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Effects of *Punica granatum* L. peel extract supplementation on body weight, cardiac function, and haematological and biochemical parameters in an animal model of metabolic syndrome

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Abstract

Introduction: Metabolic syndrome (MetS) is a cluster of pathological conditions well described in humans but still investigated insufficiently in animals. A novel approach in its management is the utilisation of nutrients from natural sources. Recent studies suggested that phenolic compounds from pomegranate peel could be a promising dietary intervention for MetS. This study evaluated the potency of polyphenol-rich pomegranate peel extract (EPP) in mitigating some MetS components in an animal model. **Material and Methods:** Zucker diabetic fatty rats (with an fa/fa missense mutation in the *Lepr* leptin receptor gene) and their healthy counterparts (fa/+) as controls were fed a high-calorie diet to induce MetS and supplemented with EPP at two doses: 100 mg/kg body weight (b.w.) and 200 mg/kg b.w. The extract was administered for eight weeks. The rats' body weights were monitored twice per week, and blood samples were taken before EPP administration after four weeks and eight weeks of study. Echocardiography measurement was performed at the beginning and at the end of the study. **Results:** The extract restrained the dynamic of weight gain. A cardioprotective effect of the highest dose of EPP supplementation was manifested in a relative decrease in heart rate and improved mid-fractional shortening, representing myocardial contractility. No improvement in fasting blood glucose or lipid profile was observed. **Conclusion:** Pomegranate peel extract possesses beneficial health properties that could be useful in dietary intervention in MetS. However, its bioavailability still requires further investigation in clinical trials in humans and animals suffering from endocrine and metabolic disorders.

Keywords: polyphenols, pomegranate peel, Zucker diabetic fatty rats, metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is a pathological condition defined as a set of several metabolic disorders comprising visceral obesity, insulin resistance, dyslipidaemia and hypertension (5). It is defined by diagnostic criteria with parametric cut-off points varying as appropriate for particular target populations. Any three out of the five following risk factors must be present to make a diagnosis: central obesity (abnormal waist circumference or body mass index), insulin resistance (impaired glucose tolerance, impaired fasting glucose or type 2 diabetes requiring treatment), dyslipidaemia (hypertriglyceridaemia, low concentration of high-density lipoprotein cholesterol (HDL) or hyperlipidaemia requiring treatment) and hypertension (high resting blood pressure or need for antihypertensive drugs) (39). Additionally, apart from the main components, MetS is also associated with impaired kidney function, polycystic ovary syndrome, hyperuricaemia, fatty liver disease, obstructive sleep apnoea and heart failure with preserved ejection fraction (EF) (47). The pathogenesis is complex and still not

completely elucidated. However, it seems that the primary roles are played by insulin resistance, chronic inflammation and neurohormonal activity (45). Early diagnosis and treatment are vital to prevent the development of more severe conditions such as atherosclerotic cardiovascular disease and to modify the risk factors. The first line treatment of MetS is based on lifestyle changes promoting physical activity, sleep hygiene, reduced alcohol consumption, and dietary intervention (12, 47). Drugs such as antihypertensives, statins and metformin are used when non-medical strategies prove ineffective. Nonetheless, their application is limited by drug-related adverse effects emerging over the course of long-term therapy (12).

In veterinary clinics, MetS is well described in horses but still requires further investigation in obese dogs and cats and in individuals with hypercortisolaemia. Some reports have already shown the connection between obesity, hyperlipidaemia and insulin resistance. The occurrence of these disorders in humans escalates the risk of cardiovascular diseases such as atherosclerosis, a fatal condition well known in humans but unusual in small animals or horses. Establishing diagnostic criteria for MetS in veterinary medicine will facilitate its diagnosis and help in the early implementation of appropriate treatment.

A novel approach to the management of MetS involves plant food supplements. Nutraceuticals are natural dietary components with proven health benefits. Studies show that compounds derived from plants display potentially propitious features in MetS (48). Polyphenols in particular, which are biomolecules present in flowers, seeds, juice, arils, roots and leaves, are believed to be integral to the future of the food industry as natural food additives (48). Studies have shown that polyphenols exhibit antioxidative, anti-inflammatory, antihypertensive, antimicrobial, antiatherogenic, antiaging and antimutagenic effects. Favourable medical features also include the promotion of weight loss, antidiabetic properties, improvement of lipid profile, and cardioprotective, hepatoprotective and nephroprotective activity (13, 48). Therefore, they are considered potential therapeutic agents in MetS. Consumption of functional food enriched with phenolic compounds could improve public well-being and help prevent diseases of affluence such as cardiovascular diseases, type 2 diabetes and obesity (22). Additionally, incorporating phenolic compounds in animal food may promote health and serve as a component of pre-emptive veterinary medicine.

Pomegranate (*Punica granatum* L.) is a rich source of phenolic compounds. Numerous studies highlight its high concentration of health-promoting phytochemicals and promising pharmaceutical properties in dietary supplementation (1, 21). It is predominantly consumed fresh or in the form of juice. Health benefits are bestowed not only by juice and the flesh of the fresh fruit but also by the uneatable parts, which contain a wide range of bioactive compounds (19, 37). Pomegranate peel, a by-product of industrial juice processing, contains a high concentration of polyphenol fractions such as phenolic acids (ellagic acid, caffeic acids, hydroxybenzoic acid, hydroxycinnamic acid and gallic acid), hydrolysable tannins (catechin, ellagitannins, gallotannins and gallagyl esters), flavonols (epicatechin and gallocatechin) and anthocyanins (9). The bioactivity of pomegranate peel is anti-inflammatory, antioxidant, antimutagenic, anticancerogenic, antidiabetic and antimicrobial (4, 7). Pomegranate peel's content of phytochemicals with nutraceutical and medical significance make it an auspicious natural source in the development of functional food products.

The main objective of the present study was to assess the potential health benefits from pomegranate peel extract (EPP) supplementation in an animal MetS model.

Material and Methods

Animals. The study was carried out on Zucker diabetic fatty rats with missense mutations in the *Lepr* leptin receptor gene (ZDF-Leprfa/Crl, fa/fa) and their healthy counterparts as controls (fa/+). The rats were purchased from Charles River Laboratories, Research Models and Services (Sulzfeld, Germany). The room where the animals were kept was maintained on a 12-h light-dark cycle at 20°C ± 2°C. After two weeks of acclimatisation, individuals were randomly assigned into five groups as follows: 1) a control group designated fa/fa H_2O (ZDF, fa/fa, n = 6) receiving only water, 2) a study group designated fa/fa 100 (ZDF, fa/fa, n = 6) receiving EPP in a dose of 100 mg/kg body weight (b.w.), 3) a study group designated fa/fa 200 (ZDF, fa/fa, n = 6) receiving EPP in a dose of 200 mg/kg b.w., 4) a study group designated fa/+ 100 (healthy controls (HC), fa/+, n = 6) receiving EPP in a dose of 100 mg/kg b.w., and 5) a study group designated fa/+ 200 (HC, fa/+, n = 6) receiving EPP in a dose of 200 mg/kg b.w. Extract of pomegranate peel was administered daily by oral gavage using water as a vehicle. All individuals were maintained on Purina 5008 (LabDiet, Richmond, IN, USA). The extract from pomegranate peel was administered for eight weeks. The study project was approved by the Ethics Committee for Experiments on Animals at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in the Polish Academy of Sciences, Wrocław, Poland (Resolution 53/2017).

