate-density lipoprotein; **LDL** – low-density lipoprotein; **SDS-PAGE** – sodium dodecyl sulfate polyacrylamide gel electrophoresis; **Sf –** Svedberg flotation rate; **TAG** – triacylglycerol; **VLDL** – very-low-density lipoprotein

Full-text PDF: http://www.medscimonit.com/fulltxt.php?IDMAN=6322
Word count: 4124
Tables: 3
Figures: 6
References: 37

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BACKGROUND

Atherosclerosis is one of the most dangerous diseases in industrial countries and the principal cause of death in Western civilization [1]. A considerable amount of epidemiological and clinical evidence has demonstrated a significantly decreased morbidity and mortality from cardiovascular and other diseases among fruit and vegetable consumers [2,3]. The positive influence of such diet is attributed to their polyphenolic compounds, and particularly flavonoids, which are known to possess antioxidant effect [4,5]. Possessing antioxidant activities, flavonoids are compounds that protect cells against the damaging effects of reactive oxygen species, such as superoxide radicals [4]. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases [5].

Studies conducted on the components of grapefruits and oranges showed the presence of high amounts of polyphenols, pectin fibers, vitamin C, and trace elements [6,7]. Pineapple is also an excellent source of vitamins A, C, pectin fibers, and bromelain [8]. Bromelain, a proteolytic enzyme found in raw pineapple, appears to have several important effects, including antithrombotic and anticoagulant activities *in vivo* [9]. Pectin fiber, on the other hand, has been shown to increase the excretion of lipids, cholesterol, and bile acids, and reduce serum cholesterol levels [10]. Pectins operate by binding with bile acids, thereby decreasing cholesterol and fat absorption [10].

Prior animal studies on miniature swine have demonstrated that pectin inhibits diet-induced hypercholesterolemia, including increases in LDL and VLDL cholesterol, while not altering HDL cholesterol [11]. Other studies revealed that grapefruit pectin did not significantly lower cholesterol levels or lipoprotein fractions of similar animals with long-standing hypercholesterolemia [12]. Studies in humans with hypercholesterolemia concluded that pectin supplementation had modest cholesterol-lowering effect [13], lowering total cholesterol by 7.6% and LDL by 10.8%. However, later human studies by Kurowska et al. [14] conducted on individuals with mild-to-moderate hypercholesterolemia showed that consumption of 750 ml, but not 250 or 500 ml, orange juice per day for 4 weeks improved plasma lipoprotein profile by significantly increasing HDL cholesterol and by reducing the LDL-HDL cholesterol ratio, an effect entirely due to changes in HDL cholesterol concentrations. This observation is in contrasts with the substantial reduction in LDL cholesterol induced in orange juice-fed, hypercholesterolemic rabbits [15]. A lack of change in plasma lipids in normocholesterolemic young men consuming unspecified doses of fresh orange juice for 2 months was also reported [16].

To our knowledge, no reports have dealt with juice effect upon apolipoprotein B synthesis, secretion, and metabolism *in vivo*. Apolipoprotein B, a surface marker of chylomicron, VLDL, IDL, and LDL molecules, allows for a better understanding of juice effect upon lipoprotein synthesis, secretion, and metabolism. Recent studies have revealed that apolipoprotein B is a better indicator of potential myocardial infarction than total cholesterol or LDL cholesterol [17], especially in individuals with low or normal LDL cholesterol [18]. In view of the controversies among previous studies, the present investigation reveals some of the important impacts of acute and chronic orange, grapefruit, and pineapple juice intake upon lipoprotein secretion and metabolism in a normolipidemic rat model, focusing on apolipoprotein B as an excellent marker for atherogenicity.

MATERIAL AND METHODS

Animals

Animal maintenance and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals [19]. All animals were sacrificed using diethyl ether at the end of the procedures described, without recovery from anesthesia. Male Sprague-Dawley rats (200–250 g) (Lebanese American University stock), maintained under a 12 h photoperiod (08.00–20.00) at an ambient temperature of 20–22°C, were fed a standard rat chow diet (Laboratory rodent starter diet no. 1, Hawa Chicken Co, Safra, Lebanon) until 18 h prior to the experiment, when food was withdrawn. The percentage composition by weight of the diet was 19% protein, 9.6% fat, 4.3% fiber, and 61% carbohydrate. Water was available *ad libitum*.

