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Original Research Article

Pomegranate seed oil influences the fatty acids profile and reduces the activity of desaturases in livers of Sprague-Dawley rats

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ABSTRACT

The aim of our study was to compare the influence of diet supplementation with pomegranate seed oil – as conjugated linolenic acids (CLnA) source, or conjugated linoleic acids (CLA) and to examine the mechanism of their activity. The content of fatty acids, levels of biomarkers of lipids' oxidation and the activity of key enzymes catalyzing lipids metabolism were measured. Obtained results revealed that conjugated fatty acids significantly decrease the activity of Δ5-desaturase ($p = 0.0001$) and Δ6-desaturase ($p = 0.0008$) and pomegranate seed oil reduces their activity in the most potent way. We confirmed that diet supplementation with pomegranate seed oil – a rich source of punicic acid leads to the increase of *cis*-9, *trans*-11 CLA content in livers ($p = 0.0003$). Lack of side effects and beneficial influence on desaturases activity and fatty acids profile claim pomegranate seed oil to become interesting alternative for CLA as functional food.

1. Introduction

Pomegranate, (*Punica granatum* L., *Punicaceae*) has been known from its health benefits since ancient times. It is a source of plenty medicine raw materials and functional foods, such as fresh and processed pomegranate juice or pomegranate cortex. The first exert antioxidant and anti-inflammatory effects resulting from the presence of flavonoids, anthocyanins and phenolic acids, which inhibit activity of inflammation activators (*e.g.* lipoxygenase (LOX) or cyclooxygenase (COX)). The second is a rich source of tannins and alkaloids, which present anti-parasite effect. Peel homogenates can inhibit proliferation of different human breast and prostate cancer lines [1]. Moreover, pomegranate flowers extract and seed oil exert a hypoglycemic effect, which in relation to pomegranate seed oil may result from the presence of punicic acid (PA) – one of conjugated linolenic acids (CLnA) [2–4].

CLnA is a common name of fatty acids (FA) – geometric and positional isomers of octadecatrienoic acid (C18:3), which occur in both plants and in animal products. These of plant origin posses three conjugated double bonds ("conjugated triene type"), while these present in ruminant-derived products are partially conjugated (also called "cojnjugated diene type") [5,6]. CLnA are natural components of oils obtained from seeds of certain plants, such as pomegranate (*Punica granatum* L., *Punicaceae*), which contains punicic acid (*cis*-9, *trans*-11, *cis*-13 C18:3, PA), bitter melon (*Momordica charantia* L., *Cucurbitaceae*) which contains α-eleostearic acid (*cis*-9, *trans*-11, *trans*-13 C18:3), catalpa (*Catalpa ovata, Bignoniaceae*) which contains catalpic acid (*trans*-9, *trans*-11, *cis*-13 C18:3), jacaranda (*Jacaranda mimosifolia, Bignoniaceae*) which contains jacaric acid (*cis*-8, *trans*-10, *cis*-12 C18:3) or pot marigold (*Callendula officinalis* L., *Compositeae*) which contains calendic acid (*trans*-8, *trans*-10, *cis*-12 C18:3) [2]. In all these seeds CLnA are prevalent of all FA (> 60%). The interest in these conjugated fatty acids (CFA) has been growing because very promising results of both animal and human studies referring to their biological activities, such as anti-diabetic and anti-cancerous properties. Also our own results proved that incorporation of pomegranate seed oil into the diet of rats did not influence negatively overall health condition of the animals [7].

