Effect of pomegranate seed oil on hyperlipidaemic subjects: a double-blind placebo-controlled clinical trial

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In vitro and in vivo studies have shown that punicic acid, a type of conjugated fatty acid and the main constituent of pomegranate seed oil (PSO), has anti-atherogenic effects. The present study aimed at determining the effect of PSO treatment on serum lipid profiles. This double-blind placebo-controlled randomised clinical trial included fifty-one hyperlipidaemic subjects, diagnosed according to National Cholesterol Education Program definition, and randomly assigned to the PSO and the control groups. The PSO and placebo groups received 400 mg PSO and placebo twice daily, respectively and were followed up for 4 weeks. Serum concentrations of lipids and lipoproteins were measured before and 4 weeks after intervention. Mean concentration of TAG and the TAG:HDL cholesterol (HDL-C) ratio were significantly decreased after 4 weeks in the PSO group as compared with baseline values (2.75 (SD 1.40) v. 3.45 (SD 1.56) mmol/l, P=0.009 and 5.7 (SD 4.6) v. 7.5 (SD 5.0), P=0.031, respectively). The treatment effect was statistically significant in the PSO group as compared with controls in diminution of cholesterol:HDL-C ratio (5.4 (SD 1.5) v. 5.9 (SD 1.4), P<0.05) adjusted for baseline values. We found a mean difference for PSO v. placebo in HDL-C concentration (0.13 v. –0.02 mmol/l) and cholesterol:HDL-C ratio (–0.42 v. 0.01, P<0.05). Serum cholesterol, LDL cholesterol and glucose concentrations and body composition variables remained unchanged. It is concluded that administration of PSO for 4 weeks in hyperlipidaemic subjects had favourable effects on lipid profiles including TAG and TAG:HDL-C ratio.

Pomegranate seed oil: Hyperlipidaemia: Randomised clinical trials

Normalisation of dyslipidaemia, a modifiable risk factor, can prevent or reduce the risk of CVD, which is a leading cause of death worldwide. In order to treat dyslipidaemia, different pharmacologic and non-pharmacologic approaches have been used. Statins are the first-line therapy for management of increased LDL cholesterol (LDL-C), despite adverse effects such as gastrointestinal symptoms and muscle aches. Although fibrates may decrease serum TAG and increase serum HDL cholesterol (HDL-C), more powerful drugs for normalisation of these lipids need to be investigated.

Conjugated fatty acids are well-known hypolipidaemic agents documented for their effect on lipid metabolism. PSO (Punica granatum) consists of about 80 % conjugated octadecatetraenoic fatty acids, with a high content of 9-cis, 11-trans, 13-cis acid or punicic acid (PA), one of the isomers of conjugated linolenic acid (CLN), animal studies report controversial results for the hypolipidaemic role of PSO.

Although Arao et al. (8) showed that PSO supplementation in obese hyperlipidaemic Otsuka—Long–Evans–Tokushima fatty rats reduces TAG accumulation, Yang et al. (9) demonstrated that PSO does not alter serum cholesterol concentration.

Considering the lack of data on the effect of PSO on lipid profiles in human subjects, the present study investigated the effect of PSO treatment on lipid profiles of hyperlipidaemic subjects.

Subjects and methods

Subjects and study design

This is a parallel, randomised, double-blind and placebo-controlled study. Subjects were recruited from an endocrine clinic in sequential fashion. Inclusion criteria were age over 20 years, not pregnant or lactating, having no diagnosed...
It was recommended that the subjects keep their regular lifestyle, including diet and physical activity during the course of study. Questionnaires were used to collect demographic data, medical history at baseline, and medication and supplement use at baseline and 4 weeks later. The modifiable activity questionnaire and three 24 h dietary recalls were collected at baseline and 4 weeks later to assess physical activity and dietary intake. BMI and waist:hip ratio were calculated at baseline and at the end of 4 weeks. Body composition including percentage of lean and fat mass was measured by bioelectrical impedance analysis, using Bodystat 1500MDD at baseline and after 4 weeks.

The present study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethical committee of the Research Institute for Endocrine Sciences of the Shahid Beheshti University of Medical Sciences. Written informed consent was obtained from all the subjects.

