Food and Nutrition Sciences, 2014, 5, 2225-2236
Published Online December 2014 in SciRes. http://www.scirp.org/journal/fns
http://dx.doi.org/10.4236/fns.2014.522236

Scientific Research

Effectiveness of Apricots (*Prunus armeniaca*), Pomegranate (*Punica granatum*) Juice and Lactic Acid Fermented Sobya on Plasma Levels of Lipid Profile Parameters and Total Homocysteine among Egyptian Adults

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Received 21 September 2014; revised 20 October 2014; accepted 16 November 2014

Abstract

Objectives: The aim of this study was to investigate the effectiveness of dietary intervention with apricots, pomegranate juice or lactic acid fermented sobya on improving the plasma levels of lipid profile parameters and total plasma homocysteine (tHcy) among healthy adults. Design of the study: Thirty-five men and women (28.9 ± 3.0 years) with mean body mass index of 23.5 ± 1.2 kg/m^2 were randomly assigned to a dietary intervention study. During the pre-feeding study records of 24-hour food intake, anthropometric measurements and blood sampling were collected for biochemical investigations. Thereafter the volunteers were divided into five groups: a control group (C); the other four groups received daily one of the following supplements: 200 g of deep yellow apricots (*Prunus armeniaca*) (AF); 250 g pomegranate (*Punica granatum*) juice (PJ); mixture of 100 g (PJ) and 150 g of fermented sobya (FS), (PJ-FS) or 165 g (FS). After completing the three-week dietary intervention, blood samples were collected and blood indices were again evaluated. Biochemical analysis of total lipids (TL), total glycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) and tHcy were completed. The low density lipoprotein-cholesterol level and three indices of risk factors were calculated using the appropriate equations. Results: Three-week dietary intervention improved the levels of lipid profile parameters TG,

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How to cite this paper: Hussein, L., *et al.* (2014) Effectiveness of Apricots (*Prunus armeniaca*), Pomegranate (*Punica granatum*) Juice and Lactic Acid Fermented Sobya on Plasma Levels of Lipid Profile Parameters and Total Homocysteine among Egyptian Adults. *Food and Nutrition Sciences*, **5**, 2225-2236. http://dx.doi.org/10.4236/fns.2014.522236

TC, HDL-C and LDL-C as compared to the respective baseline levels and the response of the control group that did not receive any supplement. Consuming (PJ-FS) had a synergistic beneficially added value on some of the above mentioned lipid profile parameters. The mean plasma tHcy concentrations; a marker for occlusive vascular disease was reduced significantly following the three week consumption of (PJ) or (FS), compared with the respective pre-feeding levels. Conclusion: Pomegranate juice (PJ) or lactic acid fermented sobya (FS) or mixture of both are potential natural agents in lowering the plasma levels of lipid profile parameters and tHcy among apparently healthy young women and men, without dyslipidemic symptoms.

Keywords

Healthy Adults, Deep Yellow Apricots, Fresh Unsweetened Pomegranate Juice, Lactic Acid Fermented Sobya, Plasma Lipid Profile Parameters, Plasma tHcy

1. Introduction

The WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World [1]. Hypercholesterolemia is a disorder characterized by high levels of blood cholesterol and elevated low density lipoprotein cholesterol (LDL-C), which represents the first stage of atherosclerosis, a risk factor for CHD [2]. Because hypercholesterolemia causes no symptoms, preventive measures and regular measurement of cholesterol levels are important for people in high-risk categories. Published reports indicate that approximately 27% of the Egyptian population are suffering from hypertension; while 20% are suffering from hypercholesterolemia, with more than double the cardiovascular death rates compared with the age matched respective figure in the United States [3]. Data from the national survey of lipid profiles indicate that the overall proportion of Egyptian adults with high total cholesterol is 46.0 percent; the proportion with high LDL-cholesterol is 18.0 percent, with high triglycerides 16 percent [4].

Analysis of the plasma of asymptomatic hypercholesterolemic Egyptian adults (total cholesterol >200 mg/dL; >5.2 mmol/l) contained significantly higher levels of lipid peroxidation (TBARS) ($P < 0.001$) and glutathione peroxidase activity (markers for oxidative stress) compared to the respective mean values obtained with the control [5].

Statins are the most commonly used drugs for lowering cholesterol [6]. Statin inhibits the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA) [7]. Muscle pain and toxicity (Myopathy) are the most troublesome adverse effect of statins [8].