Procedure for extraction of polyphenols from pomegranate peel. The polyphenolic extract was obtained from *Punica granatum* L. peel (Mollar de Eche cultivar) delivered from Spain. The dried peel of pomegranates was shredded in a Thermomix domestic kitchen appliance. The resulting material (1 kg) was extracted and re-extracted twice with 50% ethanol. The extraction process was conducted in an ultrasonic bath for 25 min. The extract was subsequently concentrated

with a Rotavapor rotary evaporator (BÜCHI Labortechnik, Flawil, Switzerland) in a water bath at 40°C. The concentrated ethanol extract from the pomegranate peel was passed through a column with Amberlite XAD-16 resin (Brenntag, Essen, Germany), and the column was washed with distilled water to rinse out the organic acids, sugars and other undesirable compounds. Polyphenols were eluted with 80% ethanol. The collected fractions were dried in an SPT-200 vacuum oven (Zeamil, Kraków, Poland).

Identification and quantification of polyphenolic compounds by liquid chromatography–mass spectrometry coupled with quadrupole time-of-flight photodiode array detection. Identification and quantification of polyphenolic compounds were performed on an Acquity ultra-performance liquid chromatography (UPLC) system, coupled with a Synapt quadrupole time-of-flight (Q-TOF) mass spectrometry (MS) instrument (Waters Corp., Milford, MA, USA), with an electrospray ionisation source and photodiode array detector. Separation was obtained on the Acquity bridged ethylene hybrid C18 column (100 mm × 2.1 mm i.d., 1.7 m, Waters Corp.). The water phase was a mixture of 0.1 % (v/v) aqueous formic acid (A) and acetonitrile (B). The gradient programme was as follows: the initial conditions were 1% B in A, 12 min of 25% B in A, 12.5 min of 100% B in A and 13.5 min of 1% B in A. The flow rate was 0.45 mL/min, and the injection volume was 10 µL. The column was operated at 30°C. Ultraviolet-visible absorption spectra were recorded online during UPLC analysis, and the spectral measurements were made in the wavelength range of 200–600 nm in steps of 2 nm. The primary operating parameters for the Q-TOF MS were set as follows: capillary voltage of 2.5 kV, cone voltage of 30 V, cone gas flow of 11 L/h, collision energy 28–30 eV, source temperature of 100°C, desolvation temperature of 250°C, argon collision gas, nitrogen desolvation gas flowing at 300 L/h, data acquisition range m/z 100–2000 Da, and negative ionisation mode. The data were collected with MassLynx v. 4.1 software (Waters Corp). The results of the analysis are presented in Table 1.

Body weight measurement. Body weight was recorded twice per week. Measurements for each individual in a single survey point were taken three times, and the mean was calculated and subsequently incorporated into the statistical analysis. The weight of individuals was measured using an SW-II certified calibrated scale (CAS Poland Sp. z.o.o., Warsaw, Poland).

Blood sampling and analyses. The blood samples were obtained at three time points: before starting EPP administration, after four weeks, and after eight weeks of study. Blood was drawn from the lateral tail vein after warming the tail to increase the obtainable blood volume, and was collected into microtubes containing 1.6 mg/mL liquid ethylenediaminetetraacetic acid (EDTA) and serum tubes. Serum samples were separated by centrifugation (4,000 × *g* for 5 min) and stored at −80°C for biochemical assays. A SCIL VET ABC animal blood counter analytical haematology system (Horiba ABX, Montpellier, France) was employed to determine the following morphological parameters: white blood cell count (WBC), red blood cell count (RBC), haematocrit, haemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT). Peripheral blood smear images were examined manually by the same laboratory technician.

Table 1. Mass spectrum characteristic and content of phenolic compounds in pomegranate peel extract

Rt (min)	MS $(M-H)^-$ (*m/z*)	MS/MS $(M-H)^-$ (*m/z*)	Name of compounds	Polyphenol content
1.67	331	271/169	Galloyl-glucose	2.00 ± 0.03
1.73	781	721/601	Punicalin α/A	3.11 ± 0.06
2.02	1083	611/331/146	HHDP-galloyl-hexoside (punicalagin)	4.20 ± 0.09
2.12	1083	781/622/301	Punicalagin isomer	14.82 ± 1.04
2.33	933	631/450/301	Ellagitannin	4.71 ± 0.40
2.87	1083	781/301	HHDP-gallagyl-hexoside (punicalagin)	93.91 ± 2.05
3.12	1085	907/783/301	Ellagic acid derivative	2.49 ± 0.53
3.69	1083	781/301	HHDP-gallagyl-hexoside (punicalagin)	157.00 ± 2.65
3.89	799	301	Granatin A	4.74 ± 0.32
5.08	783	481/301	Ellagitannin	25.86 ± 1.53
6.20	1085	933/301	Digalloyl-gallagyl-hexoside	10.37 ± 0.65
6.25	783	481/301	Ellagitannin	13.51 ± 0.99
6.38	463	301	Ellagic acid-hexoside	33.63 ± 1.23
6.89	951	907/635/301	Galloyl-HHDP-DHHDP-hex (granatin B)	2.68 ± 0.11
			Total (mg/g dry weight)	373.05

Rt – retention time; MS – mass spectrometry; $(M-H)^-$ – deprotonated molecule; *m/z* – mass-to-charge ratio; MS/MS – tandem mass spectrometry; HHDP – hexahydroxydiphenic acid; DHHDP – dehydrohexahydroxydiphenic acid

The leukocyte quantities were determined by a Schilling differential cell count. Erythrocyte abnormalities of size and shape were recorded and reticulocytes were counted in a microscopic examination. After staining EDTA blood with 1% new methylene blue with 1.6% potassium oxalate anticoagulant and 1% brilliant cresol blue in saline (ANALAB Sp. z o.o., Warsaw, Poland), the percentage of reticulocytes per 1,000 non-nucleated red blood cells was calculated. Blood biochemical indices were investigated with an Epoll 300 analyser (Alpha Diagnostic Intl. Inc., San Antonio, TX, USA). Serum levels of glucose and the lipid panel (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides) were established.