Acute juice study

To study the effect of acute juice intake on postprandial plasma lipoproteins, 32 rats were used. Animals were randomly allocated into four groups of eight animals each: the control, grapefruit, orange, and pineapple groups. After 18 h of fasting, animals received, by stomach tube, an intragastric instillation of a 5-ml emulsion of either 20% (w/v) olive oil containing 9% (w/v) sucrose in water [control group] or 20% (w/v) olive oil in either grapefruit, orange, or pineapple juice [Santal, Parmalat, Italy] for the three remaining groups. Sugar was present in the emulsion of the control group in order to replace the sugar content of the juices used. All emulsions were prepared as described previously [20] and were administered to the animals immediately after sonication. Three hours after instillation, the animals were anesthetized [sodium pentobarbital, 50 mg/kg body weight], blood samples collected from the inferior vena cava, and plasma concentrations of TAG, total cholesterol, HDL cholesterol, and glucose were determined. Similarly, TAG, cholesterol, phospholipid, and apoB concentrations were measured in the lipoprotein fractions CM (Sf >400), VLDL (Sf 20–400), and LDL (Sf 0–20) after density gradient ultracentrifugation of the plasma samples.

To determine the effect of juice on gastric emptying in animals receiving the intragastric load, the stomach of each animal was ligated at the distal esophagus and proximal duodenum just after blood withdrawal, removed, opened, and the liquid content was estimated after blotting with a pre-weighed filter paper. The TAG adsorbed to the filter paper was extracted according to the method of Folch et al. [21].

Chronic juice study

With the aim of studying the effects of chronic juice intake (6 months) on blood lipid profile in the fasted state, 64 male Sprague-Dawley rats, weighing 200–220 g (Lebanese American University stock) were randomly allocated into four groups

of 16 animals each: the control, grapefruit, orange, and pineapple groups. The animals of all groups were fed the same rat chow diet (6.5 g/100 g BW) with the three experimental groups receiving drinking water containing either grapefruit, orange, or pineapple juice in a ratio of 1:1. In order to maintain an isocaloric intake, the animals of the control group received drinking water containing 4.5% by weight sucrose. The animals were weighed on a weekly basis, and modifications in their diets were adjusted accordingly. The animals were maintained on the above regimen for 6 months until 18 hours prior to the experiment, when the fasted animals were anesthetized [sodium pentobarbital, 50 mg/kg body weight] and blood samples collected from the inferior vena cava for blood lipid analyses and lipoprotein fractionation.

Fecal study

During the last month of the chronic juice study, stool samples from the different groups were collected, dried in the oven for 24 hours at 60°C, and subjected to lipid extraction [21] and lipid analysis.

Isolation of TAG-rich lipoprotein fractions

In the acute and chronic studies, about 9 ml of blood were withdrawn from the inferior vena cava using a 10-ml syringe containing Na_2EDTA (1 mg/ml), and transferred to 10-ml tubes. After centrifugation (2000 *g*; 20 min; 4°C), plasma was collected for lipid analysis and isolation of the different lipoprotein fractions. To minimize proteolytic degradation of apoB48 and apoB100, the following were added: 5 µl/ml plasma of aprotonin (Fluka, Switzerland), 2 mg/liter, and 5 µl/ml plasma of phenylmethylsulfonyl fluoride [PMSF], 5 mM in 2-propanol. Lipoprotein fractions (Sf >400, Sf 20–400, and Sf >20) were isolated by density gradient ultracentrifugation according to the method described by Karpe et al. [22] and as described in detail previously by Daher et al. [23]. Total apoB (apoB48 and apoB100) content in the plasma of the fasted animals was estimated in the lipoprotein fraction (d <1.063 g/ml) that includes CM, VLDL, intermediate-density lipoprotein [IDL], and low-density lipoprotein (LDL). Briefly, 2 ml of plasma were put in a 10 ml polycarbonate ultracentrifuge tube (Sorvall, Kendro Laboratory Products) and 140 mg/ml of solid NaCl was added to increase the density to 1.1 g/ml. The plasma sample was overlayered with 5 ml of NaCl solution [d=1.063 g/ml] containing 0.01% w/v Na_2EDTA and 0.02% w/v NaN_3 [pH=7.4]. The top 0.5 ml lipoprotein layer was collected after 48 h of centrifugation at 28,000 rpm at 15°C [Sorvall RC 28S centrifuge; Supraspeed F-28/13 fixed-angle rotor].

