ORIGINAL SCIENTIFIC PAPER

The Effects of Olive and Pumpkin Seed Oil on Serum Lipid Concentrations

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Summary

Low-fat diets increase serum triacilglycerol and decrease HDL-cholesterol concentrations, thereby potentially adversely affecting cardiovascular disease (CVD) risk. The present study compared the CVD risk profile of a high-MUFA diets (olive oil) and high-PUFA diets (pumpkin seed oil). The most significant difference between the two groups was that the atherogenic index in groups given olive oil was significantly lower (for approximately 60 %) than atherogenic index recorded in control group, while in the group receiving pumpkin seed oil this reduction was approximately 40 %. Collectively, these findings point to the fact that both a high MUFA and PUFA diet may be preferable to a low-fat diet because of more favorable effects on the CVD risk profile.

Keywords: cholesterol, triglycerides, HDL and LDL-cholesterol, olive oil, pumpkin seed oil

Sažetak

Prehrana sa niskim udjelom masti povećav koncentraciju triacilglicerola i snižava koncentraciju HDL-kolesterola u serumu, što može imati negativan utjecaj na faktor rizika za razvoj kardiovaskularnih bolesti. U ovom radu je uspoređen utjecaj prehrane sa povećanim udjelom mononezasićenih masnih kiselina (maslinovo ulje) i prehrane sa povećanim udjelom polinezasićenih masnih kiselina (bučino ulje) na faktor rizika za razvoj kardiovaskularnih bolesti. Prehrana sa povećanim udjelom mononezasićenih masnih kiselina snizila je aterogeni indeks za 60%, a prehrana sa povećanim udjelom polinezasićenih masnih kiselina za 40% u odnosu na kontrolnu grupu, što je ujedno najznačajnija zabilježena razlika između ove dvije prehrane. Na osnovu rezultata prikazanih u ovom radu može se zaključiti da je dodatak oba ispitivana ulja u prehrani bolji nego prehrana bazirana na niskom sadržaju masnoća zbog povoljnijeg utjecaja na profil lipida u krvi i faktore rizika za razvoj kardiovaskularnih bolesti.

Ključne riječi: kolesterol, triacilglicerol, HDL i LDL kolesterol, maslinovo ulje, bučino ulje

INTRODUCTION

Dietary modification is the foundation of population strategies for the prevention and treatment of coronary heart disease (CHD), so it is imperative to identify alternative diets that can effectively modify the blood lipid profile, and thus reduce CHD risk.

Dietary fat is often divided into saturated and unsaturated fat. It has been shown that saturated fats increase the risk for coronary artery diseases, while unsaturated fatty acid consumption reduces this risk (Demaison et al., 2002). Examples through trials based on animal models have strengthen the concept that the decrease in food lipid intake is associated with a reduction of the blood cholesterol level as well as with a decrease of the risk of CVD (Keys et al., 1996; Nagyova et al., 2003; Trautwein et al., 1999).

Scientific Advisory of the American Heart Association (Kris-Etherton, 1999) reported that high monounsaturated fatty acid (MUFA) diets tend to raise HDL and lower triacylglycerol concentration compared with low fat, carbohydrate-rich, cholesterol-lowering diets (Grundy, 1986; Mensink and Katan, 1987). Mesnik et al. (2003) examined the effects of carbohydrates and various fats on blood lipid levels. In trials in which polyunsaturated and monounsaturated fats were eaten in place of carbohydrates, these good fats decreased levels of harmful LDL and increase protective HDL (Mensink et al., 2003). Randomized trial known as the Optimal Macronutrient Intake Trial for Heart Health (Omni Heart) showed that replacing a carbohydrate-rich diet with one rich in unsaturated fat, predominantly monounsaturated fats, lowers blood pressure, improves lipid levels, and reduces the estimated cardiovascular risk (Appel et al., 2005).

MUFA- and PUFA-rich diets decrease the levels of total plasma cholesterol and LDL-cholesterol and increase serum HDL-cholesterol in healthy normolipidemic subjects (Grundy, 1986; Grundy and Denke, 1990) and in mouse models of atherosclerosis (George et al., 2000).

Detailed description on lipid physiology and impact of dietary fats on lipid profile has been given in review by Landeka et al. (2011).