Cardiac function. Heart size and function assessment was performed before EPP administration and eight weeks after it. Echocardiography measurements were performed in the Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats in the Faculty of Veterinary Medicine at Wroclaw University of Environmental and Life Sciences. The echocardiographic measurements were taken by the same researcher over at least three consecutive cardiac cycles using an Arietta echocardiograph (Aloka Company, Tokyo, Japan) and a 7.5–10 MHz transducer according to the guidelines of the American Society for Echocardiography. The following cardiac dimensions were determined: the relative left atrial size (from the left atrial diameter to aortic root diameter ratio (LA/Ao), end-diastolic and end-systolic thickness of the interventricular septum, left ventricle posterior wall, and the internal left ventricular dimensions at end diastole and end systole (LVIDd and LVIDs). Estimates of left ventricular systolic function were obtained from the index of circumferential myocardial contraction and fractional shortening (FS) using the Teicholz formula ((LVIDd−LVIDs/LVIDd) × 100%). Estimates of left ventricular end-diastolic volume, end-systolic volume, stroke volume, and EF were calculated by the echocardiographic software.

Statistical analysis. The results are displayed as a mean ± standard deviation. All statistical analyses were performed using the R statistical computing environment (version 4.1.1.; 32). If the data followed a normal distribution, parametrical tests were used. The non-normal data were analysed with the use of nonparametric tests. For three or more groups of variables with normal distribution, one-way analysis of variance was applied, and to compare two groups of normal data, Welch's *t*-statistic was calculated. Welch's formula for one-way ANOVA was applied in order to preserve type I error robustness for unequal variances of groups. The effect size was estimated applying Field's convention for the comparison of more than two groups (estimator ω^2_p) and Cohen's criteria for the comparison of two groups (estimator $\hat{g}_{Hedges}$). Violin plots were drawn to visualise the distribution of the numerical data. To examine the effect size of repeated measurements associated with differences between interventional and control groups, the multilevel growth model proposed by Feingold was applied. Statistical analyses were conducted at a significance level of $P < 0.5$.

Results

Effect of pomegranate peel extract on body weight. Body weight assessments were analysed at 16 time points. No significant differences were noted at the last time point between the control group fa/fa H_2O (mean = 407.14 g, standard deviation (SD) = 32.68 g) and the experimental group fa/+ 200 (mean = 337.69 g, SD = 29.34g), F_{Welch} = 4.56 using the value 4 as adjustment for degrees of freedom, 11.68 as adjustment for error degrees of freedom, and 0.02 as the rectified p-value according to the Welch formula. Similarly, no significant effect of pomegranate peel extract on body weight in other groups was observed (Table 2 and Fig. 1).

The dynamic of body weight increase was registered at 16 time points. Graphic representations of data are presented in Fig. 2. The data show clear tendencies, despite slight differences between individuals at the beginning of the study. Body weights in all groups trended upwards. However, in groups without MetS (fa/+), the increase was more pronounced than in rats predisposed to the syndrome (fa/fa), and the gain in these groups was considerably quicker (Fig. 3). Experimental groups with MetS were characterised by smaller weight gains than the group of control rats with MetS administered only water. The body weight gain at each time point in group fa/fa 100 was 1.37 g less and in group fa/fa 200 was 1.22 g less than that in group fa/fa H_2O. In groups of rats without MetS, the body mass increase was higher at each time point in comparison to the increase in group fa/fa H_2O, in group fa/+ 100 being so by about 3.09 g, and in group fa/+200 by about 2.25 g.

Table 2. Mean body weights for individual groups at the final 16th time point

group	fa/fa H_2O	fa/fa 100	fa/fa 200	fa/+ 100	fa/+ 200
n	6	6	6	6	6
Mean	407.14	382.08	392.75	358.97	337.69
Standard deviation	32.68	35.48	36.28	12.90	29.34

fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP

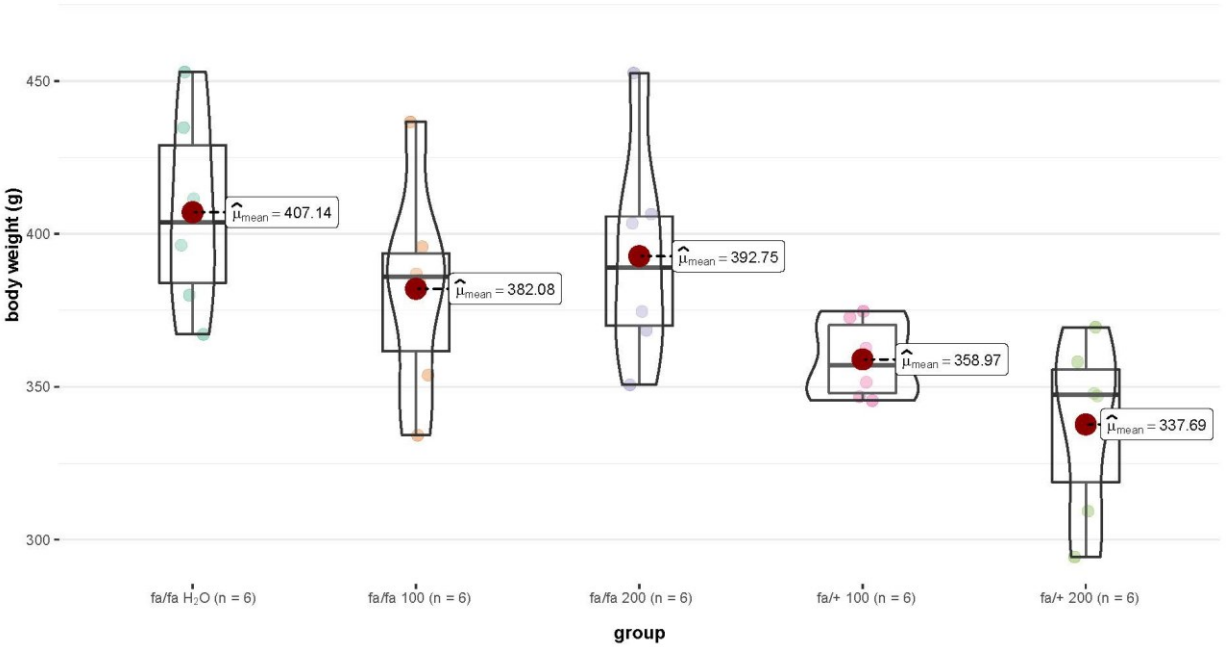


Fig. 1. Violin plots of body weight with regard to individual groups at the final 16th-time point. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; $\hat{\mu}$ – predicted value of group means