Samples containing the different lipoprotein fractions were delipidated in a methanol-diethyl ether solvent system [22]. The protein material was dissolved in 0.15M sodium phosphate, 12.5% v/v glycerol, 2% w/v sodium dodecyl sulfate (SDS), 5% v/v mercaptoethanol, and 0.001% w/v bromomophenol blue, pH=6.8, at room temperature for 30 min, denatured at 90°C for 4 min, and centrifuged for 4 min at 15,680 *g*. Samples, frozen at –20°C, were subjected to 4–20% SDS-PAGE within 3 days and then analyzed for apoB48 and apoB100 concentrations [23]. All samples were run in duplicate.

Human apo B100 was used as standards for rat apo B48 or apo B100 quantification. It has been shown that human apoB100 and rat apo B48 and apo B100 have similar chromogenicity when stained with Coomassie Blue R-250 [24,25]. Hence validating the use of human apolipoprotein B100 as a standard. Apolipoprotein B100 standards were prepared from human LDL (1.030< d <1.040 g/ml) isolated from fasting human plasma samples by the density gradient ultracentrifugation procedure [22].

In the Sf >400 lipoprotein fraction, only CMs of intestinal origin having apoB48 as a molecular marker are collected, while small intestinal CMs and VLDLs newly secreted by the liver are collected in the Sf 20–400 lipoprotein fraction. In the human model, small CMs and VLDLs can be differentiated by their apoB48 and apoB100 markers, respectively. However, a liver secretion of VLDL with either apoB48 or apoB100 as a surface marker distinctively characterizes the rat species [26]. Consequently, the assessment of the number of small CMs in the Sf 20–400 lipoprotein fraction is not feasible. Therefore, apoB48 of the latter lipoprotein fraction was considered to be a VLDL constituent.

Measurement of lipids and glucose

Total serum cholesterol and LDL cholesterol levels were measured using a cholesterol ester/oxidase enzymatic procedure. HDL cholesterol levels were measured directly using an enzymatic colorimetric method that incorporated polyethylene glycol-modified cholesterol ester oxidase. TAG levels were measured using a glycerol kinase-based enzymatic procedure. Phospholipid levels were measured using a phospholipase/choline oxidase-based enzymatic procedure. Glucose levels were measured using a glucose oxidase-based enzymatic procedure.

VLDL size

VLDL size was expressed as the ratio of VLDL TAG (calculated as triolein) to VLDL apoB.

Statistical analysis

Values are presented as means ±SEM. The effect of lipid loading in each group was analyzed statistically by analysis of variance. The comparison between two groups was made by independent *t* test. An α level of.05 was considered significant for both the *t* test and analysis of variance.

RESULTS

Effects of acute juice intake

Three hours after intragastric instillation of the corresponding emulsions in the four groups [grapefruit, pineapple, orange, and control], the animals were anesthetized and blood samples were collected. Concentrations of plasma TAG and total cholesterol were determined (Figure 1) as well as the concentrations of TAG and cholesterol in the different lipoprotein fractions CM (Sf >400), VLDL (Sf 20–400), and LDL (Sf 0–20) (Table 1). The results showed that all juice groups caused a decrease in mean plasma TAG, CM TAG, and CM cholesterol concentrations with respect to the control group, but significance was only reached with the pineapple and grapefruit groups. The mean plasma cholesterol concentrations were similar in all groups. The mean VLDL

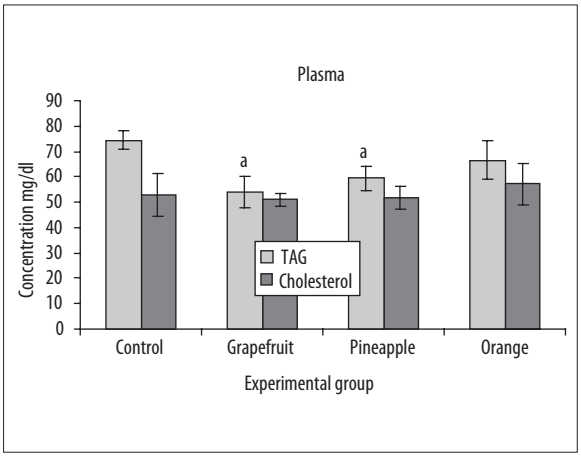

Figure 1. Plasma TAG (mg/dl) and plasma cholesterol (mg/dl) concentrations as determined 3 hours after intragastric instillation of a 5-ml emulsion of either 20% (w/v) olive oil containing 4.5% (w/v) sucrose in water [controls] or 20% (w/v) olive oil in either grapefruit, orange, or pineapple juice. Values denote mean ±SEM (n=8). [a] $p<0.05$ compared with controls.