The aim of this study was to compare the impact of regular dietary intake of olive oil and pumpkin seed oil produced in Croatia on serum lipid profile. Olive oil and pumpkin seed oil differ in PUFA and MUFA composition. Nutritional effects of olive oil on fat metabolism are well known and described (Acin et al., 2005; Kris-Etherton et al., 1999; Rocio et al., 1999) while, on the other hand, pumpkin seed oil is less described in literature.

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Pumpkin seed oil is a regular component of meals in Northern Croatia (Delaš, 2009) whereas olive oil is a regularly used in Mediterranean part of Croatia. In recent years olive oil use increased but the results of these study show that pumpkin seed oil also may have beneficial influence on lipid levels and profile.

MATHERIALS AND METHODS

Animals and treatment

Experiments were carried out according to the guidelines force in Croatia (Law on the Welfare of Animals, NN# 19, 1999) and in compliance with the Guide for the Care and Use of Laboratory Animals, DHHS Publ. # (NIH) 86-123. Inbred Swiss mice 90±5 days, from the mouse colony of Faculty of Science, University of Zagreb were used. The animals were maintained on a formulated commercial pellet diet and water was provided *ad libitum*. The animals were maintained under 12L:12D hour light-dark regime at 60% humidity. Food pellets were a Standard mice and rat diet 4RF21 certificate (Mucedola, Italy; Batch No. 238603, shape 12 mm) composed of wheat, maize, soybean dehulled, corn gluten feed, wheat strow, fish meal, dicalcium phosphate, calcium carbonate, sodium chloride, whey powder, soybean oil, yeasts, hazelnut skins. Analytical components were 12% moisture, 18.50% protein, 3% fats, 6% crude fibers, 7% crude ash, supplemented with E672 (vitamin A), E671 (vitamin E), E1 (Fe), E5 (Mn), E6, (Zn), E4, (Cu), E2 (I), E3 (Co) added as sulphates or carbonates.

Through the whole experiment on the days of treatment the body weight of the animals was recorded. Each group contained 7 male animals. The first group was a control group. The control group was receiving physiological solution (vehicle). The second group received olive oil. The third group was treated with pumpkin seed oil. All treated groups received by gauge a total volume of 0.2 ml/animal/day, repeatedly for 30 days. To avoid the false changes influenced by excessive blood loss caused by frequent sampling the whole blood samples were collected on the 30th day of the experiment respectively. On the 30th day the animals were anesthetized with izofourane (2% in a flow of oxygen) and exsangunated by intra-cardiac puncture without anticoagulant, as described in EMPReSS, SOP (Standard Operating Procedure) (Green at al., 2005). After coagulation the unhemolysed serum was collected and frozen at -80°C until further processing that did not take more than five days. Control group data were within the normal reference ranges at the end of the experiment and they were the reference point for comparison with the treated groups (Matsusawa et al., 1998; James 1993). The whole experiment was repeated twice and the statistical analysis showed no difference between the first and the second experimental setup (repetition).

Biochemical analysis

Total triglyceride, cholesterol and high density lipoprotein (HDL) were measured using Hitachi 717 automatic analyzer (Hitachi, Japan) (Concensus Conference, 1985). The low density cholesterol (LDL) concentrations were calculated from the Friedewald equation: LDL-cholesterol: [LDL] = [C] - [HDL] - [TG/5], according to the manufacturer's instructions (Friedwald at al., 1972).

Statistical analysis

Statistical analyses were performed using Statistica 8.0 software (Stat-Soft, Tulsa, USA). Each sample was characterized by the mean (± standard deviation of the mean). The unit of measurement was the animal. Multiple comparisons between control and treated groups were done by on log-transformed data in order to normalize the distribution and to equalize the variances. Level of statistical significance was set at $p \leq 0.05$. Post-hoc analysis of differences was conducted by Scheffé test to establish between the group differences (Zar, 1999).

RESULTS

There were no statistically significant difference in weight gain between treated animals and the control group (Figure 1). Over the course of experimental period none of the experimental animals had died nor did they show abnormal food intake or behavioral changes.