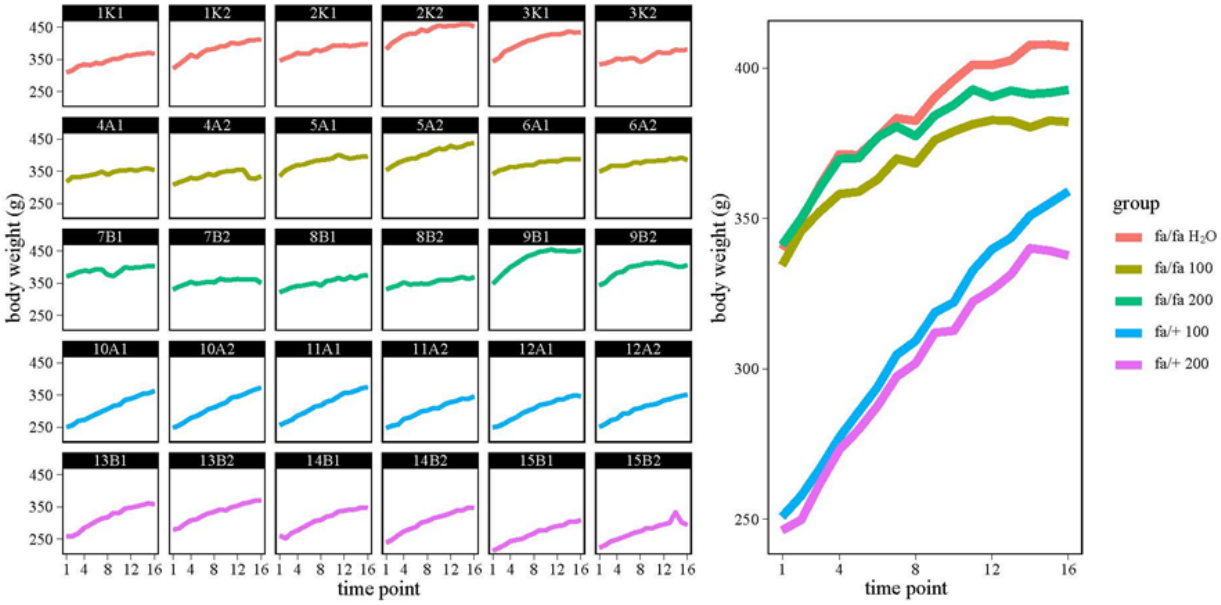


Fig. 2. The dynamics of body weight increases of individual rats (left side) and of group mean weight increases (right side) during the study period. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP

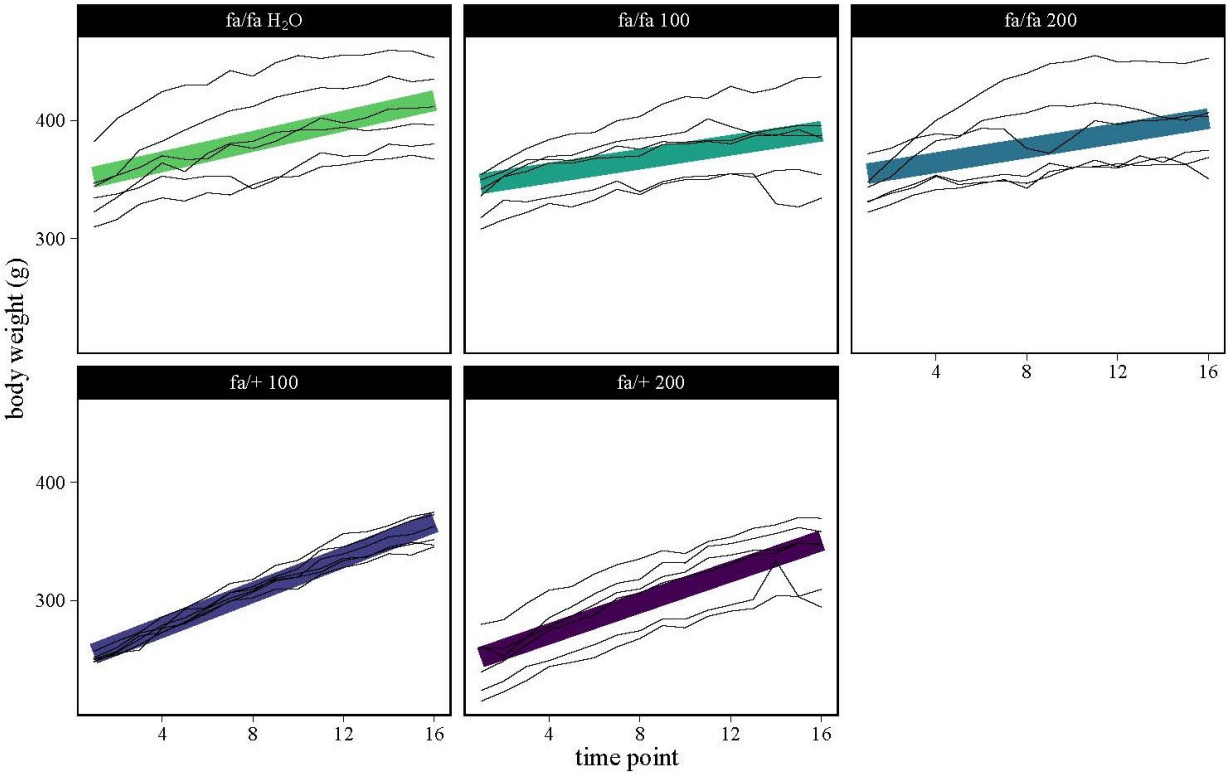


Fig. 3. Trajectories of individual rats' body weight variation and group average variations. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP

Effects of pomegranate peel extract on echocardiographic parameters. Echocardiographic parameters were determined to evaluate the influence of EPP administration on cardiac function. The measurements were made twice: before the beginning of the study and after its termination (Table 3). The analysis was based on the design control group pretest-posttest approach. The results indicate that there were no significant differences between groups with EPP supplementation and the group receiving only water in most of the analysed parameters. However, some of them showed changes versus the fa/fa H_2O group. At the end of the study, the heart rate in experimental groups was significantly lower – by 54.67 bpm (95% CI: −88.19–−21.15, P = 0.002) – than at the beginning. Moreover, the heart rate in the fa/fa 200 group was markedly decreased – by 43.83 bpm – compared to the control group (CI: −79.29–−8.38, P = 0.017). The value of end-diastolic volume in the fa/fa 200 group was significantly altered when compared with the fa/fa H_2O group (95% CI: 0.03–0.45, P = 0.026). Cardiac output was diminished in all groups after eight weeks by 0.03 L/min (95% CI −0.06–−0.01, P = 0.015). Also LA/Ao was lower in all groups by 0.11 without regard to EPP supplementation (95% CI: −0.19–−0.04, P = 0.005). Mid-wall fractional shortening (mFS) representing myocardial contractility was better by 7.65% in the fa/fa 200 group in comparison to the control group (95% CI: 0.69%–14.61%, P = 0.032).