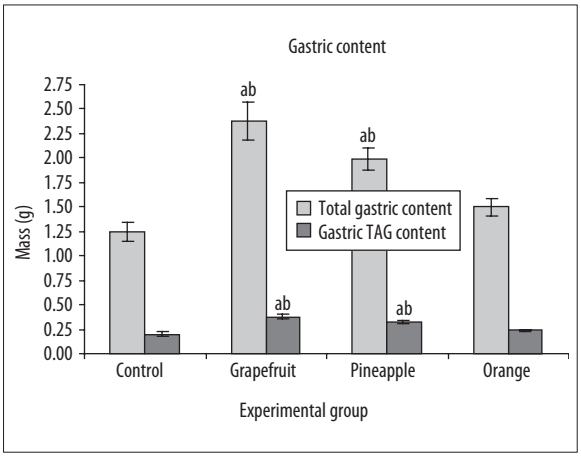

Figure 2. Total gastric content (g) and gastric TAG content [g] as determined 3 hours after intragastric instillation of a 5-ml emulsion of either 20% (w/v) olive oil containing 4.5% (w/v) sucrose in water [controls] or 20% (w/v) olive oil in either grapefruit, orange, or pineapple juice. Values denote mean ±SEM (n=8). [a] $p<0.05$ compared with controls, [b] $p<0.05$ compared with orange.

Table 1. Chylomicron TAG (mg/dl), chylomicron cholesterol (mg/dl), VLDL TAG (mg/dl), VLDL cholesterol (mg/dl), LDL TAG (mg/dl), and LDL cholesterol (mg/dl) concentrations as determined 3 hours after intragastric instillation of a 5-ml emulsion of either 20% (w/v) olive oil, 4.5% (w/v) sucrose in water (controls), or 20% (w/v) olive oil in either grapefruit, orange, or pineapple juice.

	CM TAG (mg/dl)	CM Cholesterol (mg/dl)	VLDL TAG (mg/dl)	VLDL Cholesterol (mg/dl)	LDL TAG (mg/dl)	LDL Cholesterol (mg/dl)
Controls	134.5±19.5	6.1±0.8	36.2±3.9	17.1±1.2	7.8±1	8.8±0.7
Grapefruit	95.4±7.5[a]	5.3±0.5[a]	35.3±3.4	14.7±1.3	7.8±1.5	7.9±0.4
Pineapple	92.6±12.0[a]	5.3±0.4[a]	38.6±4.4	18.2±1.4	7.8±1.1	8.2±1.2
Orange	105.5±14.8	5.9±0.8	35.2±3.0	18.1±1.6	7.5±0.9	8.4±1.1

Values denote mean ±SEM (n=8); [a] $p<0.05$ compared with control.

TAG, VLDL cholesterol, LDL TAG, and LDL cholesterol concentrations in all groups were similar and no significant changes were detected.

The mean gastric contents retained in the stomach 3 hours after instillation of the intragastric load are shown in Figure 2. The data showed that increased gastric retention was associated with juice intake. The grapefruit group showed the highest gastric retention, while the orange group showed the least gastric retention among the different juice groups. This increase in gastric retention reached significance when comparing the grapefruit and pineapple groups with the orange and control groups. Significance was not attained between the orange and control groups. Determination of gastric TAG content retained in the stomach 3 hours following the intragastric load also revealed an increased TAG retention in the stomach of all juice groups with respect to the control group (Figure 2). However, significance was only reached when comparing the grapefruit and pineapple groups with the orange and control groups. There was no significant difference between the orange and control groups.