Dietary approaches and some natural products are effective hypolipidemic agents; lowering TC, LDL-C and TG [9] and are important to the general population, who do not yet have dyslipidemic symptoms. Among the natural hypolipidemic fruits, pomegranate and derived products have been acknowledged with health-beneficial effects from ancient times [10]. The therapeutic effect of PJ was attributed to high polyphenolic compounds and the presence of anthocyanin & ellagitannins pigments, mainly punicalagins [11] [12]. The hypocholesterolaemic effect of PJ was attributed to its inhibitory effect on the activities of 3-hydroxy-3-methylglutaryl-CoA reductase and sterol O-acyltransferase, two key enzymes in cholesterol metabolism [13].

The use of food probiotics as agents for the management of hypercholesterolemia had been repeatedly reported and the accepted proposed mechanism is mediated by the probiotic bile salt hydrolase (BSH) activity, which hydrolyzes conjugated glycodeoxycholic acid and taurodeoxycholic acid. The hydrolysis products inhibit the absorption of cholesterol and suppress the reabsorption of bile acid (Bsh) [14]. Patients with plasma cholesterol (TC) levels ranging between 5.4 - 8.3 mmol/L) consuming Lactobacillus acidophilus L1 in yoghurt for 10 weeks were reported to have significant reduction in their plasma TC level [15]. In another clinical trial, the 4-week ingestion of *Bifido longum* BL1 (10^8 CFU per gram of yoghurt) to hypercholesterolemic patients (plasma TC level 5.7 - 7.2 mmol/l^{-1}) reduced TC significantly ($P < 0.05$), with concomitant 14.5% increase in HDL-C, compared to the respective changes in the control group consuming yoghurt without *B. longum* BL1. Similar positive findings were obtained after daily consumption of the probiotic *L. plantarum* strains at dosages of 10^7 to 10^{11} CFU [16].

The aim of the present investigation is to assess the effectiveness of some natural pre- and probiotic products

on plasma levels of lipid profile parameters and tHcy among apparently healthy young women and men.

2. Materials and Methods

Participants: The study subjects were 35 healthy adults, who satisfied the inclusion criteria: young adults with age ranging between 20 - 34 years, without metabolic diseases, not using any medication for the last 6 weeks, and with no signs of allergy or hypersensitivity to any food or material. Subjects were laboratory staff from the National research center. Compliance with the supplementation in all subjects was satisfactory as assessed by daily contact. All subjects continued their habitual diets during the study. The research protocol received approval from the institution review board and all subjects provided written, informed consent before participation in the study.

Dietary survey: Estimated dietary intake was assessed by 3 repeated food records one week before enrollment in the trial. The average portion size consumed and composition data values from nutrient composition of the food were combined to assess average daily energy and nutrient intakes by the nutrisurvey software program. The characteristics of the voluntary subjects enrolled in the study and the mean daily intake of energy and selected macro nutrients are presented in the table (**Table 1**).

Supplements: Apricots and pomegranate were purchased in bulk from the Obour public market. The apricot fruits were washed and the fruits were packed in 218 g packages, which is equivalent to 200 g net weight without stones. The pomegranate fruits were peeled and the juice was obtained by using a laboratory pilot press (Braun, Germany). The juice was distributed in aliquots of 100 or 250 grams in air tight polyethylene bottles, protected from light and saved frozen at −20˚C. Under this storage condition, the pomegranate polyphenols were fully stable [17]. Sobya Lactic acid fermented porridge was purchased twice a week from the retail market (Brand name Elrahmany, Sayeda Zeinab, Cairo) and saved in the refrigerator. Sobya is fermented rice containing per gram 3×10^7 cfu diverse lactic acid bacteria (LAB) and 1×10^7 cfu *Sacharomyces cerivisiae*. Other ingredients: added milk, sugar and grated coconut. **Table 2** presents the proximate chemical composition and the bacterial counts of the apricot fruits (AF), pomegranate juice (PJ) and fermented sobya (FS).

Table 1. Characteristics of the study subjects at the pre-feeding phase.

