

EFFECT OF PRUNE *Prunus domestica* CONSUMPTION ON BLOOD LIPID PROFILE IN PATIENTS WITH MODERATE HYPERCHOLESTEROLEMIA

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ABSTRACT

Most of chemical and nutritional plum properties presented in the literature relate to cultivars grown in warm climates. The aim of the study was to assess the effect of prune consumption on blood lipid profiles in patients with moderate hypercholesterolemia. It used plums originating from temperate climates, characterized by lower sugar and higher polyphenol content than fruits grown in areas with greater sunlight. The study was conducted using *Prunus domestica* cv. 'Valor' plums, dried using the pulsed fluid bed method at the temperature of 80°C. Subjects were given 100 g prunes daily for 6 weeks. During that time, biochemical and morphological blood indexes, anthropometric parameters and blood pressure were monitored. The intake of nutrients and energy value was determined based on 24-hour diet recall interviews. Consumption of prunes resulted in a significant reduction in total cholesterol, LDL fraction and the LDL/HDL atherogenicity index. Dried plums of temperate climate origin exhibit a health-promoting effect on individuals with a disturbed blood lipid profile.

Key words: prunes, dietary fiber, hypercholesterolemia, LDL, HDL cholesterol

INTRODUCTION

Plums (*Prunus domestica*) are considered the fruits with a high or medium antioxidant capacity, resulting from their relatively high level of polyphenols. In studies determining the antioxidant capacity in different types of fruit, plums proved to be highly beneficial e.g. in comparison to oranges, kiwi, grapes or apples [Wang et al. 1996]. More of the chemical and nutritional plum properties presented in the literature relate to cultivars grown in warm climates. It is known that plants grown in temperate or cold climate are characterized by higher levels of phenolic compounds and antioxidant activity [Ksouri et al. 2008].

Plums are fruits with a high nutritional and dietary value when consumed both fresh and in their dried form as prunes. They are a source of saccharides, organic acids, polyphenolic compounds, dietary fiber, minerals (potassium, phosphorus, calcium, magnesium) and they exhibit high antioxidant activity. Potassium plays highly significant role among macronutrients found in plums due to its involvement in the regulation of blood pressure [Beals and Fulgoni 2005], while among microelements, boron plays an important role, since it reduces calcium excretion with urine, thus preventing the osteoporosis [Arjmandi et al. 2002, Stacewicz-Sapuntzakis 2013]. Predominant phenolic

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compounds found in plums include derivatives of caffeic acid, e.g. 3-O-caffeicquinic (neochlorogenic) acid, 5-O-caffeicquinic (chlorogenic) acid, 4-O-caffeicquinic (cryptochlorogenic) acid and caffeic acid, as well as anthocyanins (derivatives of cyanidin, peonidin), flavanols (catechin, epicatechin, proanthocyanins), flavonols (quercetin) [Nakatani et al. 2000, Tomas-Barberan et al. 2001, Kim et al. 2003]. Chlorogenic acid exhibits high antioxidant activity, has an advantageous effect on the level of blood LDL cholesterol and glucose levels, exhibits antimutagenic and anticarcinogenic action and inhibits the formation of conjugated dienes of linolic acid [Bouayed et al. 2007]. Many compounds found in plums, particularly polyphenols, dietary fiber, macro- and microelements, participate in the regulation of important life processes.

Numerous studies have confirmed the beneficial effect on the human organism of a diet rich in natural antioxidants, e.g. fruit and vegetables, by reducing blood pressure, improving the blood lipid profile, decreasing the incidence of cancer, promoting proper functioning of the alimentary tract, regulating bowel movement, and reducing body weight, as well as supporting treatment of arthritis, cataracts and dysfunctions of the immune system [Kris-Etherton et al. 2002, Lucas et al. 2004, Mateos et al. 2005, Śmidowicz and Reguła 2015a, Śmidowicz and Reguła 2015b]. According to the literature data, there is a strong correlation between polyphenolic content in fruit and their antioxidant activity [Bermudez-Soto and Tomas-Barberan 2004, Cevallos-Casals et al. 2006, Diaz-Mula et al. 2009].