Effects of long-term chronic juice intake

The effects of a six-month period of chronic juice intake upon blood lipid profile in the fasted state was also assessed. Data (Table 2) revealed that when compared with the grapefruit group, the control, orange, and pineapple groups showed a significant increase in their mean plasma TAG concentrations. All the latter groups showed similar plasma TAG concentrations. The circulating concentrations of plasma cholesterol as determined in the four groups did not show any significant change, and the same applies to plasma HDL cholesterol and plasma glucose concentrations (Table 2).

The mean VLDL TAG concentrations (Figure 3) in both the grapefruit and control groups were significantly lower than both the pineapple and orange groups. Also, VLDL TAG concentrations of the grapefruit group showed a significant decrease when compared with the control group. No significant difference was observed between the orange and the pineapple groups. Compared with the control and grapefruit groups, the pineapple group showed a significant in-

Table 2. Plasma TAG (mg/dl), glucose (mg/dl), total cholesterol (mg/dl), HDL cholesterol (mg/dl), LDL cholesterol (mg/dl) concentrations, and LDL cholesterol / HDL cholesterol ratio as determined in fasted rats after a 6-month period of chronic juice intake (grapefruit, orange, and pineapple) in drinking water (50% v/v).

	TAG (mg/dl)	Glucose (mg/dl)	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl) [d<1.063 g/ml]	LDL Cholesterol / HDL Cholesterol
Controls	50.8±4.3	140.5±11.5	64.0±2.9	43.1±3.0	26.7±2.4	0.62
Grapefruit	33.1±3.1[a]	136±11.0	58.7±4.4	41.3±2.7	28.2±2.5	0.68
Pineapple	54.4±4.1	123.3±8.6	67.9±4.2	39.8±2.2	30.2±3.9	0.76
Orange	54.1±4.9	123.1±8.2	63.2±4.1	39.4±4.2	25.0±3.3	0.63

Values denote mean ±SEM (n=16); [a] $p<0.05$ compared with controls.

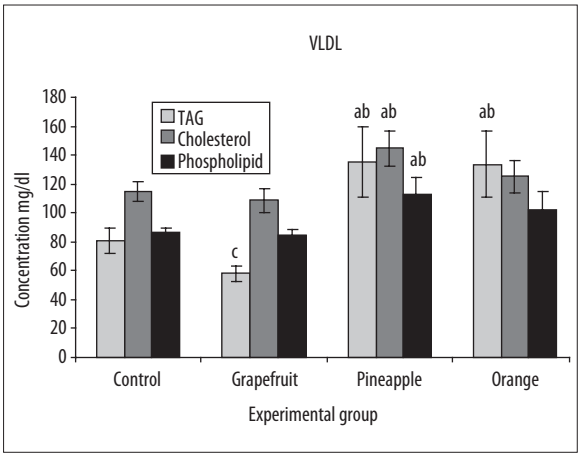

Figure 3. TAG (mg/dl), cholesterol (mg/dl), and phospholipid (mg/dl) concentrations in plasma VLDL (Sf 20–400) lipoprotein fractions as determined in fasted rats after a 6-month period of chronic juice intake (grapefruit, orange, or pineapple) in drinking water (50% v/v). Values denote mean ±SEM (n=16). [a] $p<0.05$ compared with controls, [b] $p<0.05$ compared with grapefruit, [c] $p<0.05$ compared with controls, orange, and pineapple.

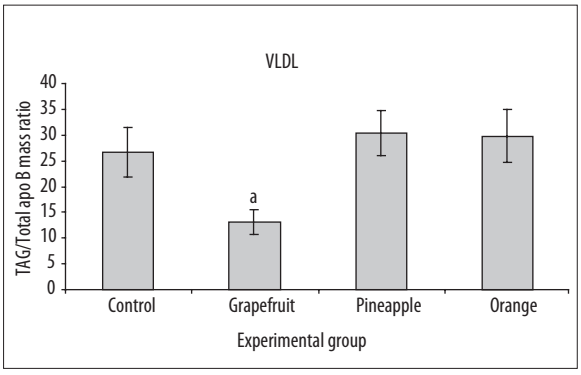

Figure 5. TAG/Total apo B mass ratio in plasma VLDL (Sf 20–400) lipoprotein fractions as determined in fasted rats after a 6-month period of chronic juice intake [grapefruit, orange, or pineapple] in drinking water (50% v/v). Values denote mean ±SEM (n=16). [a] $p<0.05$ compared with controls, orange, and pineapple.