Parameter	Unit	Mean ± SE	Range	Normal Range
Estimated Dietary Fiber Intake	g/d	29.2 ± 4.0	24.4 - 33.2	≤25
Estimated Energy Intake	Kcal/d	2349.3 ± 227	2114.2 - 2877.4	2200 - 2600
Dietary Fat	g	61.4 ± 6.7	27 - 84	50 - 70
Males	%	79	-	-
Age	years	24.4 ± 0.7	20 - 30	-
BMI	$Kg \cdot ml^{-2}$	23.7 ± 0.9	18 - 30	25
Biochemical Analysis of Plasma				
Total Lipid	mg/dl	712.9 ± 45	580.1 - 747.6	400 - 1000
Triglycerides	$mmol \cdot l^{-1}$	1.3 ± 0.1	0.6 - 2.0	0.45 - 1.71
Total Cholesterol	$mmol \cdot l^{-1}$	5.0 ± 0.0	4.31 - 6.5	<5.2
HDL-C	$mmol \cdot l^{-1}$	1.0 ± 0.0	0.7 - 1.5	>0.91
LDL-C	$mmol \cdot l^{-1}$	3.3 ± 0.1	2.2 - 4.9	<3.4
VLDL-C	$mmol \cdot l^{-1}$	0.6 ± 0.2	0.2 - 0.9	0.128 - 0.645
LDL-C/HD L C ratio		2.8 ± 0.06	1.5 - 5.3	1.5
Atherogenic Index		3.4 ± 0.17	2.0 - 6.6	-
Total Chol/HDL-C Ratio		4.4 ± 0.17	3.0 - 7.5	≤4
Albumin	$g \cdot l^{-1}$	40.8 ± 1.1	34.5 - 42.9	35 - 50
tHcy	$\mu mol \cdot l^{-1}$	18.03 ± 4	10.5 - 31.8	5.9 - 15.3
IgA	mg/dl	127.45 ± 44.1	30 - 301	45 - 300

Table 2. Composition of the supplements.

		Apricot	Pomegranate Juice	PG-FS	Fermented Sobya
Parameter	Unit	Portion Served	Portion Served	Portion Served	Portion Served
Portion Size	g	200*	250*	100 - 150	167
Total Solids	g	-	25	64	60
Water	g	-	214.75	202	130
Protein	g	2.8	0.5	3.6	4
Lipid	g	0.8	0.75	4.3	4.6
Carbohydrate	g	22	32.75	59	51.1
Energy	Kcal	100	135	290	263
pH		-	-	-	3.5
Total Bifidobacterium	CFU	-	-	-	-
Total Lactobacilli	CFU	-	-	4.5×10^9	5×10^9
Yeast	CFU	-	-	1.4×10^9	1.67×10^9
Dietary Fiber	g	4	0.25	48.6	54
Vitamin C	mg	20	0.25	0.1	-
Total-ORAC	umol TE/200g	2230	5852.5	1170.5	-
Phyto-Nutrients	µg	2612	-	-	-
Tannin	mg	-	500	200	-

*Apricots (*Prunus armeniaca*), fresh, Nutritive Value per 100 g, Total-ORAC µmol Trolox equivalent (TE)/100 g - 1115) pomegranate (*Punica granatum*) 2341 µmol TE/100 g. Source: USDA [52].

Design of the Study

A randomized controlled trial was conducted and the protocol is outlined in the **Flow Chart 1**. At prefeeding, the subjects were assigned into five groups of equal number using computer-based randomization. Group (1) served as the control and didn't receive any supplement. Group (2) received daily 200 g (AF); group (3) a bottle containing 250 g of (PJ) equivalent to; group (4) a mixture of (PJ) (100 g) and (FS) (150 g) and the last group received (FS) 167 g. The volunteers in each group consumed the supplement daily between 5 - 6 pm.

3. Blood Sampling and Laboratory Investigation

Polyphenol measurement: The total polyphenol concentration of the PJ was determined spectrophotometrically with phosphomolybdic phosphotungstic acid reagent [18] and gallic acid as a standard.