### Table 1. Anthropometric, body composition, blood pressure and biochemical variables at baseline and after 4 weeks in the pomegranate seed oil (PSO) and placebo groups

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>PSO (n 23)</th>
<th></th>
<th>Placebo (n 22)</th>
<th></th>
<th>P for treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
<td>Baseline</td>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.79</td>
<td>0.81</td>
<td>6.92</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>3.45</td>
<td>1.56</td>
<td>2.75*</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.90</td>
<td>1.09</td>
<td>4.39</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.25</td>
<td>0.39</td>
<td>1.38</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Ox-LDL (mU/l)</td>
<td>167</td>
<td>43</td>
<td>170</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Cholesterol:HDL-C ratio</td>
<td>3.37</td>
<td>1.24</td>
<td>3.41</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>TAG:HDL-C ratio</td>
<td>5.87</td>
<td>1.67</td>
<td>5.45</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>7.57</td>
<td>3.18</td>
<td>7.9</td>
<td>3.36</td>
<td></td>
</tr>
<tr>
<td>Insulin:glucose ratio</td>
<td>0.08</td>
<td>0.04</td>
<td>0.09</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.68</td>
<td>0.89</td>
<td>1.80</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>36.6</td>
<td>6.8</td>
<td>36.1</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>27.2</td>
<td>7.9</td>
<td>26.8</td>
<td>9.1</td>
<td></td>
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<tr>
<td>Lean mass (kg)</td>
<td>47.0</td>
<td>7.9</td>
<td>43.4</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.83</td>
<td>0.07</td>
<td>0.81</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2</td>
<td>3.1</td>
<td>27.0</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

**LDL-C, LDL-cholesterol; HDL-C, HDL cholesterol; Ox-LDL, oxidised LDL; HOMA-IR, homeostasis model for insulin resistance.**

Mean values were significantly different within the groups using paired t-test: *P* < 0.005, **P** < 0.001, ***P*** < 0.001.

† All PSO variables were not significant at baseline, in comparison to placebo.

‡ Analysis of covariance with 4-week values as dependent variables, baseline values as covariates and treatment group as a fixed factor.
ELISA kits (Mercodia AB Company, Uppsala, Sweden); insulin resistance was calculated by the homeostasis model for insulin resistance (HOMA-IR) model, using the formula:

\[
\text{HOMA-IR} = \frac{\text{glucose (mmol/l)} \times \text{insulin (mU/l)}}{22.5}
\]

### Statistical method

To have a clinically significant effect on lowering serum cholesterol\(^{(11)}\), we based our sample calculation to detect a difference of 200 mg/l and an SD of 200 mg/l in endpoint cholesterol concentrations between the PSO and placebo groups, and we found that a sample size of 21 was sufficient with 95% CI and 90% power in each group. Fifty-one hyperlipidaemic subjects were enrolled to compensate for eventual study dropouts.

SPSS (version 16.0; SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Differences between the two groups at baseline were tested with Student’s t test and the Mann–Whitney test. Paired Student’s t test and Wilcoxon ranked test were used to compare the baseline and 4-week values in each group. To distinguish treatment effect between the groups, following adjustment of their baseline values, analysis of covariance test was used.

### Results

Forty-five of fifty-one patients enrolled in our study were eligible for final analysis, as six withdrew and did not provide 4-week blood samples. Mean values for baseline body weight and age were 74.2 (SD 10.0) and 75.7 (SD 12.2) kg, and 51 and 4-week blood samples. Mean values for baseline body weight were 74.2 (SD 10.0) and 75.7 (SD 12.2) kg, and 51 and 55 (SD 9) years for the PSO and placebo groups, respectively. No significant differences between the groups were seen for age, sex, weight, height, consumption of lipid lowering drugs or \(n\)-3 supplements and smoking. All the subjects maintained good health throughout the study, without any major adverse events.

A comparison of dietary intake and physical activity variables at baseline and after 4 weeks between the PSO and placebo groups revealed no significant differences. Energy intake and dietary cholesterol decreased in the placebo group during the treatment period (\(P<0.05\)).