Literature reports show that consumption of plums, either fresh or as prunes, has a beneficial effect on several life functions. Prune consumption improved the blood lipid profile and reduced bone mass loss in women [Lucas et al. 2004], decreased the blood LDL cholesterol level and reduced the level of bile acids in men [Tinkler et al. 1991], as well as improved metabolism of lipids and glucose [Tinkler et al. 1994], and also prevented the development of coronary heart disease and atherosclerosis [Chai et al. 2012]. Numerous nutrition studies have confirmed the beneficial effect of prunes in maintaining the appropriate bone condition and in preventing the osteoporosis [Arjmandi et al. 2002,

Hooshmand and Arjmandi 2009, Hooshmand et al. 2011], as well as reducing the risk of cancer, e.g. of the alimentary tract, the reproductive system and breast cancer [Yang and Gallaher 2005, Stacewicz-Sapuntzakis 2013].

The aim of this study was to assess the potential use of temperate climate origin prunes as a component of the diet for individuals with a disturbed blood lipid profile as a measure to prevent the hypercholesterolemia.

MATERIAL AND METHODS

Scheme of a nutrition study

The study was conducted with the participation of 48 individuals, qualified on the basis of a total cholesterol level in blood serum ≥ 200 mg/dl, while an additional condition was no administration of hypolipemic drugs. The mean age of the participants was 58 ± 15 years, while the mean height was 169 ± 8.4 cm. All patients gave informed consent to participate in the research.

The experiment was conducted for 7 weeks, of which stage I (preliminary) lasted for 1 week before the incorporation of prunes to the diet, and stage II (experimental) 6 weeks of incorporation of prunes into the diet. While introducing no changes to their previous diet, the patients additionally consumed prunes made from plum cv. Valor at 100 g daily for 6 weeks. The protocol of the study was approved by the Research Ethics Committee of the Poznan University of Medical Sciences and registered at No. 983/06.

In the preliminary period and in the first, third and sixth week of prune incorporation into the diet, a 24-hour diet recall interview was conducted to assess the intake levels of nutrients and energy value based on tables of the nutritive value of foodstuffs. The nutritional status was assessed at the beginning, in the middle and at the end of the experiment using the waist to hip ratio (WHR), the body mass index (BMI) and fat mass (%FM), determined based on measurements of skinfolds thickness ($\%FM = \left(\frac{4.95}{d} - 4.50\right) \times 100$) [Durnin and Womersley 1974]. During the second stage of the experiment, on the first day, after 3 and 6 weeks, biochemical and morphological blood parameters such as total cholesterol and HDL and LDL fractions, triacylglyc-

erols, glucose, hemoglobin, white blood cells (WBC) and red blood cells (RBC), and hematocrit were recorded. For 7 weeks, i.e. before and during the incorporation of prunes into the diet, blood pressure was monitored in patients three times daily at the same time each day. The study participants' eating habits and physical activity were determined based on a nutrition questionnaire.

Preparation of experimental material

The nutrition experiment was conducted using *Prunus domestica* cv. 'Valor' plums, originating from the experimental orchard of the Department of Pomology at Poznan University of Life Sciences (Agricultural and Fruit Farming Experimental Station in Przybroda; 52°31'N, 16°38'E). The fruit was from trees at full fruiting, growing on the Węgierka Wangenheimia rootstock, on grey-brown podzolic soil, at a 5 × 3 m spacing. In the orchard, irrigation was performed as indicated by the tensiometer. Agricultural practices and protection of plants were carried out according to the indications for orchard production (State Inspectorate of Plant Health and Seed in Poland). The harvest date was determined based on the sensory evaluation of fruit firmness and skin color. Immediately after harvesting, the fruits were processed. The plums were washed, pitted and dried using the pulsed fluid bed method at the temperature of 80°C until the 80% dry matter content in the product was reached.