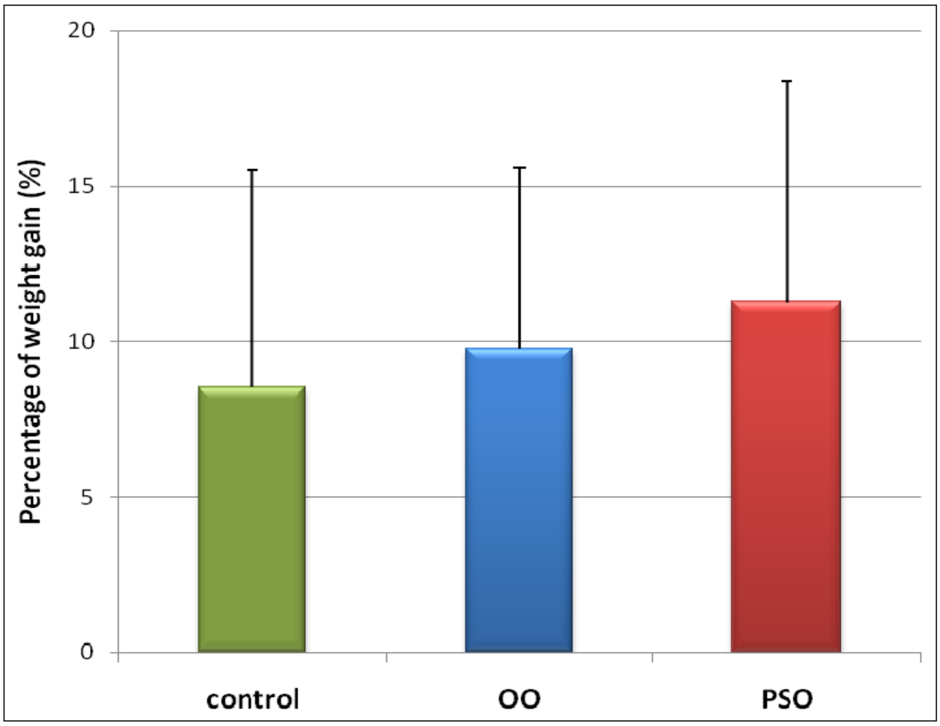

Figure 1. *The mean percentage of weight gain (± S.D.) from the beginning of the experiment in control animals, animals receiving olive oil (OO) and pumpkin seed oil (PSO). Between groups there were no statistically significant differences.*

Effect of olive and pumpkin seed oil on serum triglycerides and cholesterol in mice fed for 30 d. In comparison to the control group are presented in Table 1. and Figure 2. The groups receiving olive oil had significantly lower triglyceride levels in serum (10.90%) compared to control. Cholesterol levels were 5.78% lower than in control animals. HDL cholesterol was 19.04% higher compared to control and LDL was 14.79% lower than in control animals. In the group receiving pumpkin seed oil diet cholesterol level was decreased for 2.68% compared to control and the triglyceride level in this group was significantly lower (for 6.64%) than in the control group. HDL cholesterol level was significantly higher for 16.70% compared to control group. LDL levels in serum significantly descend (for 10.60%) compared to the controls.

Table 1. *Effect of olive oil and pumpkin seed oil diet on mice serum lipid composition - after 30 days of diet.*

Parameters	Groups		
	Control (C)	Olive Oil (OO)	Pumpkin Seeds Oil (PSO)
HDL (mg/dl)	41.91±3.86[a,b]	49.89±3.97[#]	48.91±3.62[#]
[1]LDL (mg/dl)	94.18±3.78[a,b]	79.18±3.75[#]	84.19±3.44[#]
Cholesterol (mg/dl)	152.64±3.09	143.82±3.22	148.55±2.73
Triglyceride (mg/dl)	82.74±5.06[a,b]	73.71±4.87[#]	77.25±5.1[#]
LDL/HDL	2.25±0.32[a,b]	1.59±0.29[#]	1.72±0.32[#]
Cholesterol/HDL	3.64±0.36	2.88±0.36	3.03±0.34
[2]Atherogenic index	0.98±0.24[a,b]	0.37±0.22[#,b]	0.58±0.38[#,a]

[1] LDL-cholesterol: [LDL] = [C] - [HDL] - [TG/5]
[2] Atherogenic index: [T] - [HDL] / [HDL]
[#] - significantly different from control
[a] - significantly different from Olive oil
[b] - significantly different from Pumpkin seed oil

Both oils lowered the atherogenic index compared to the control. However, the most significant difference between the two groups was that the atherogenic index in groups given olive oil was significantly lower (for approximately 60%) than atherogenic index recorded in control group, while in the group receiving pumpkin seed oil this reduction was approximately 40% of the atherogenic index recorded in control group.