Effects of pomegranate peel extract on blood morphology and smear results. Regarding the results of blood morphology, no explicit differences between tested groups were found (Table 4). However, in all groups, some tendencies were revealed. During the course of the study, the WBC, HGB, PLT, MCV, MCH, MCHC and lymphocytes decreased, in contrast to the RBC and neutrophil count, which increased. The only marked difference noted in blood morphology between the control and experimental groups was the eosinophilic granulocyte count, which was relevantly elevated in individuals obtaining EPP. The results of blood smears and determination of abnormal cells showed a higher number of acanthocytes and schistocytes in rats without MetS (fa/+ 100 and fa/+ 200) than in individuals with genetically programmed MetS (fa/fa H_2O, fa/fa 100 and fa/fa 200) regardless of EPP supplementation. The findings are presented in Figs 4 and 5. Images of peripheral blood smears containing acanthocytes and schistocytes are shown in Fig. 6. No blast cells or other abnormalities were reported.

Table 3. Echocardiographic parameters at two time points: at the beginning of the study (t1) and after eight weeks of pomegranate peel supplementation (t2)

Index	fa/fa H_2O		fa/fa 100		fa/fa 200		fa/+ 100		fa/+ 200	
	t1	t2	t1	t2	t1	t2	t1	t2	t1	t2
HR (bpm)	289.00 ± 28.08	234.33 ± 37.03	265.00 ± 21.02	232.25 ± 45.85	245.17 ± 21.34	226.83 ± 32.62	264.60 ± 20.40	230.60 ± 46.55	275.20 ± 11.82	245.80 ± 31.40
LVIDs (mm)	2.52 ± 0.54	2.65 ± 1.03	3.35 ± 1.61	2.65 ± 1.03	3.35 ± 1.23	2.90 ± 1.01	3.36 ± 0.69	2.36 ± 1.19	2.80 ± 0.70	2.28 ± 0.42
LVIDd (mm)	6.48 ± 0.51	6.08 ± 1.15	6.65 ± 0.83	5.82 ± 0.74	6.87 ± 1.25	6.52 ± 0.95	6.08 ± 1.13	5.56 ± 1.39	5.84 ± 0.77	5.68 ± 0.69
LVPWs (mm)	2.93 ± 0.32	3.00 ± 0.39	2.72 ± 0.43	3.00 ± 0.55	2.62 ± 0.39	2.77 ± 0.67	2.68 ± 0.49	3.32 ± 0.72	2.38 ± 0.49	3.18 ± 0.72
LVPWd (mm)	1.80 ± 0.39	1.95 ± 0.42	1.78 ± 0.39	2.22 ± 0.38	1.88 ± 0.28	2.03 ± 0.43	1.98 ± 0.56	1.86 ± 0.19	1.62 ± 0.30	1.94 ± 0.42
IVSs (mm)	3.32 ± 0.38	3.15 ± 0.62	3.10 ± 0.55	3.67 ± 0.71	3.68 ± 0.55	3.22 ± 0.41	3.04 ± 1.10	3.24 ± 0.58	3.30 ± 0.71	3.38 ± 0.36
IVSd (mm)	1.77 ± 0.16	1.77 ± 0.29	1.73 ± 0.13	2.00 ± 0.27	1.92 ± 0.23	1.80 ± 0.43	1.96 ± 0.55	1.62 ± 0.46	1.78 ± 0.19	1.86 ± 0.30
LVEDV (mL)	0.63 ± 0.15	0.55 ± 0.26	0.68 ± 0.22	0.48 ± 0.22	0.80 ± 0.38	0.65 ± 0.26	0.54 ± 0.26	0.44 ± 0.37	0.50 ± 0.16	0.44 ± 0.15
LVESV (mL)	0.05 ± 0.05	0.07 ± 0.05	0.15 ± 0.17	0.05 ± 0.10	0.13 ± 0.12	0.08 ± 0.08	0.10 ± 0.07	0.04 ± 0.09	0.06 ± 0.09	0.02 ± 0.04
SV (mL)	0.60 ± 0.14	0.50 ± 0.24	0.58 ± 0.10	0.40 ± 0.22	0.65 ± 0.31	0.57 ± 0.20	0.44 ± 0.21	0.40 ± 0.28	0.40 ± 0.16	0.38 ± 0.13
CO (L/min)	0.17 ± 0.04	0.12 ± 0.07	0.15 ± 0.02	0.09 ± 0.04	0.16 ± 0.07	0.14 ± 0.06	0.12 ± 0.06	0.09 ± 0.04	0.11 ± 0.04	0.10 ± 0.03
EF (%)	92.85 ± 3.36	89.83 ± 6.38	83.50 ± 14.01	84.12 ± 18.64	85.80 ± 8.92	89.55 ± 5.71	80.68 ± 7.18	90.22 ± 6.40	87.00 ± 7.26	92.64 ± 1.78
FS (%)	61.25 ± 6.78	57.72 ± 12.38	51.52 ± 18.55	53.75 ± 21.09	52.07 ± 12.09	56.62 ± 10.47	44.80 ± 8.12	58.58 ± 13.68	52.18 ± 8.29	59.94 ± 3.22
mFS (%)	25.40 ± 3.67	22.05 ± 3.24	21.50 ± 7.14	18.10 ± 12.31	20.95 ± 4.67	25.25 ± 3.25	18.84 ± 3.43	15.22 ± 3.37	19.92 ± 1.76	19.80 ± 7.22
LAD (mm)	4.22 ± 0.71	3.57 ± 0.47	4.34 ± 1.08	3.82 ± 0.36	4.87 ± 0.53	3.83 ± 0.69	4.30 ± 0.58	4.15 ± 0.42	4.58 ± 0.42	3.76 ± 0.29
AOD (mm)	3.37 ± 0.38	3.23 ± 0.35	3.34 ± 0.73	3.44 ± 0.25	3.85 ± 0.23	3.12 ± 0.51	3.28 ± 0.39	3.65 ± 0.83	3.44 ± 0.22	3.12 ± 0.33
LA/Ao	1.25 ± 0.19	1.10 ± 0.08	1.30 ± 0.11	1.12 ± 0.13	1.26 ± 0.11	1.24 ± 0.17	1.31 ± 0.08	1.17 ± 0.18	1.34 ± 0.18	1.21 ± 0.13

fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; HR – heart rate; LVIDs – left ventricular internal dimension at end systole; LVIDd – left ventricular internal dimension at end diastole; LVPWs – left ventricular posterior wall thickness at end systole; LVPWd – left ventricular posterior wall thickness at end diastole; IVSs – interventricular septum thickness at end systole; IVSd – interventricular septum thickness at end diastole; LVEDV – left ventricular end-diastolic volume; LVESV – left ventricular end-systolic volume; SV – stroke volume; CO – cardiac output; EF – ejection fraction; FS – fractional shortening; mFS – mid-wall fractional shortening; LAD – left atrial diameter; AOD – aortic diameter; LA/Ao – left atrial to aortic root ratio. Values are presented as mean ± standard deviation

Table 4. Blood cell counts at two time points: at the beginning of the study (t1) and after eight weeks of EPP supplementation (t2)