Desaturases, as enzymes involved in FA transformation and in metabolism of both n-3 and n-6 fatty acids, are known to be responsible for conjugated linoleic acids (CLA) synthesis. Activity of Δ6- and Δ5-

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; CFA, conjugated fatty acids; CLA, conjugated linoleic acids; CLnA, conjugated linolenic acids; COX, cyclooxygenase; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; D5D, Δ5-desaturase; D6D, Δ6-desaturase; EPA, eicosapentaenoic acid; FA, fatty acids; FAME, fatty acids methyl esters; GC, gas chromatography; GLA, γ-linolenic acid; HPLC, high-performance liquid chromatography; LA, linoleic acid; LOX, lipoxygenase; MUFA, monounsaturated fatty acids; OLA, oleic acid; OL, group of animals which were fed standard diet and water *ad libitum* and they received vegetable oil given *via* gavage in the amount of 0.15 ml/day; PA, punicic acid; PI, peroxidability index; PUFA, polyunsaturated fatty acids; RA, rumienic acid; SFA, saturated fatty acids; TBARS, thiobarbituric acid reactive substance

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desaturase is the main factor controlling the conversion of dietary linoleic acid to arachidonic acid, and many dietary factors can influence this activity [8–10]. Rumenic acid (*cis*-9, *trans*-11 CLA) can be endogenously formed in human body from *trans*-11 octadecenoic acid (vaccenic acid) by the action of Δ9-desaturase [11]. Our previous results revealed that the cancerous process increases the activity of Δ6-desaturase, which in consequence may cause a more rapid formation of prostaglandin E2 (with known effects). The decrease of Δ6-desaturase activity, resulting from the presence of CLA in the animals' diet, is one of mechanisms of the anticancer properties of these isomers [12]. CLA also influences fatty acids profile in livers and changes the oxidative status of different tissues [13,14]. Due to the high similarity in structure between CLA and CLnA the main aim of present study was to examine potential mechanisms of CLnA physiological activity and to compare whether they are similar to these observed for CLA. The objective of the present study was to compare the influence of diet supplementation with sources of different CFA (pomegranate seed oil as a natural source of CLnA or dietary supplement as a source of CLA) on FA profile of liver and activity of key enzymes catalyzing lipids metabolism and intensity of lipids' oxidation in livers of rats.

2. Materials and methods

2.1. Conjugated linoleic acids

Commercially available dietary supplement containing CLA mixture in form of gel capsules – Bio-C.L.A. was obtained free of expense from Pharma Nord Denmark (Pharma Nord, Warsaw, Poland). This preparation contained 600 mg of CLA in each capsule. Mean content of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA was (31.4 ± 0.0%) and (33.3 ± 0.1%) respectively (performed with GC analysis thoroughly described in 2.6). It was stored at room temperature, according to the manufacturer's instruction. Oil filling of capsule was expressed before administration to the animals.

2.2. Vegetable oil

Applied oil was kindly donated by Pharma Nord Denmark (Pharma Nord, Warsaw, Poland), where it is used as the substrate for Bio-C.L.A. synthesis. It does not contain CLA, and its main FA were C18:1 n-9 (64.5 ± 0.1%) and C18:2 n-6 (25.5 ± 0.0%) (performed with GC analysis thoroughly described in 2.6).

2.3. Pomegranate seed oil

Commercially available cold pressed unrefined oil from seeds of pomegranate fruits was purchased from the local market (Zielony Klub, Kielce, Poland). It was stored at 8°C before administration to animals. It contains *cis*-9, *trans*-11, *cis*-13 CLnA, which is the predominant fatty acid and constitutes 32.7 ± 1.6% of all FA (performed with GC analysis thoroughly described in 2.6).

2.4. Animals

The whole experiment as well as the guiding principles in the use and care of laboratory animals were approved by The 2nd Local Ethical Committee on Animal Experiments in Medical University of Warsaw (Warsaw, Poland) (approval number: 56/2013). Female Sprague–Dawley rats (n = 30, age – 30 days) were purchased from Division of Experimental Animals, Department of General and Experimental Pathology (Medical University of Warsaw, Warsaw, Poland). They were kept in animal room at 21°C, in a 12 h light: 12 h dark cycle. During the whole experiment animals were fed *ad libitum* water and a standard laboratory fodder Labofeed H (Feed and Concentrates Production Plant, A. Morawski, Kcynia, Poland), which is composed of 22.0% protein, 4.0% fat, 30.0% starch, 5.0% fibre, 6.5% minerals (caloric value 1.28 MJ/306.2 kcal per 100 g). Detailed composition of Labofeed H fodder is given in Table 1. After 1-week adaptation animals were randomly divided into 5 groups of 6 individuals each (n = 6). The total characteristics of experimental groups is shown below:

Table 1
Composition of Labofeed H fodder.