Determination of antioxidant activity and selected bioactive compounds contents in plums and prunes tested

Antioxidant activity was determined by spectrophotometry using the ABTS* (2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonic acid)) radical according to the method of Re et al. [Re et al. 1999], with results expressed in $\mu\text{mol Trolox/g dm}$. Absorbance was measured using a Helios Alpha spectrophotometer (Thermo Electron Corporation, USA). Polyphenol content was assayed by HPLC according to the method of Podsędek et al. [Podsędek et al. 2006], applying detection from 240 to 540 nm, with readings at wavelengths of 280, 320 and 520 nm. The separation was performed in the reversed phase system on a Poroshell 120 column, SB-C18, with a particle size

of 2.7 μm and dimensions of 4.6 × 150 mm. Total polyphenol content was expressed as equivalents of chlorogenic acid in mg/100 g d.m. Carotenoids content was determined by HPLC according to the method of De Sa and Rodriguez Amaya [De Sa and Rodriguez-Amaya 2003], on a Waters Spherisorb 5 μm ODS2 column of 4.6 × 250 mm, with the results expressed in β -carotene in mg/100 g d.m. Chromatographic determinations were performed using an LC Agilent 1200 Rapid Resolution system. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were determined using the enzymatic method [Asp et al. 1983]. Total dietary fiber (TDF) was the sum of SDF and IDF.

Analyses of blood morphological and biochemical indices

Blood morphological indices (haematocrit, blood cells, hemoglobin) were determined using a Symex K-1000 hematological analyzer (TAO Medical Electronics Co., Kobe, Japan). Blood biochemical indices (cholesterol, HDL and LDL fractions, glucose) were analyzed using a Vitalab Flexor (Vital Scientific NV, Dieren, the Netherlands). All determinations were performed according to generally accepted recommendations described in the text.

Statistical analysis

Statistical analysis of obtained results was conducted using the Statistica PL 9.0 software (StatSoft) based on the analysis of variance for factorial systems, while differences between groups were assessed by the post-hoc HSD Tukey's test.

RESULTS

Plums used in the experiment were found to contain high amounts of total dietary fiber (10.95 g in 100 g product), of which almost 42% was soluble dietary fiber (Tab. 1). Plums of cv. 'Valor' had very high polyphenol content and high antioxidant activity, 1059 mg/100 g of the product and 83.8 $\mu\text{mol Trolox/g}$ of the product, respectively. The prunes used also constituted a good source of carotenoids (Tab. 1).

Table 1. Biochemical characteristic of prunes (plum cv. ‘Valor’) used in the nutrition experiment

Component	Contents in fresh mass of product	Contents in dry mass of product
Insoluble dietary fiber (g/100 g)	6.38 ±0.31	7.97 ±0.38
Soluble dietary fiber (g/100 g)	4.57 ±0.25	5.71 ±0.31
Total dietary fiber (g/100 g)	10.95 ±0.40	13.69 ±0.50
Total polyphenol contents (mg/100 g)	1059 ±87.2	1324 ±109
including:		
Neochlorogenic acid	708 ±28.8	886 ±36.5
Antioxidant activity (µmol Trolox/g)	83.8 ±10.2	104.8 ±12.8
Carotenoid contents (mg/100 g)	2.25 ±0.17	2.81 ±0.21

Table 2. Energy and nutritive value of daily diets of the study participants

Parameter	Study stage I	Study stage II
Energy (kcal)	2309 ±620 ^a	2390 ±530 ^a
Total protein (g)	80.6 ±19.9 ^a	76.8 ±14.2 ^a
Energy from protein (%)	14.1 ±1.49 ^a	12.6 ±1.94 ^a
Total carbohydrates (g)	313 ±74.6 ^a	351 ±65.8 ^a
Energy from carbohydrates (%)	52.3 ±8.23 ^a	55.8 ±8.72 ^a
Total fat (g)	89.5 ±32.2 ^a	84.4 ±30.2 ^a
Energy from fat (%)	33.6 ±5.37 ^a	31.6 ±6.00 ^a
Dietary fiber (g)	25.3 ±4.44^a	34.4 ±4.18^b
Cholesterol (mg)	294 ±96.0 ^a	278 ±90.9 ^a
Sodium (mg)	1290 ±324 ^a	1299 ±284 ^a
Potassium (mg)	3445 ±594^a	4060 ±626^b
Calcium (mg)	683 ±252 ^a	663 ±232 ^a
Phosphorus (mg)	1291 ±324 ^a	1299 ±284 ^a
Magnesium (mg)	308 ±84.5 ^a	323 ±61.9 ^a
Iron (mg)	11.9 ±2.77 ^a	12.0 ±2.18 ^a
Zinc (mg)	10.5 ±2.57 ^a	9.76 ±1.64 ^a
Retinol equivalent (µg)	1006 ±372 ^a	1012 ±367 ^a