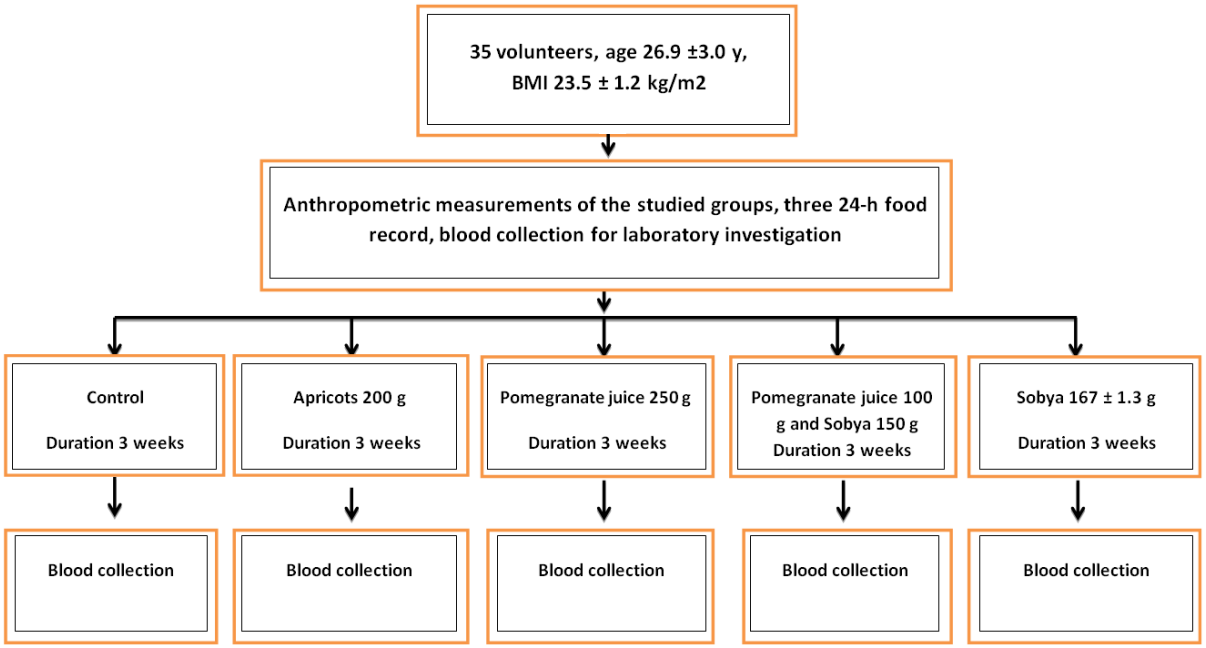
Blood sampling: Blood samples were taken from the antecubital vein and were collected in vacutainer tubes coated with sodium citrate. After centrifugation at 3000 rpm in the cold, the plasma samples were saved frozen at −70˚C. Total cholesterol (TC) and triglyceride (TG) were assayed enzymatically by microplate methods [19] with a kit (Biodiagnostics-Egypt). HDL-cholesterol (HDL-C) was assayed after precipitation of the apo β-containing lipoproteins with phosphotungstic acid and magnesium ions with commercial kit (Biodiagnostics, Egypt). The performance of these assays was regularly certified by reference plasma samples The Friedewald equation [20] was used for evaluation of plasma LDL-Cholesterol

Plasma LDL-C (mmol·l^{-1}) = TC (mmol·l^{-1}) – (HDL-C mmol·l^{-1} + Triglycerides mmol·l^{-1}/5).

Plasma tHcy was separated by tandem spectrophotometry [21]; the plasma albumin was determined colorimetrically by the bromocresol green method with commercial kit (Bio diagnostics, Egypt). Plasma immunoglobulin A was assayed by the radial immune diffusion (RID) method according to instructions given by the manufacturer (FAR, Verona, Italy).

4. Statistical Analysis

Quantitative data were expressed as arithmetic means with standard error of the mean. All evaluated variables are presented as means ± SE. Statistical analysis was performed with the use of Excel sheet. Differences between



Flow Chart 1. Illustrating the design of the randomized controlled trial.

treatments were examined for significance by one way ANOVA and P = 0.05 was considered statistically significant.

ANOVA test was used to compare between groups whether at baseline or subsequent to treatment, while, paired t-test was used to compare within groups between baseline and subsequent to treatment. P < 0.05 indicates significant statistical differences.

5. Results

Table 1 presents characteristics of the participants and the baseline mean plasma levels of lipid profile parameters and tHcy. The mean plasma levels of TC (5.0 mmol·l^{-1}); LDl-C (3.3 mmol·l^{-1}) and HDL-C (1.0 mmol·l^{-1}) were within the respective reference norms of <5.2; 2.6 - 3.3 and ≥1.04 mmol/l·mmol·l^{-1} (NCEP, 2001). However, 15% of the subjects were presented with hypercholesterolemia and 24% were presented with LDL-C above 3.3 mmol·l^{-1}; while 87% of the participants had initial plasma HDL-C less than the norm of 1.0 mmol·l^{-1}.