To assess how changes in plasma lipids correspond to changes in CVD risk status, we estimated the change in risk expected for the lipid and lipoprotein responses as follows: a 1% decrease in LDL cholesterol decreases CVD risk by 1.5% (Davis et al., 1990). The average 12.7% reduction in LDL cholesterol achieved with olive and pumpkin seed oil would be expected to decrease CVD risk by 19% (Figure 2).

DISCUSSION

A low-fat and low-cholesterol diet has been proposed as healthful eating for decades. It is assumed that there is a linear relationship between an overconsumption of saturated fatty acids and atherogenesis, promoting CVD as a primary cause of mortality in industrialized countries. Recommendations in these countries are based on a collection of experimental, epidemiological, or nutritional data published during the last 20 years, showing that the CVD incidence is positively correlated with the total cholesterol (TC) concentration and negatively correlated with the high-density lipoprotein (HDL) threshold (Baudet et al., 1984; Esteva et al., 1986). Growing evidence shows that the structure of fatty acids and the number of double bonds between carbon atoms play a central role in the etiology of numerous chronic diseases. Saturated fatty acid consumption increases the risk of coronary artery diseases, while unsaturated fatty acid consumption reduces this risk (Demaison and Moreau, 2002; Nagyova et al., 2003). Various studies suggest a protective role of PUFA and MUFA, and

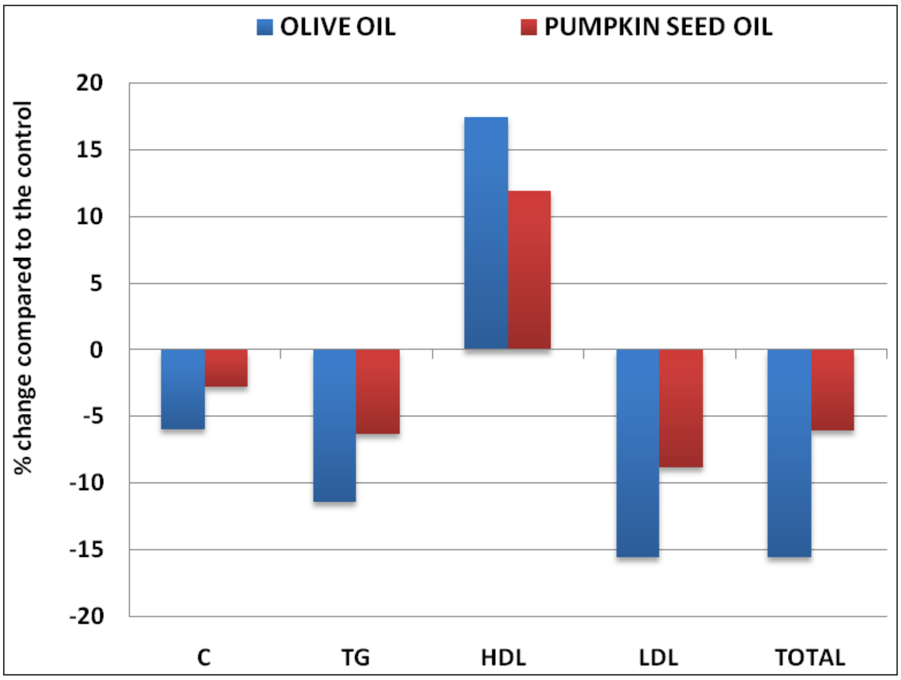

Figure 2. *Effects of LDL-cholesterol, HDL-cholesterol, total cholesterol (C) and triacilglycerol (TG) concentrations on cariovascular disease risk reduction in response to olive oil and pumpkin seed oil diets.*

a harmful effect of SFA in many other diseases affecting the aging population. This finding raises a question about ″healthful eating″ guidelines concerning fat and cholesterol in diet and, from a public health perspective, it is now time to reevaluate what the optimal diet is for lowering risk of CVD.

It is generally accepted that higher-fat diets that are high in MUFAs and low in SFAs lowers total and LDL cholesterol to a degree similar to that observed for a lower-fat, cholesterol-lowering diet (Grundy, 1996; Grundy et al., 1988; Kris-Etherton and Yu 1997; Mensink and Katan 1988). The Mediterranean diet, with its hallmark of high levels of utilization of olive oil (high in content with MUFA:PUFA=5:1), had a pivotal role in many studies. The Food and Drug Administration recommends using about 2 tablespoons (23 grams) of olive oil a day instead of other dietary fats to get its heart-healthy benefits (Covas et al., 2006; Covas et al., 2008). Trichopoulou et al. (2005) suggested significant reduction in mortality among patients with known coronary heart disease who adhered to a traditional Mediterranean diet. Fito et al. (2005) studied the effect of virgin olive oil, rich in phenolic compounds, and found that it significantly decreased oxidized low-density lipoprotein (LDL) and lipid peroxide levels. Olive oil contains a potent mix of antioxidants that can lower "bad" (LDL) cholesterol but leave "good" (HDL) cholesterol untouched.