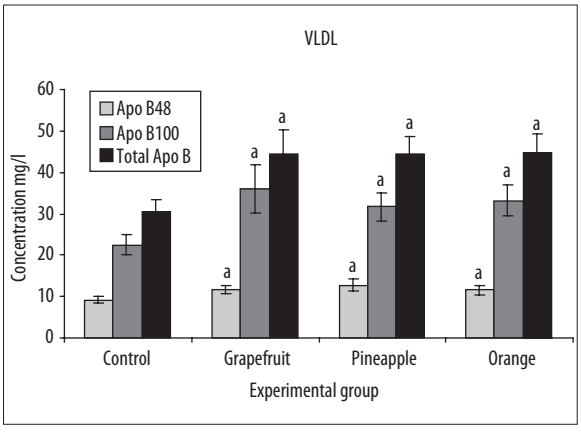

Figure 4. Apo B48 (mg/l), apo B100 (mg/l), and total apo B [apo B100 + apo B48] (mg/l) concentrations in plasma VLDL (Sf 20–400) lipoprotein fractions as determined in fasted rats after a 6-month period of chronic juice intake (grapefruit, orange, or pineapple) in drinking water (50% v/v). Values denote mean ±SEM (n=16). [a] $p<0.05$ compared with controls.

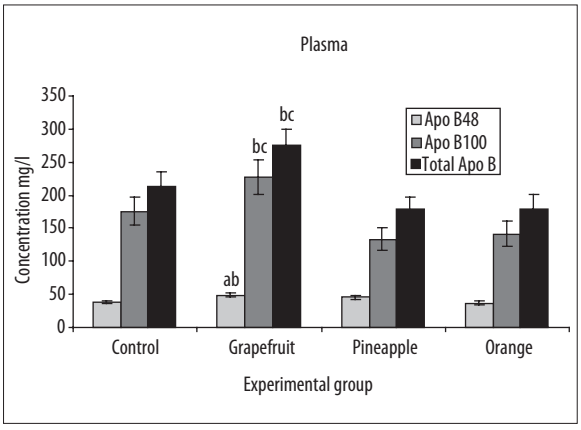

Figure 6. Plasma total apo B48 (mg/l), total apo B100 (mg/l), and total apo B (mg/l) concentrations as determined in fasted rats after a 6-month period of chronic juice intake [grapefruit, orange, or pineapple] in drinking water (50% v/v). Values denote mean ±SEM (n=16). [a] $p<0.05$ compared with controls, [b] $p<0.05$ compared with orange, [c] $p<0.05$ compared with pineapple.

Table 3. Fecal TAG (mg/g), fecal cholesterol (mg/g), and body weight changes (g) as determined in fasted rats after a 6-month period of chronic juice intake (grapefruit, orange, or pineapple) in drinking water (50% v/v).

	Fecal TAG (mg/g)	Fecal Cholesterol (mg/g)	Body Weight Change (g)
Controls	0.75±0.09	1.8±0.2	72±4
Grapefruit	0.88±0.12	1.9±0.3	74±6
Pineapple	0.73±0.07	1.9±0.1	81±5
Orange	0.97±0.12	2.1±0.2	76±3

Values denote mean ±SEM (n=16).

crease in mean VLDL cholesterol concentrations (Figure 3). No significant differences were observed among the grapefruit, orange, and control groups. Similar findings were observed for VLDL phospholipid levels (Figure 3).

Determination of VLDL apo B100 and apo B48 concentrations showed similar concentrations in the different juice groups (Figure 4); however, all the juice groups exhibited a significant increase in their VLDL apo B100 and apo B48 concentration compared with the control group. Calculation of total apo B (apo B48 + apo B100) concentration (Figure 4) revealed a significant increase in VLDL apo B secretion with juice intake irrespective of the juice type when compared with the control group (Figure 4). Estimation of VLDL size as VLDL TAG/Total apo B (Figure 5) showed that the liver of the grapefruit group secreted by far the smallest mean VLDL particle size with respect to all other groups. Although slightly larger, neither the orange nor pineapple groups had any significant effect on particle size with respect to the control group.