The three week nutritional intervention improved the plasma levels of the lipid profile parameters considerably (**Table 3**) as compared to the respective changes among the control group that did not receive any supplement. Analysis of variance showed high statistical significant reduction in mean plasma TC following the consumption of PJ (P < 0.007) a mixture of PJ and FS (P < 0.001) or FS (P < 0.02), compared with the respective prefeeding levels. The three week dietary intervention with the four supplements led to significant increase in the mean HDL-C concentrations compared with the respective prefeeding levels with P values ranging from (P < 0.014) after consumption of AF to P < 0.000 following the consumption of PJ, mixture of PJ-FS or FS (**Table 3**). The three atherogenic indices, *i.e.*, TC/HDL-C; LDL/HDL ratio and the AI improved and responded positively following the three week dietary intervention with the four supplements with P values ranging between (0.05 > P < 0.1) after the consumption of apricots to P < 0.000 following the consumption of PJ , mixture of PJ-FS or FS. The plasma levels of the lipid profile parameters remained stable among the control group at the end of the three week period compared with the mean initial levels (**Table 3**).

Initial mean TC/HDL-C ratio (4.4 ± 0.17) was above the optimum ratio of ≤3.5 - 1, and was reduced to mean ratios of 3.4 (0.05 > P < 0.1); 2.3 (P < 0.001); 2.65 (P < 0.003) and 2.68 (P < 0.005) following the consumption of AF, PJ, PJ-FS or FS, respectively (**Table 3**).

The initial overall LDL-C/HDL-C ratio averaged 2.8 ± 0.1; 20% of the volunteers had elevated ratio above the norms (3.4:1). This ratio was reduced significantly following the consumption of AF (0.05 > P < 0.1); PJ (P <

Table 3. Mean initial and terminal plasma levels of lipid profile parameters, tHcy, Albumin, IgA following three week consumption of different supplements.

	Control			Apricots			Pomegranate			Pomegranate + Sobya			Sobya		
	Initial	Terminal		Initial	Terminal		Initial	Terminal		Initial	Terminal		Initial	Terminal	
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	P	$\bar{x} \pm SE$	$\bar{x} \pm SE$	P	$\bar{x} \pm SE$	$\bar{x} \pm SE$	P	$\bar{x} \pm SE$	$\bar{x} \pm SE$	P	$\bar{x} \pm SE$	$\bar{x} \pm SE$	P
Total lipid, mg/dl	641.0 ± 47	629.5 ± 40	0.328	713.0 ± 21	678.0 ± 27	0.025	712.8 ± 8.0	700.6 ± 10	0.944	684.5 ± 18	682.8 ± 15	0.061	649.4 ± 17	638.7 ± 15	0.306
TG, mmol·Γ^{1}	1.39 ± 0.0	1.4 ± 0.0	0.205	1.4 ± 0.3	1.0 ± 0.3	0.09	1.8 ± 0.2	1.6 ± 0.2	0.001	1.5 ± 0.1	1.1 ± 0.0	0.412	1.2 ± 0.2	1.0 ± 0.2	0.118
T-Cho, mmol·Γ^{1}	5.38 ± 0.0	5.3 ± 0.1	0.795	5.0 ± 0.3	4.5 ± 0.3	0.108	4.6 ± 0.1	4.2 ± 0.1	0.007	4.8 ± 0.1	4.5 ± 0.1	0.001	5.0 ± 0.3	4.5 ± 0.3	0.026
HDL-C, mmol·Γ^{1}	1.73 ± 0.0	1.8 ± 0.4	0.803	1.0 ± 0.2	1.4 ± 0.2	0.014	1.1 ± 0.1	1.8 ± 0.1	0.001	1.2 ± 0.1	1.7 ± 0.1	0.001	1.0 ± 0.1	1.7 ± 0.1	0.002
LDL-C, mmol·Γ^{1}	3.01 ± 0.0	2.8 ± 0.5	0.763	3.4 ± 0.3	2.6 ± 0.3	0.056	2.7 ± 0.1	2.1 ± 0.2	0.253	3.0 ± 0.2	2.8 ± 0.2	0.017	3.5 ± 0.3	2.7 ± 0.4	0.005
VLDL-C, mmol·Γ^{1}	0.35 ± 0.0	0.3 ± 0.0	0.803	0.6 ± 0.1	0.4 ± 0.1	0.09	0.8 ± 0.1	0.7 ± 0.1	0.001	0.7 ± 0.0	0.5 ± 0.0	0.223	0.6 ± 0.1	0.5 ± 0.1	0.118
Total Chol/ HDL-C ratio	3.08 ± 0.2	3.0 ± 0.2	0.227	5.4 ± 0.9	3.4 ± 0.6	0.078	4.3 ± 0.4	2.3 ± 0.1	0.001	4.3 ± 0.3	2.6 ± 0.1	0.003	5.1 ± 0.5	2.7 ± 0.2	0.001
LDL: HD L ratio	1.74 ± 0.0	1.6 ± 0.6	0.912	3.7 ± 0.7	2.0 ± 0.5	0.072	2.5 ± 0.3	1.2 ± 0.1	0.004	2.6 ± 0.3	1.6 ± 0.1	0.001	3.6 ± 0.5	1.7 ± 0.2	0.001
Atherogenic index (AI)	2.08 ± 0.2	2.0 ± 0.2	0.227	4.4 ± 0.9	2.4 ± 0.6	0.078	3.3 ± 0.4	1.3 ± 0.1	0.001	3.3 ± 0.3	1.6 ± 0.1	0.003	4.1 ± 0.5	1.8 ± 0.2	0.001
Albumin, g/dl	41.8 ± 0.7	41.5 ± 0.1	0.677	40.9 ± 0.9	40.0 ± 1.0	0.454	40.5 ± 0.5	39.6 ± 0.7	0.303	39.0 ± 0.8	40.1 ± 0.4	0.23	39.5 ± 0.9	40.6 ± 0.6	0.332
IgA, mg/dl	122.0 ± 62	112.2 ± 82	0.71	127.5 ± 50	182.7 ± 99	0.478	247.7 ± 27	361.3 ± 97	0.06	179.4 ± 77	299.2 ± 85	0.219	189.9 ± 45	219.7 ± 44	0.343
tHcy, µmol/L	18.2 ± 3.9	18.43 ± 0.8	0.966	14.3 ± 2.1	13.4 ± 1.9	0.2	16.7 ± 1.1	12.0 ± 0.7	0.107	31.4 ± 11	20.9 ± 5.3	0.001	16.5 ± 1.2	12.3 ± 0.8	0.003