Index	fa/fa H_2O		fa/fa 100		fa/fa 200		fa/+ 100		fa/+ 200	
	t1	t2	t1	t2	t1	t2	t1	t2	t1	t2
WBC (K/µL)	10.40 ± 1.63	7.05 ± 1.96	10.50 ± 1.26	6.02 ± 2.33	11.26 ± 2.56	6.40 ± 1.29	8.52 ± 0.86	5.00 ± 1.41	9.38 ± 0.99	3.73 ± 1.25
RBC (G/µL)	7.49 ± 0.21	8.36 ± 0.69	7.63 ± 0.23	8.59 ± 0.44	7.60 ± 0.13	8.19 ± 0.53	7.53 ± 0.64	8.07 ± 0.54	7.69 ± 0.24	7.82 ± 0.63
HGB (g/L)	16.13 ± 0.37	14.53 ± 0.99	16.10 ± 0.37	14.80 ± 0.65	15.90 ± 0.34	14.32 ± 0.73	15.70 ± 0.73	13.88 ± 0.88	16.05 ± 0.42	13.90 ± 0.35
HCT (%)	43.47 ± 1.25	43.23 ± 3.31	39.18 ± 12.49	44.52 ± 2.32	44.12 ± 0.92	42.22 ± 2.66	41.88 ± 3.76	41.86 ± 2.61	42.75 ± 1.48	39.75 ± 3.18
PLT (K/µL)	1,069.33 ± 207.16	748.00 ± 76.58	1033.83 ± 74.09	658.67 ± 67.82	1078.80 ± 76.59	718.20 ± 52.11	905.80 ± 53.27	656.60 ± 244.39	911.67 ± 60.07	738.00 ± 39.33
MCV (fL)	58.00 ± 1.10	51.83 ± 0.75	57.67 ± 0.52	51.83 ± 0.41	58.00 ± 1.00	51.60 ± 0.55	55.60 ± 1.14	51.80 ± 0.84	55.50 ± 0.55	51.00 ± 0.00
MCH (pg)	21.55 ± 0.58	17.43 ± 0.63	21.15 ± 0.22	17.25 ± 0.29	20.88 ± 0.40	17.50 ± 0.42	20.90 ± 1.07	17.18 ± 0.26	20.90 ± 0.19	17.87 ± 1.11
MCHC (g/dL)	37.15 ± 0.69	33.65 ± 1.13	36.50 ± 0.49	33.30 ± 0.62	36.02 ± 0.29	33.92 ± 0.73	37.62 ± 2.00	33.20 ± 0.17	37.58 ± 0.42	35.08 ± 2.23
RET (%)	2.17 ± 0.87	1.85 ± 1.56	2.72 ± 0.31	1.15 ± 0.82	3.60 ± 0.67	0.92 ± 0.33	2.34 ± 0.61	1.12 ± 1.11	1.48 ± 0.69	0.53 ± 0.30
NEU (%)	13.33 ± 6.92	25.50 ± 4.76	19.33 ± 8.04	21.50 ± 4.59	20.40 ± 5.08	33.20 ± 5.72	16.25 ± 7.37	20.75 ± 3.95	12.83 ± 5.53	18.67 ± 4.50
LYM (%)	81.17 ± 5.00	71.50 ± 5.68	76.83 ± 10.26	74.83 ± 6.11	78.40 ± 5.13	61.80 ± 4.76	81.00 ± 8.68	75.75 ± 5.68	84.67 ± 4.63	79.50 ± 4.46
MONO (%)	2.50 ± 3.51	0.83 ± 0.98	1.00 ± 0.89	0.33 ± 0.52	0.20 ± 0.45	2.00 ± 1.58	0.50 ± 1.00	0.25 ± 0.50	1.83 ± 1.33	0.17 ± 0.41
EOS (%)	1.83 ± 0.75	0.67 ± 0.52	1.50 ± 1.38	2.33 ± 2.42	0.60 ± 0.55	1.60 ± 1.34	2.00 ± 1.15	2.50 ± 1.29	0.33 ± 0.52	1.33 ± 1.03

fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; WBC – white blood cell count; RBC – red blood cell count; HGB – haemoglobin concentration; HCT – hematocrit; PLT – platelet count; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular hemoglobin concentration; RET – percent of reticulocytes; NEU –- percent of neutrophils; LYM – percent of lymphocytes; MONO – percent of monocytes; EOS – percent of eosinophils. Values are presented as mean ± standard deviation

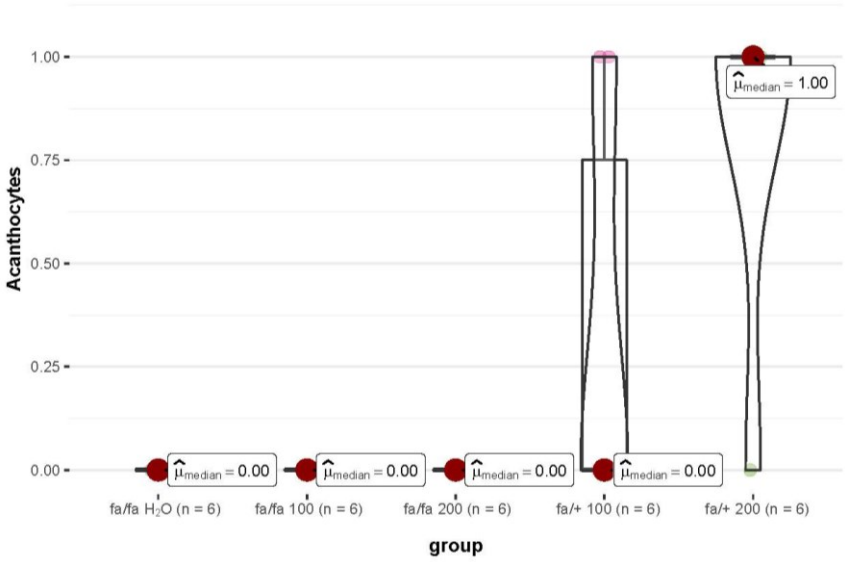


Fig. 4. Violin plot of the level of acanthocytes by group after eight weeks of pomegranate peel supplementation. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; $\hat{\mu}$ – predicted value of group means

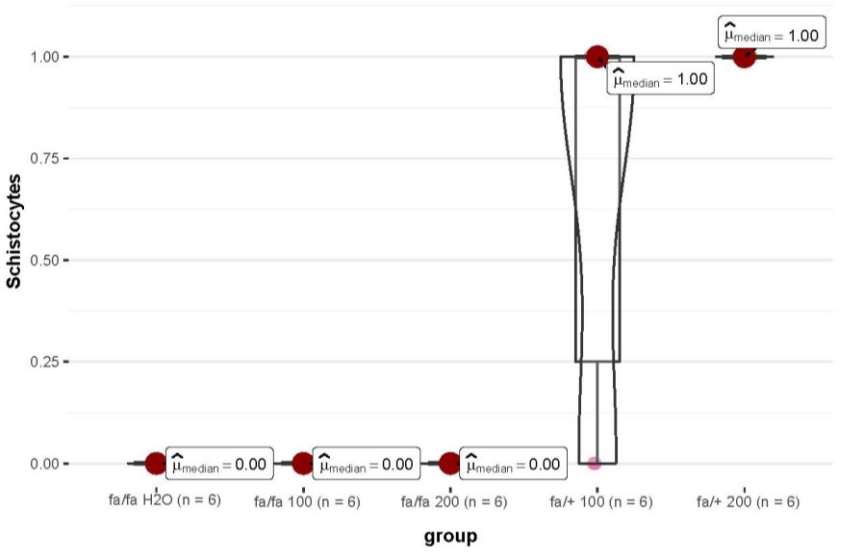


Fig. 5. Violin plots of the level of schistocytes by group after eight weeks of pomegranate peel supplementation. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; $\hat{\mu}$ – predicted value of group means