The circulating concentrations of total apo B100, apo B48, and total apo B (d <1.063 g/ml) were determined in the four groups (Figure 6). The grapefruit group showed the highest apo B100 concentration among all the groups. A significant difference was reached between the grapefruit group and the other two juice groups (orange and pineapple). The latter two groups did not show any significant difference from the control group. The mean total apo B48 concentration in the grapefruit group showed a significant increase compared with the control and orange groups. However, neither the orange nor pineapple groups had any significant difference from the control group. Calculations of the total apo B concentration (apo B100 + apo B48) revealed that the grapefruit juice group had by far the highest apo B concentration among all the groups. However, significance was only reached when comparing the grapefruit group with the orange and pineapple groups. Although apo B concentrations of the control group were substantially lower than in the grapefruit group, significance was not reached (p<0.09).

The concentrations of fecal TAG and fecal cholesterol were also determined in the four groups as shown Table 3. Comparison of the mean fecal TAG concentrations of all groups did not show any significant difference. Similarly, no significant differences in fecal cholesterol were observed among all groups. Determination of the mean body weight changes showed that all juice types had no impact on body weight changes over the six months of the study period (Table 3).

Discussion

The effects of a six-month period of juice intake were investigated in normolipidemic rat model. The data showed that, compared with the control group, none of the juices used had a significant effect on blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and the ratio LDL cholesterol to HDL cholesterol. Surprisingly, only animals of the grapefruit group showed a drastic decrease in plasma TAG concentrations with respect to all other groups. This decrease in mean plasma TAG concentration appeared to be the result of a direct effect of grapefruit juice on liver TAG secretion, since VLDL particles of the grapefruit group carried by far the least amount of TAG. Unlike the grapefruit group, both the pineapple and the orange groups significantly increased VLDL TAG secretion with respect to the control and grapefruit groups, indicating different impacts of juice types on liver TAG secretion. To better understand the mechanism involved, the concentrations of total apo B and VLDL apo B were determined. Knowing that each VLDL particle contains one apo B molecule [27,28], measuring plasma apo B as apo B100 or apo B48 reflects exactly the total number of newly secreted VLDL particles. The present data have shown that all juices increased significantly total VLDL apo B secretion, indicating that irrespective of the juice type an increase in VLDL secretion is observed. However, determination of total plasma apo B [representing VLDL, IDL, and LDL] reveals that the grapefruit group had the highest apo B concentrations among all the groups, while both the orange and pineapple groups had the lowest apo B concentrations. This indicates that, unlike grapefruit, chronic consumption of either pineapple or orange juice increases the rate of metabolism and clearance of lipoprotein particles from the blood. Since apolipoprotein B is a better indicator of potential myocardial infarction than total cholesterol or LDL cholesterol [17] especially in individuals with low or normal LDL cholesterol [18], chronic orange and pineapple juice consumption, but not grapefruit, may be recommended as a means to somehow improve (about 18%) total plasma apo B concentration in normolipidemic subjects. Why chronic intake of grapefruit juice did not speed the metabolism and clearance of lipoprotein particles remains a subject for further studies. Early studies showed that large lipoprotein particles [29,30] are cleared more rapidly than smaller ones. Thus a possible explanation may reside in the small-sized VLDL particles originally secreted by the liver of the grapefruit group.

Previous *in vitro* studies in HepG2 human hepatoma cells [31] have shown that the flavonoids hesperetin and naringenin,

present in orange and grapefruit juices, respectively, drastically reduced net apo B secretion. HepG2 human hepatoma cells [31], a commonly used model of human hepatocytes, have a defect in the second step of lipoprotein assembly which allows the addition of only small quantities of TAG to the primordial particle [32]. Consequently, these cells secrete small lipoprotein particles with a density similar to that of LDL [33]. On the other hand it is reported in the same *in vitro* study [31] that both naringenin and hesperetin failed to inhibit net apo B secretion after 4 h in the presence of oleate. *In vivo*, hepatocytes are constantly exposed to TAG present in the blood even in the fasted state. Consequently, the inhibition observed in the *in vitro* study [31] in HepG2 cells in the absence of TAG may not be applicable to our *in vivo* study.