Data represent mean ± standard error. N = 7 volunteers for each dietary group. For differences in mean values, $P < 0.05$ is considered significantly different.TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AI: Atherogenic index; NM: Normal diet group; A S: Normal diet supplemented with 200 g apricots; PG J: Normal diet with 250 g pomegranate juice; PG J-F S: Normal diet with 100 g pomegranate juice and 150 g fermented sobya; FS: Normal diet with 167 ± 1.3 g fermented sobya.

0.005); PJ-FS ($P < 0.001$) or FS ($P < 0.001$). The overall initial atherogenic index averaged 3.4 and responded also in the same manner as the above mentioned two other indices (**Table 3**). The overall initial plasma total tHcy (tHcy) level averaged 18.03 ± 4 μmol/L and the mean level decreased following the consumption of mixture of PJ-FS ($P < 0.000$) or FS ($P < 0.003$). All four supplements increased the mean level of plasma Ig A compared to the respective initial level, however significant increase was attained only following the consumption of PJ ($P < 0.06$).

The mean plasma Ig A levels remained stable among the control group and averaged 122 and 112 mg/dl at day zero and day 21, respectively.

6. Discussion

The promotion of a healthy lifestyle with non-pharmacological means is a preventive measure strategy aiming at ideal CV health [22] and to lower LDL-C levels to the updated guidelines (100 mg/dL ≤ 2.6 mmol/l) [23]-[25].

Certain foods phytochemicals can achieve this strategy and can lead to better health by altering metabolism and gene expression [26]. The pomegranate tree (*Punica granatum*) is known for centuries for its protective effects as a folk medicine. The world pomegranate production is estimated to be 1,500,000 tons [27]; while in the United Kingdom pomegranate juice is consumed at a rate of 500,000 liters per week [28]. Pomegranate juice (PJ) might be useful as an adjunctive therapy for the management of hypertension on top of other non-pharmacological interventions in patients with hypertension and high oxidative burden such as those with diabetes, obesity, metabolic syndrome or who smoke [29]. PJ is one of the richest sources in the polymeric polyphenols ellagitannins (ETs) (2.4 g/l); punicalagin (1.56 g/l); free anthocianin, (0.39 g/l), Ellagic acid (0.12 g/l), hydrolysable tannin (0.42 mg/l) and total phenolic (2.5 g/l) [11]. The phenolic content of pomegranate juice prepared from "Wonderful" variety grown in California ranged between 1800 - 2100 mg/l and 2487 mg/l for experimental and commercial products, respectively [11]. The phenolic contents of pomegranate cultivars grown in Iran ranged between 1598 - 9842 mg per kilogram fresh weight [30]. The PJ ellagic acid appears in the blood of healthy volunteers consuming 180 ml PJ containing 25 mg ellagic acid and 318 mg ellagitannins as a peak after one hour of consumption with a level of 32 micrograms per liter plasma and disappeared completely at 4 hours [31]. Another trial demonstrated that the appearance of ellagic acid peak and in vivo antioxidant capacity was maximum after 0.5 h of the consumption of 800 mg of pomegranate extract. The daily consumption of 200 ml PJ by hypercholestolemic patients for 4 weeks was associated with significant decreases in plasma TC and LDL-C [32]. The total polyphenols intake with the PJ estimated to be 30 g at the end of the three week intervention study and was associated with significant reduction in all plasma lipid profile parameters except LDL-C.