Such results were confirmed in this work with animals receiving olive oil. Animals receiving olive oil had 10.90% lower triglyceride levels in serum, 5.78% lower cholesterol levels and 14.79% lower LDL level compared to control. HDL cholesterol was even 19.04% higher compared to control.

For treatment group edible virgin olive oil (Zvijezda, Croatia) was used (ratio of unsat/sat = 4.8, 14% palmitic acid, 2.8% stearic acid, 72.9% oleic acid, 8.6% linoleic acid) (Žanetić and Gugić, 2006) and pumpkin seed oil (Zvijezda, Croatia) was used (ratio of unsat/sat = 4.8, 11.2% palmitic acid, 5.2% stearic acid, 35% oleic acid, 47% linoleic acid) (Neđeral Nakić et al., 2006).

Results of the present study provide further evidence that the oils rich in PUFA may have similar effect. This finding has not been typically reported. The major ω-6 fatty acid in the diet is linoleic acid, which serves as the precursor for arachidonic acid (20:4n-6), which has important biological effects in the body. Linoleic acid is not synthesized by the body and is therefore an essential fatty acid (Siguel et al., 1987). Pumpkin seed oil is rich in linoleic acid. Linoleic acid clearly has a hypocholesterolemic effect when substituted for dietary saturated fatty acids, reducing LDL cholesterol concentrations as well. Pumpkin seed oil is a natural product commonly used in folk medicine. It was shown in several countries that the incidence of hypertension, atherosclerosis and prostatic hypertrophy was reduced in people regularly consuming the seed (Harvath and Bedo, 1988). Such effects were demonstrated in this work with animals receiving pumpkin seed oil. Cholesterol level in this group of animals was decreased for 2.68% compared to control, the triglyceride level was 6.64% lower and LDL level in serum was descended for 10.60% compared to the controls. On the other hand HDL cholesterol level was significantly higher (for 16.70%) compared to control group. Such results might be a consequence of rich linoleic acid content of pumpkin seed oil. This findings show that food source rich in PUFAs (i.e. pumpkin seed oil) could also be used in designing diets for reduction of CVD risk.

An important finding reported in the present study is that a high-MUFA and a high-PUFA diet gave 40 to 60% reduction of atherogenic index in mice compared with an average diet. The average 12.7% reduction in LDL cholesterol achieved with olive and pumpkin seed oil would be expected to decrease CVD risk by 19%.

On the basis of the results of the present study, it appears that a high-MUFA, cholesterol-lowering diet is superior to a low-fat diet such as the Step II diet. Although the reduction in CVD risk due to a decrease in LDL cholesterol is similar for a high-MUFA diet and a low-fat diet (Step II diet), a high-MUFA diet lowers triacylglycerol and does not decrease HDL cholesterol. In contrast, a Step II diet increases triacylglycerol and lowers HDL cholesterol, thereby possibly undone some of the beneficial effects of reducing LDL cholesterol.

CONCLUSIONS

Both high-MUFA and high-PUFA diets showed decrease in cholesterol, triglyceride and LDL level, and on the other hand increase in HDL level in serum of treated animals compared to the controls. High-MUFA and a high-PUFA diet gave 40 - 60% reduction of atherogenic index in mice compared with an average diet. The average 12.7% reduction in LDL cholesterol achieved with olive and pumpkin seed oil would be expected to decrease CVD risk by 19%. According to this results high-MUFA and high-PUFA, cholesterol-lowering diet may be preferable to a low-fat diet because of more favorable effects on the CVD risk profile.

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Selected Abbreviations and Acronyms:

MUFA - monounsaturated fatty acid
PUFA - polyunsaturated fatty acid
SFA - saturated fatty acid
TG – total triglyceride
C – total cholesterol
VLDL – very low density lipoprotein
HDL – high density lipoprotein
LDL – low density lipoprotein
CHD – coronary heart disease
CVD - cardiovascular disease
SOP – Standard Operating Procedure
C – control
OO – Olive oil
PSO – Pumpkin seed oil

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