Study stage I – diet without prunes (week I – preliminary study). Study stage II – diet with prunes (during 6 weeks of the experiment). Different letter superscripts in columns denote significant difference between means at $p < 0.05$

Table 3. Anthropometric parameters in patients consuming diet with prunes during the experiment

Parameter	Beginning of the experiment*	After 3 weeks	After 6 weeks
Body mass (kg)	73.4 ±14.0 ^a	73.3 ±16.3 ^a	73.4 ±18.7 ^a
BMI (kg/m ²)	25.2 ±4.10 ^a	25.2 ±3.94 ^a	25.2 ±4.24 ^a
WHR	0.89 ±0.09 ^a	0.89 ±0.10 ^a	0.89 ±0.08 ^a
Fat mass (%)	34.6 ±5.45 ^a	34.8 ±5.20 ^a	34.4 ±5.19 ^a

* first day of prune consumption, fasting

Different letters in columns denote significant difference between means at $p < 0.01$

Table 4. Morphological indices and glucose level in blood of patients consuming diet with prunes during the experiment

Parameter	Beginning of the experiment*	After 3 weeks	After 6 weeks
WBC ($10^9/l$)	6.62 ±1.47 ^a	6.32 ±1.50 ^a	6.24 ±1.56 ^a
RBC ($10^{12}/l$)	4.80 ±0.48 ^a	4.77 ±0.49 ^a	4.79 ±0.47 ^a
Hemoglobin (mg/dl)	14.3 ±1.08 ^a	14.2 ±1.17 ^a	14.4 ±1.22 ^a
Haematocrit (%)	41.9 ±3.28 ^a	41.8 ±3.62 ^a	42.1 ±3.54 ^a
Glucose (mg/dl)	102 ±20.4 ^a	102 ±30.1 ^a	103 ±28.8 ^a

Explanations as in Table 3

Table 5. Blood pressure measurements in patients consuming diet with prunes

Parameter	Beginning of the experiment*	After 3 weeks	After 6 weeks
Systolic pressure (mm Hg)	133 ±18.7 ^a	131 ±11.9 ^a	127 ±13.7 ^a
Diastolic pressure (mm Hg)	83 ±8.88 ^a	81 ±6.32 ^a	78 ±7.70 ^a

Explanations as in Table 3

Table 6. Level of total cholesterol and its fraction and atherogenicity index of patients consuming diet with prunes during dietary intervention

Parameter	Beginning of the experiment*	After 3 weeks	After 6 weeks
Total cholesterol (mg/dl)	254 ±19.5^b	246 ±25.9^b	234 ±34.8^a
LDL cholesterol (mg/dl)	166 ±12.2^b	153 ±16.2^b	142 ±24.8^a
HDL cholesterol (mg/dl)	60.2 ±13.6 ^a	62.5 ±16.4 ^a	64.4 ±12.3 ^a
Triacylglycerol (mg/dl)	138 ±81.2 ^a	154 ±87.8 ^a	139 ±62.2 ^a
Indicator LDL/HDL	2.91 ±0.03^b	2.57 ±0.02^b	2.32 ±0.09^a

Explanations as in Table 3

Based on the diet recall interviews, the level of intake was calculated for nutrients and energy value in the diet of the participants before (stage I) and during the period of the diet including prunes (stage II of the study) (Tab. 2). A lack of significant differences in the intake of basic nutrients (except for dietary fiber and potassium, $p < 0.05$ for both) and energy value between stages I and II indicates that patients did not change their diet during the experiment. This is reflected in the values of blood morphology indices and anthropometric parameters, which analogously showed no significant differences throughout the study period

(Tabs. 3 and 4). Low physical activity was observed for the study participants based on data supplied in the diet recall questionnaires. On this basis, it may be stated that the intake of macronutrients, level of energy intake and physical activity of patients in the course of the study did not differ significantly, i.e. they did not result in changes in blood biochemical indices recorded during the period of diet including prunes.