The effects of PSO on biochemical, anthropometric, body composition and blood pressure variables are given in Table 1; PSO administration resulted in a decrease in serum TAG concentration (2.75 (SD 1.40) v. 3.45 (SD 1.56) mmol/l, \(P<0.01\)) and a non-significant increase in HDL-C concentration (1.38 (SD 0.44) v. 1.25 (SD 0.39) mmol/l, \(P=0.081\)) compared with baseline values; TAG:HDLC ratio decreased significantly within the PSO group (5.73 (SD 4.55) v. 7.49 (SD 4.95), \(P<0.031\)). Comparison of treatment effects between the groups with baseline values as covariates revealed lower values for cholesterol:HDLC (5.45 (SD 1.51) v. 5.89 (SD 1.43), \(P<0.05\)) and higher, but non-significant HDLC concentrations (1.38 (SD 0.44) v. 1.25 (SD 0.26) mmol/l, \(P=0.059\)) in the PSO group as compared with the placebo group, respectively. Other lipid profile variables including cholesterol, LDL-C, oxidised LDL and LDL-C:HDLC ratio revealed no significant differences within or between the groups. Fat mass, BMI and waist:hip ratio decreased in the PSO group, but not significantly. PSO had no effect on systolic and diastolic blood pressure within or between the groups (data not shown). Means for differences were significant in HDLC concentration (0.13 v. –0.02 mmol/l) and choles- terol:HDLC ratio (–0.42 v. 0.01) when the PSO group was compared with the placebo group (\(P<0.05\) for both) (Fig. 1).

![Fig. 1. Mean differences compared with baseline in the pomegranate seed oil (PSO; ●) and placebo groups (■). O-\(x\)-LDLC, oxidised LDL; TC, total cholesterol; HDLC, HDL cholesterol; LDL-C, LDL cholesterol; HOMA-IR, homeostasis model for insulin resistance. \(^*P<0.05\).](https://www.cambridge.org/core/terms)
found a significant difference in cholesterol:HDLC ratio after adjusting for baseline values, in PSO group vs placebo group.

A recent study in healthy human subjects reported Trichosanthes kirilowii seeds, naturally occurring sources of PA, had no significant effect on serum lipid 

contrary to our findings which may be attributable to the pre-existence of hyperlipidaemia in the present study. Current data on the effect of PSO on lipid concentration and metabolism are controversial. Similar to our findings, the liver accumulation of TAG was reduced by PSO administration, a plausible mechanism, demonstrated in an in vitro study, that 9-cis, 11-trans, 13-cis CLN suppressed cellular synthesis of TAG in HepG2 cells.

One study suggests a metabolic pathway for 9-cis, 11-trans, 13-cis CLN is its conversion to 9-cis, 11-trans conjugated linoleic acid (CLA) in a rat model; this conversion coefficient is about 12% for PSO. Also two recently published articles have demonstrated that PA from T. kirilowii seed oil incorporated into different tissues and serum metabolises to CLA in rats and human subjects. Hence, the physiological activities of PSO may be attributable to 9-cis, 11-trans CLA derived endogenously or to PA itself. Scientific evidence reveals the inconsistent effects of 9-cis, 11-trans CLA on lipid profiles, alterations that may occur mostly through the carnitine palmitoyltransferase activity-enzyme involved in fatty acid ß-oxidation, as mitochondrial and peroxisomal carnitine palmitoyltransferase activity is reported to increase with PSO and CLN; therefore, there is no conclusive evidence to confirm the effect of CLN and CLA on lipid profiles, but it does seem that the isomer type and amount of CLN and CLA could be determining factors.

In the present study, no significant changes were observed in body composition (fat or lean mass), BMI or waist:hip ratio following treatment. Since energy intake, a main contributor to weight gain, did not change during the 4 weeks, the decrease in body composition indices is attributable either to weight loss, did not change during the 4-week study period. Further studies of larger samples with longer durations need to confirm the effects of PSO and related mechanisms.

To conclude, the present study found that PSO consumption in hyperlipidaemic subjects did not alter cholesterol and LDL-C but did reduce TAG and TAG:HDLC ratio during 4-week study period. The present study was supported by a financial grant (no. 234) from the Research Institute of Endocrine Sciences, Shahid Beheshti University Medical Sciences and Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Iran. None of the authors had any personal or financial conflicts of interest. P. M. was involved in design, implementation and analysis; M. R. F. was involved in preparation of PSO capsules and implementation; G. A. was involved in review of the literature, implementation and analysis; A. S. supervised technical issues in the preparation of PSO and design; F. A. supervised overall project, design, implementation and analysis.

References