Fresh PJ (150 ml/day) consumed by hypertensive Iranian patients (aged 30 - 67 years) for 2 weeks was associated with significant reductions in systolic blood pressure, but with no significant effect on serum levels of lipid profile parameters in any of the study groups [33].

Supplementation with 20 - 80 ml PJ/d, equivalent to 0.54 - 2.16 mmol total polyphenols/d for 10 wk by healthy subjects didn't affect the plasma levels of lipid profile parameters [34] The supplementation of 1 liter of PJ to healthy subjects for 5 days had no effect on antioxidative indices [17]. In another study, the effect of pomegranate juice (PJ) (400 ml) equivalent to 2.7 g polyphenols supplementation for 5 weeks on 30 patients with stable chronic obstructive pulmonary disease (COPD), did not affect any of the 18 serobiochemical parameters evaluated.

The daily consumption of pomegranate juice (240 ml/day) for 3 months in patients who had CHD and myocardial ischemia improved stress-induced myocardial ischemia, didn't show significant differences in any of the plasma levels of lipid profile parameters [35].

A study carried out on adolescents (12 - 15 years) with metabolic syndrome showed that the daily consumption of 240 ml natural home-made pomegranate juice without adding any sweetener declined inflammation after one month without changes in conventional biochemical parameters of lipid profile [36]. A meta analysis of 19 randomized controlled trials comprising a total of 618 subjects, who consumed different fruit juices, suggested that the fruit juice consumption reduced TC concentration in low-median intake of total polyphenols group, but had no effect on HDL-C, LDL-C concentrations or systolic blood pressure [37]. Investigators from Western countries confirm the promising data obtained following the consumption of PJ; yet issues such as the high prices and un-availability worldwide should be considered [38].

The null effect of PJ on ameliorating the plasma lipid profile parameters reported in the literature could be at-

tributed to the qualitative characteristics of commercial manufactured PJ. Commercial juice preparations subjected to enzymatic treatment with pectinase to hydrolyze the 1,4-galacturonide bonds when served to healthy subjects at portions of 20 - 80 mL PJ/d (0.54 - 2.16 mmol total polyphenols/d) for 10 wk failed to lower the plasma total cholesterol level and didn't affect the plasma levels of lipid profile parameters [33]. Another commercial pomegranate juice was prepared by industrial hydrostatic processing, which includes concentrating the PJ to 65% soluble solids and before use, the juice was prepared by reconstituting the preparation to 16% soluble solids with water [11]. Furthermore, POMx is prepared by extracting PJ ellagitannins in significant amounts, subsequently enriching pomegranate juice with at least 2 g/l of punicalagins and this juice is a standardized proprietary for use in clinical studies as a dietary supplement [39]. The polyphenol composition of POMx consists of 77% oligomers composed of 210 repeating units of gallic acid, ellagic acid and glucose, 19% ellagitannins. Pomegranate polyphenols ranged between 652 - 948 mg/237ml [40]. After consumption of large amounts of POMx, no PJ ellagitannins could be detected in human systemic circulation suggesting that it was not hydrolyzed in the gut and the aglycone was not liberated [39]. In another clinical trial, the consumption of POMx (237 ml) with a high-fat meal by young healthy men, effectively suppressed the postprandial increase in systolic blood pressure following the high-fat meal but POMx couldn't decrease postprandial plasma triacylglycerides concentrations [40].