Effects of pomegranate peel extract on glucose and lipid profile. The levels of fasting blood glucose and serum lipidic indices are presented in Table 5. Regarding the glucose level, the EPP supplementation did not improve the glycaemic status of rats. The glucose concentration in individuals with MetS (fa/fa H_2O, fa/fa 100 and fa/fa 200) was significantly elevated compared to rats without MetS (fa/+ 100 and fa/+ 200), as was to be expected (Fig. 7). The same pattern was observed in the lipid profiles. No significant differences ($P > 0.05$) were found in the total cholesterol (CHOL), LDL, HDL or triglyceride concentrations among the tested groups (Fig. 8).

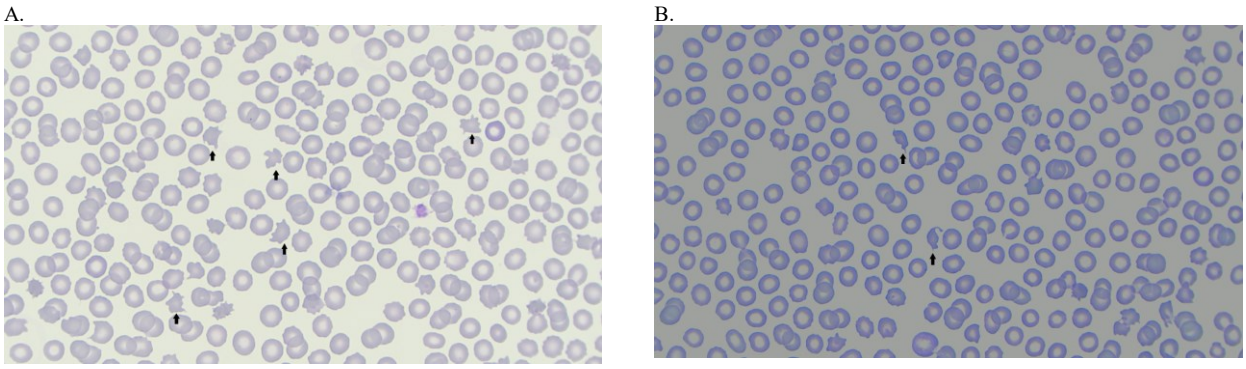


Fig. 6. A – Acanthocytes and B – schistocytes found in smears of rat peripheral blood. Abnormal cells are indicated by arrows

Table 5. Glucose and lipid profile at the beginning of the study (t1) and after eight weeks of pomegranate supplementation (t2)

Index	fa/fa H_2O		fa/fa 100		fa/fa 200		fa/+ 100		fa/+ 200	
	t1	t2	t1	t2	t1	t2	t1	t2	t1	t2
GLU (mmol/L)	17.50 ± 5.90	59.40 ± 11.30	21.40 ± 6.40	63.00 ± 12.80	21.10 ± 8.50	57.80 ± 8.50	7.60 ± 0.60	23.00 ± 3.70	7.70 ± 1.20	22.90 ± 2.80
CHOL (mmol/L)	4.30 ± 0.30	5.10 ± 0.70	4.30 ± 0.20	4.80 ± 0.50	4.10 ± 0.10	5.10 ± 1.00	3.40 ± 0.20	2.20 ± 0.30	3.60 ± 0.10	2.40 ± 0.10
LDL (mmol/L)	2.50 ± 0.10	2.90 ± 0.40	2.40 ± 0.20	3.00 ± 0.40	2.40 ± 0.20	3.20 ± 0.60	1.70 ± 0.10	1.30 ± 0.20	1.80 ± 0.10	1.40 ± 0.10
HDL (mmol/L)	2.20 ± 0.10	2.80 ± 0.20	1.90 ± 0.10	2.80 ± 0.20	1.90 ± 0.20	2.80 ± 0.40	1.20 ± 0.20	1.20 ± 0.20	1.20 ± 0.10	1.10 ± 0.10
TG (mmol/L)	11.50 ± 2.70	5.60 ± 5.60	12.70 ± 3.40	5.70 ± 2.90	12.50 ± 2.80	5.90 ± 1.30	1.30 ± 0.30	1.20 ± 0.60	1.30 ± 0.30	0.90 ± 0.60

fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; GLU – glucose concentration; CHOL – total cholesterol concentration; LDL – low-density lipoprotein concentration; HDL – high-density lipoprotein concentration; TG – triglyceride concentration. Values are presented as mean ± standard deviation