Protein [g]	210.0	Caloric value [MJ]	12.8
Fat [g]	39.2	Caloric value [kcal]	3062
Fiber [g]	43.2		
Strach [g]	300.0		
Ash [g]	55.0		
Vitamin A [IU]	15000	Lysine [g]	14.5
Vitamin D_3 [IU]	1000	Methionine [g]	4.1
Vitamin E [mg]	90.0	Tryptophan [g]	3.0
Vitamin K_3 [mg]	3.0	Threonine [g]	7.4
Vitamin B_1 [mg]	21.0	Isoleucine [g]	17.5
Vitamin B_2 [mg]	16.0	Valine [g]	11.0
Vitamin B_6 [mg]	17.0	Histidine [g]	6.0
Vitamin B_{12} [μg]	80.0	Arginine [g]	13.0
Pantothenate [mg]	30.0	Phenylalanine [g]	10.0
Folic acid [mg]	5.0	Tyrosine [g]	7.8
Nicotinic acid [mg]	133.0	Choline [mg]	2750.0
Biotin [mg]	0.4		
Calcium [g]	10.0	Iron [mg]	250.0
Phosphorus total [g]	8.17	Manganese [mg]	100.0
Phosphorus saturated [g]	4.5	Zinc [mg]	76.9
Magnesium [g]	3.0	Copper [mg]	21.3
Potassium [g]	9.4	Cpbalt [mg]	2.0
Sodium [g]	2.2	Iodine [mg]	1.0
Chlorine [g]	2.5	Selenium [mg]	0.5
Sulfur [g]	1.9		

Declared data are expressed per kg of diet.

CON – control group, fed standard diet and water *ad libitum*,

OL – animals were fed standard diet and water *ad libitum* and they received vegetable oil given *via* gavage in the amount of 0.15 ml/day,

CLA1 – animals were fed standard diet and water *ad libitum* and they received conjugated linoleic acid (Bio-C.L.A. (Pharma Nord Denmark)) given *via* gavage in the amount of 0.15 ml/day,

CLA2 – animals were fed standard diet and water *ad libitum* and they received conjugated linoleic acid (Bio-C.L.A. (Pharma Nord Denmark)) given *via* gavage in the amount of 0.30 ml/day,

PSO – animals were fed standard diet and water *ad libitum* and they received pomegranate oil given *via* gavage in the amount of 0.15 ml/day.

Table 2 shows the fatty acids composition of applied diets (performed with GC analysis thoroughly described in 2.6). Dietary supplementation was conducted for the following 21 weeks. Rats were daily monitored for any specific signs of welfare disorders (*e.g.* appetite loss, ruffling, sluggishness, apathy, hiding, curling up). Weekly they were also checked by the veterinarian for any specific signs of health deterioration and weighed. In the 21st week of the experiment all animals were decapitated and exsanguinated without anesthesia and the weight of internal organs were determined.

2.5. Preparation of experimental material

Samples of liver were collected during necropsy and stored at −70°C before the analysis.

Hepatic microsomes were prepared immediately after decapitation according to the slightly modified method of Kłyszejko-Stefanowicz [15] which was thoroughly described previously [13].

2.6. Fatty acids analysis

FA analysis was made with gas chromatography (GC) using gas chromatograph (GC-17A gas chromatograph, Shimadzu, Kyoto, Japan)