Naringenin was found to inhibit the lipidation and subsequent secretion of apo B-containing lipoproteins in HepG2 human hepatoma cells [34]. Although such a finding may explain the reduced VLDL TAG secretion in the grapefruit group, it seems somehow unlikely, since naringin failed to affect plasma lipids and apolipoprotein levels when supplemented to normolipidemic subjects [35]. Recent evidence in our lab has shown a high correlation between liver fat content and VLDL TAG secretion. However, this seems not to be the case in the present study, since a one-month period of chronic grapefruit juice intake did not affect the fat content in the liver (4.51% of the control group versus 4.43% of the grapefruit group). Thus the reason behind reduced liver TAG secretion is still unclear and studies are underway in order to clarify the mechanism involved. As a preliminary conclusion, chronic grapefruit juice intake, unlike orange and pineapple juice intake, seems to inhibit the addition of TAG to the preformed VLDL particles without a concomitant inhibition of the number of particles to be secreted.

Studies on miniature swine with long-standing hypercholesterolemia and ingesting a lipid-rich diet revealed that grapefruit pectin did not significantly lower cholesterol levels or lipoprotein fractions [12]. However, the same study [12] showed that pectin either regressed atherosclerosis induced by 1 year of sustained hypercholesterolemia or interfered with lesion progression. This indicates that pectin, a major component of juices used in the present study, may inhibit atherosclerosis via a mechanism independent of a direct effect on lipid levels. On the other hand, the disagreement among the different studies dealing with the effects of pectin [11,13] or juice [14,15,16] on blood cholesterol levels may be attributed to several possible reasons, such as species differences, doses of juice, length of study and, most importantly, the state of being normo- or hypercholesterolemic. It appears that the ability of chronic juice intake to improve blood lipid profile is mainly correlated with the degree of hypercholesterolemia, where subjects with the highest blood cholesterol levels would benefit the most, while those with normal blood cholesterol may not benefit at all. This is supported by the present study and other studies in humans [14,16] where chronic juice intake failed to affect blood cholesterol levels in normocholesterolemic subjects.

Dietary pectins have previously been shown to increase the excretion of fecal neutral steroids in rabbits [10], and thus contribute indirectly to reduction of blood cholesterol levels. Other studies in hypercholesterolemic rabbits [15], however, showed that both orange and grapefruit juices, both rich in pectin, did not increase fecal cholesterol excretion but, on the contrary, significantly decreased its excretion in the feces. In the present study we have shown that chronic juice [orange, grapefruit, and pineapple] intake did not significantly affect fecal excretion of cholesterol and TAG in the rat model. Differences in results might be attributed to species differences (rat versus rabbit) and to different amounts of pectin received by the animals. The role of soluble dietary fiber in reducing plasma cholesterol levels via increasing fecal cholesterol excretion needs further studies and characterizations.

The effect of acute juice consumption on blood lipid levels 3 hours postprandially was also investigated. This period falls within the steady-state period of lipid absorption [20]. Our data revealed that both pineapple and grapefruit groups had significantly lower plasma TAG concentrations with respect to the control group. This effect appeared to be the result of a reduced rate of gastric emptying. A delayed gastric emptying may help moderating postprandial hypertriglyceridemia, especially in patients unable to clear postprandial TAG from the blood rapidly. However, grapefruit juice is well known to interact with a large number of prescribed drugs by inhibiting a special enzyme in the intestine responsible for the natural breakdown and absorption of many medications. Among these drugs are Zocor (Simvastatin) [36] and Lipitor (Atorvastatin) [37], the most commonly used drugs by hyperlipidemic subjects. Consequently, pineapple juice would be the juice of choice rather than grapefruit in order to avoid drug interaction complications. Unlike the chronic study, the acute study does not support the possibility of a rapid direct effect of juices on liver (3 h), since VLDL TAG concentrations of all groups were similar.

Historically, grapefruit juice intake has been associated with weight loss. Grapefruit itself has no direct effect on weight loss, as observed in the present and previous studies [15]. However, grapefruit juice may possibly help people on a diet by delaying gastric emptying, extending satiety, and reducing the feeling of empty stomach.

CONCLUSIONS

A six-month period of chronic juice intake in normolipidemic rat showed a modest effect on blood lipid profile. Chronic grapefruit juice intake had a direct inhibitory effect on liver TAG secretion; however, this was accompanied by a reduced metabolism and clearance of lipoprotein particles. In contrast, both orange and pineapple juice appeared to increase relatively the metabolism and clearance of the lipoprotein particles from the blood. Intake of grapefruit or pineapple juices together with the diet may moderate sharp increases in postprandial plasma TAG concentrations accompanying peak digestion and absorption, an effect related to delayed gastric emptying.

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