At the beginning of the experiment and during it, the mean body weight of patients was about 73 kg. Nutrition status indices, i.e. BMI and WHR, fell within the limits of the standards, although they were in the

upper area in terms of its optimal values (Tab. 3). Mean BMI was about 25 kg/m² and WHR about 0.89.

Values of all blood morphological indices of study participants were appropriate (Tab. 4). The mean glucose level was excessively high according to Diabetes Poland (>99 mg/dl). Moreover, it was constant throughout the experiment, which means that the consumption of prunes did not affect the value of this parameter.

When analyzing patients' food rations, no significant differences were found in the course of experiment, except for dietary fiber and potassium in the intake levels of basic nutrients potentially affecting the blood lipid profile (Tab. 2). An excessive intake of sodium and potassium was observed.

Values for blood pressure of patients at the beginning of the experiment were on average 133 ±18.7 mm Hg for systolic pressure and 83 ±8.9 mm Hg for diastolic pressure. After 3 and 6 weeks of the experiment, values of systolic and diastolic blood pressure decreased slightly in the patients; however, these differences were statistically non-significant (Tab. 5).

Patients participating in the diet recall experiment reported elevated levels of total cholesterol and its LDL fraction. The mean value of total cholesterol was 254 mg/dl, its LDL fraction 166 mg/dl and HDL fraction 60.2 mg/dl. In the course of the experiment, it was found that significant ($p < 0.01$) reduction for total cholesterol (by 8%) and the LDL fraction (by 14%) (Tab. 6) in the patients was recorded as a result of prunes consumption for 6 weeks. As a consequence of changes in the concentration of the cholesterol fraction, an advantageous reduction of 20% was found for the atherogenicity index (LDL/HDL).

DISCUSSION

In a view of the nutritive value and sensory attributes, as well as relatively high contents of bioactive compounds, plums should be a permanent ingredient of our diet, both in their fresh and processed form. The health-promoting value of plums, including prunes, results from their high dietary fiber content and antioxidant compounds, such as phenolic acids, anthocyanins and other flavonoids [Nakatani et al. 2000, Kahlon and Smith 2007]. They are sources of readily available energy and carbohydrates; however, their consumption does not cause a rapid increase in

blood glucose levels, due to their high content of dietary fiber, fructose and sorbitol [Stacewicz-Stapuntzakis et al. 2001, Stacewicz-Stapuntzakis 2013].

A relatively high content of dietary fiber, including soluble fraction, is connected with the ability of prunes to reduce the blood cholesterol level by binding bile acids [Tinkler et al. 1991, Lucas et al. 2004, Kahlon and Smith 2007]. 'Valor' plums are a rich source of polyphenol compounds, that can prevent oxidation of cholesterol, and inhibit the activity of oxidative enzymes. Phenolic acid content in plums, as presented in the literature, falls within a wide range of values, i.e. neochlorogenic acid 45–87 mg/100 g f.w., chlorogenic acid 4–15 mg/100 g f.w., cryptochlorogenic acid 0.2–1.1 mg/100 g f.w. [Nakatani et al. 2000, Tomas-Barberan et al. 2001]. Piga et al. [2003] studied (*Prunus domestica*) cv. 'President' plums belonging to the same group of varieties as cv. 'Valor' and which were convectively dried at 85°C. They recorded a total polyphenol content of 420 mg/100 g d.m., including neochlorogenic acid at 370 mg/100 g d.m. These values are 2 to 3 times lower than for 'Valor' prunes used in this experiment. Carotenoids, that are components of these plums, also exhibit antioxidant activity, which is confirmed by Del Caro and Piga [2013]. The presence of such high amounts of bioactive compounds and relatively high antioxidant activity affects several health-promoting functions, which are ascribed to prunes [Sagar and Suresh 2010, Del Caro and Piga 2013].