The complex interaction between ellagitannins and microbiota in the intestinal environment is determined by the abundance and richness of gut bacteria [41]. PJ punicalagins and ellagic acid are transformed by human gut bacteria to the dibenzo-pyranone-type urolithins; while the remaining ellagitannins and ellagic acid retained unabsorbed in the gut lumen [39]. Individuals with a low gut bacterial richness at different proportions are characterized by more marked overall dyslipidaemia, when compared with high bacterial richness individuals [42].

There is general agreement in the literature on the beneficial effectiveness of PJ as non-pharmacological agent against the development of dyslipidemia.

Immune modulatory actions of PJ had been confirmed in the present study and the mean level of plasma IgA increased significantly after three week consumption of PJ compared to prefeeding level ($P < 0.05$) is in good agreement with previous reports [43] [44].

Mann and Spoerry [45] were the first researchers to illustrate the hypo-cholesterolemic effect of wild Lactobacillus-fermented milk. Evidences demonstrate that the consumption of selected bacteria strains with probiotic activities in the diet reduces the concentration of total cholesterol (TC); LDL-C in plasma [45]. Lactobacillus plantarum strains are potential probiotic cultures with cholesterol-lowering activity for the management of hypercholesterolemia [16].

The daily consumption of 300 g/day of yoghurt supplemented with *L. acidophilus* 145 and *B. longum* 913 by 29 women for 21 weeks was associated with significant increase in the level of HDL-cholesterol with a concomitant significant decrease in the ratio of LDL/HDL-C [46].

Several mechanisms of cholesterol reduction by probiotics have been proposed and the high bile salt hydrolase (BSH hypothesis) activity of the probiotics are among the proposed mechanisms [47]. Sobya is a lactic acid fermented milk rice based porridge with diverse lactic acid bacteria and yeasts and has been increasingly recognized as a functional food [48]. Throughput gene sequencing shows that Lactobacillus rhamnosus GG was the predominant LAB strain in sobya (Unpublished data).

The present finding that the consumption of either sobya or a mixture of PJ-FS effectively reduced the TC level, LDL-C and the three atherogenicc indices denotes that this product can lend itself in the dietary management of disorders of lipid metabolism.

The synergistic action of PJ and FS on reducing the plasma lipid profile parameters is interesting, since earlier reports showed that the consumption of pomegranate products enhance the growth of Bifidobacterium and Lactobaccilli with no effect on clostridium histoloticum [39].

The overall initial plasma tHcy averaged 18.03 ± 4 μmol/l is higher than the respective mean values of 7.5 and 9.8 μmol/l reported for American women and men 20 - 29 years, respectively [49]. Much higher values amounting to 80 μmol/l were reported among Asian Indians, suggesting cobalamin deficiency [50]. Blood tHcy is a very important biomarker of cardiovascular diseases and high levels of tHcy are considered an independent risk factor for heart disease. Hyperhomocysteinaemia in rural Indian patients was attributed to genetic factors and is associated with the development of ischaemic heart disease in Indian patients [51].

Dietary intervention with either PJ ($P < 0.05$) or with FS ($P < 0.001$) effectively reduced the plasma tHcy in comparison to respective mean plasma level obtained at baseline.

Although the sample size in this study was relatively small, the strength of the design and the use of natural home-made PJ without adding any sweetener effectively improved the plasma levels of lipid profile parameters suggesting that daily consumption of pomegranate juice may have important clinical benefits in the Egyptian population.

The present study was carried out on apparently healthy subjects, therefore, the results cannot be extrapolated to dislipidemic subjects. Long term well-designed studies are anticipated to study the effectiveness of the same supplements on restoring the plasma lipid profile parameters among patients with afflictions such as dyslipidemia.

Acknowledgements

All authors: intellectual contribution, the interpretation of the results and the writing of the manuscript and acceptance of the final version. The authors wish to thank all volunteers, who participated in the trial. Thanks are also due to Prof Amal Saad El Din, Department Environmental Medicine and NRC for providing technical assistance with the statistical analysis on SPSS program.

Competing Interest

The authors declare that they have no competing interests.

Conflict of Interest

None of the authors had a personal or financial conflict of interest.

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List of Abbreviations

AF: Apricots.
BMI: Body mass index.
CHD: Coronary heart disease.
HDL-C: High density lipoprotein cholesterol.
LDL-C: Low density lipoprotein cholesterol.
PJ: Pomegranate juice.
PJ-S: Pomegranate juice-Lactic acid fermented sobya.
FS: Lactic acid fermented sobya.
T-Cho: Total cholesterol.
TG: Triglyceride.
tHcy: Total plasma homocysteine.
VLDL-C: Very low density lipoprotein.

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