Energy value of the diet and macronutrients intake in the patients during the nutrition study did not differ significantly. A BMI index value of about 25 kg/m², as was found in the experiment, indicates that the study participants were not overweight. A WHR value of around 0.9 indicates an android type of fat distribution.

Values of all blood morphological indices in patients were appropriate, within limits of the standard [Dembińska-Kieć and Nastalski 2009], and did not differ significantly during the experiment.

It was found that patients consumed adequate amount of sodium in their diet, although simultaneously low intake of potassium in I study stage. In II study stage, intake of potassium met the standards. An appropriate Na to K ratio in the diet was observed

in both study stages [Jarosz 2012]. Significant differences in the intake of dietary fiber and potassium in the course of the experiment were connected with the incorporation of prunes into the diet at the amount of 100 g a day. These fruits are a very rich source of these nutrients [Ertekin et al. 2006]. Despite the high intake of dietary fiber, patients did not report any undesirable effects of the diet on the functioning of their alimentary tract, which was confirmed by studies conducted by Lucas et al. [2004] and Howarth et al. [2010]. Blood pressure values of patients were appropriate for these individuals' age (58 ± 15), according to the recommendations of the Polish Society of Hypertension and consistent with the guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) [Mancia et al. 2013]. The blood pressure values recorded in individual patients fell within the limits of normal arterial pressure ($<130/85$ mm Hg) or high normal arterial pressure (prehypertension), which corresponds to systolic blood pressure of 130–139 mm Hg and diastolic blood pressure of 85–89 mm Hg (according to ESH/ESC). The protective action of prunes, and also peaches and nectarines, in preventing and reducing the risk of hypertension and other cardiovascular diseases, is confirmed by numerous research reports [Beals and Fulgoni 2005].

An appropriate total cholesterol level is assumed to be <200 mg/dl, LDL <130 mg/dl, HDL >40 mg/dl, while that of triacylglycerols <150 mg/dl [Reguła 2009]. A decrease in the total and LDL fraction of cholesterol and a reduction in the atherogenicity index is probably connected with the absorption of cholesterol and bile acids by soluble dietary fiber contained in plums [Kahlon and Smith 2007]. This is advantageous for preventing the development of hypercholesterolemia and cancer. It is assumed that secondary bile acids, i.e. lithocholic acid (LCA) and deoxycholic acid (DCA), may exhibit the mutagenic and carcinogenic activity [Costarelli et al. 2002]. A reduction in cholesterol and bile acid concentrations in blood serum as a result of prune consumption was confirmed by many authors [Tinkler et al. 1991, Tinkler et al. 1994, Lucas et al. 2000, 2004].

Advantageous changes in the blood lipid profile as a result of prune consumption are also attributed to the antioxidant activity and high polyphenol content, particularly chlorogenic acids, in this fruit [Mateos

2005]. Plums of the cv. 'Valor' had high values of these parameters (Tab. 1). Due to the reduction of free radicals, polyphenols prevent oxidation of cholesterol, particularly its LDL fraction, and inhibit oxidation of endogenous antioxidants [Reguła 2009].

CONCLUSION

In conclusion, our findings make it possible to state that a diet with addition of dried *Prunus domestica* cv. 'Valor' plums can decrease the level of total cholesterol and its LDL fraction in patients' blood. This facilitates a reduction in the risk of cardiovascular diseases. This is associated with high level of dietary fiber and antioxidant activity of polyphenols in prune composition. It is important to note that at the same time there was no undesirable effects of a diet rich in dietary fiber on the functioning of the alimentary tract. Results of our studies make it possible to recommend *Prunus domestica* cv. 'Valor' prunes derived from the moderate climate as a filling snack with health-promoting properties.

ACKNOWLEDGEMENTS

This work was partly supported by the State Committee for Scientific Research of Poland Grant No. NN312 1497 33.

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. All procedures involving human subjects have been approved by the Poznan University of Medical Sciences, registered at No. 983/06 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study.

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