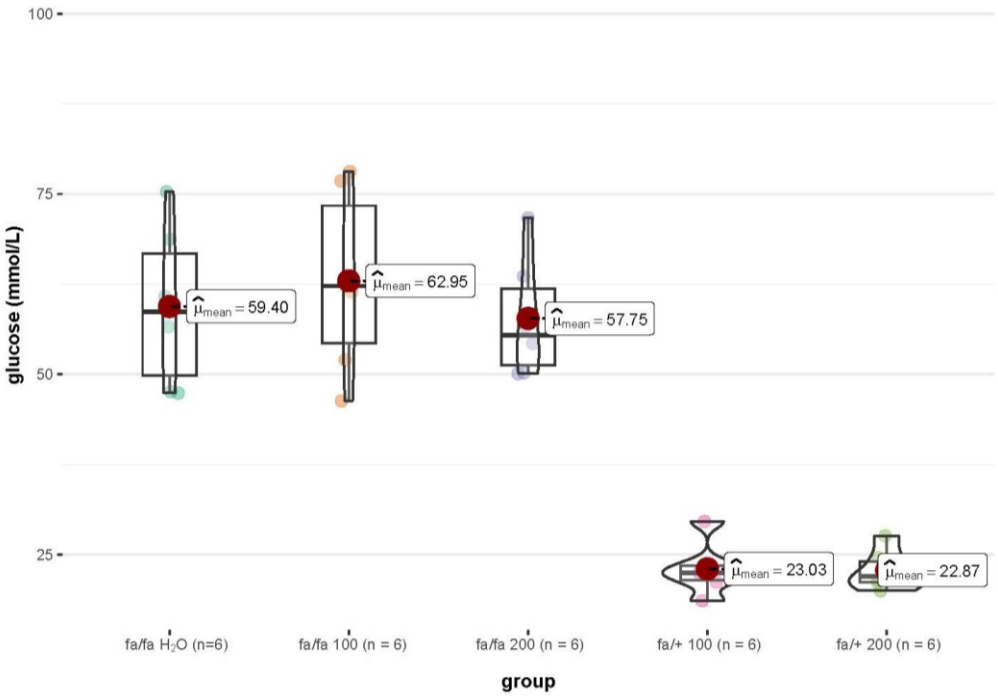
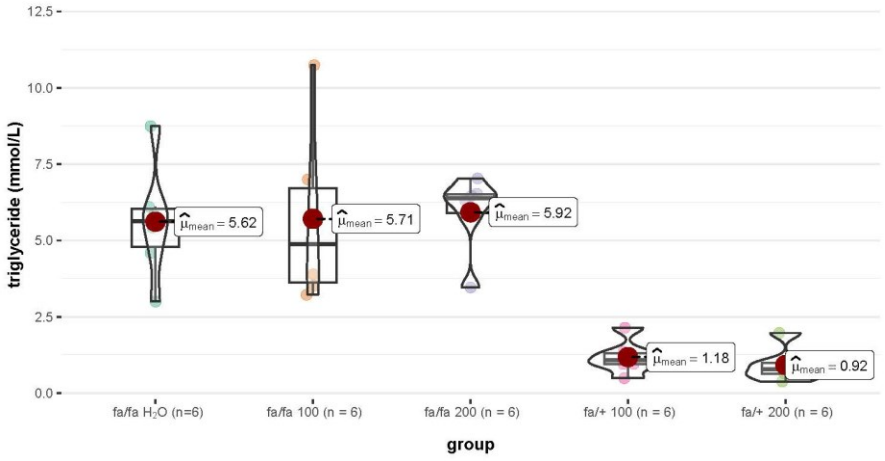
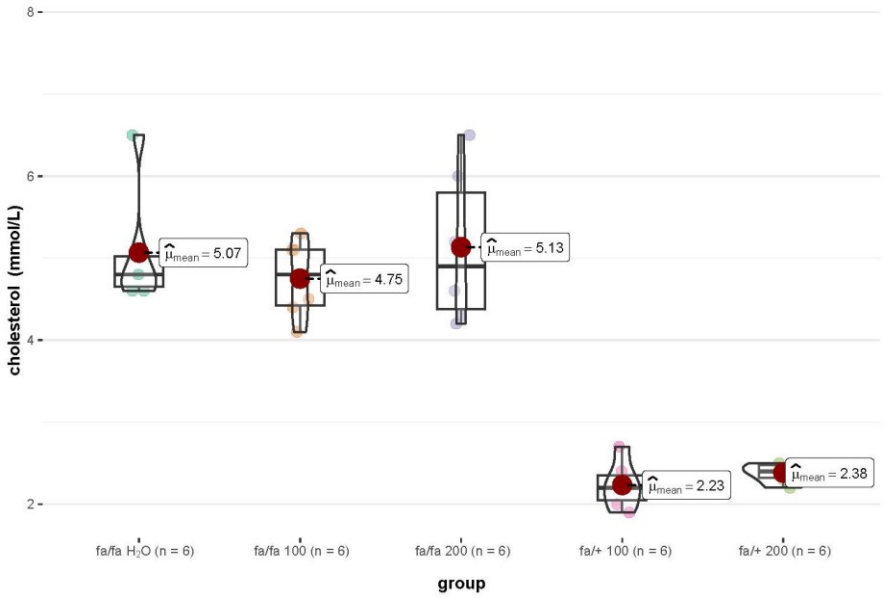


Fig. 7. Violin plots of fasting blood glucose concentrations by group after eight weeks of pomegranate peel supplementation. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; $\hat{\mu}$ – predicted value of group means

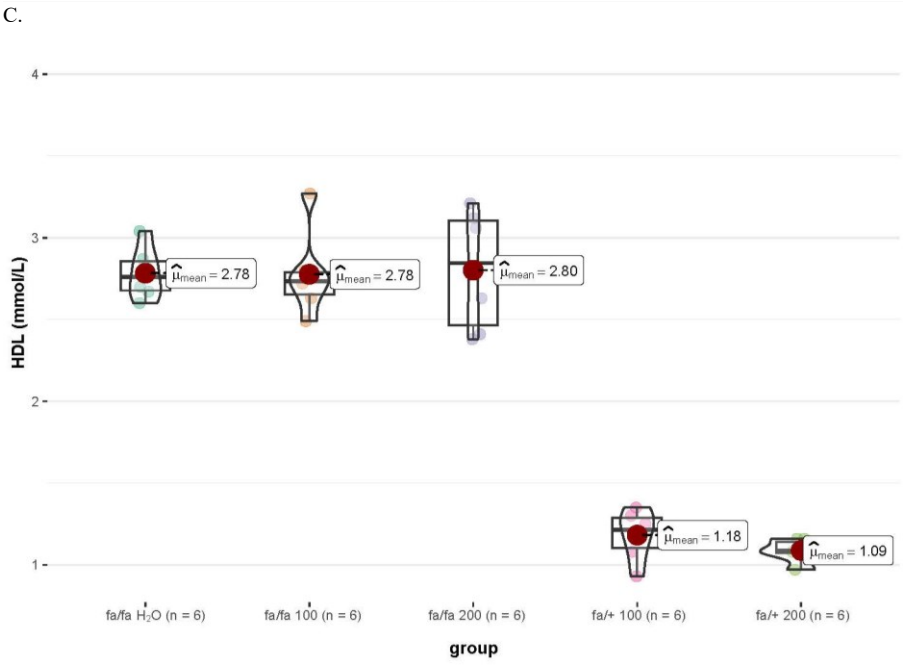
A.


triglyceride (mmol/L)
12.5
10.0
7.5
5.0
2.5
0.0
μmean = 5.62
μmean = 5.71
μmean = 5.92
μmean = 1.18
μmean = 0.92
fa/fa H2O (n=6)
fa/fa 100 (n = 6)
fa/fa 200 (n = 6)
fa/+ 100 (n = 6)
fa/+ 200 (n = 6)
group

B.


cholesterol (mmol/L)
8
6
4
2
μmean = 5.07
μmean = 4.75
μmean = 5.13
μmean = 2.23
μmean = 2.38
fa/fa H2O (n = 6)
fa/fa 100 (n = 6)
fa/fa 200 (n = 6)
fa/+ 100 (n = 6)
fa/+ 200 (n = 6)
group

C.


HDL (mmol/L)
4
3
2
1
μmean = 2.78
μmean = 2.78
μmean = 2.80
μmean = 1.18
μmean = 1.09
fa/fa H2O (n = 6)
fa/fa 100 (n = 6)
fa/fa 200 (n = 6)
fa/+ 100 (n = 6)
fa/+ 200 (n = 6)
group

D.

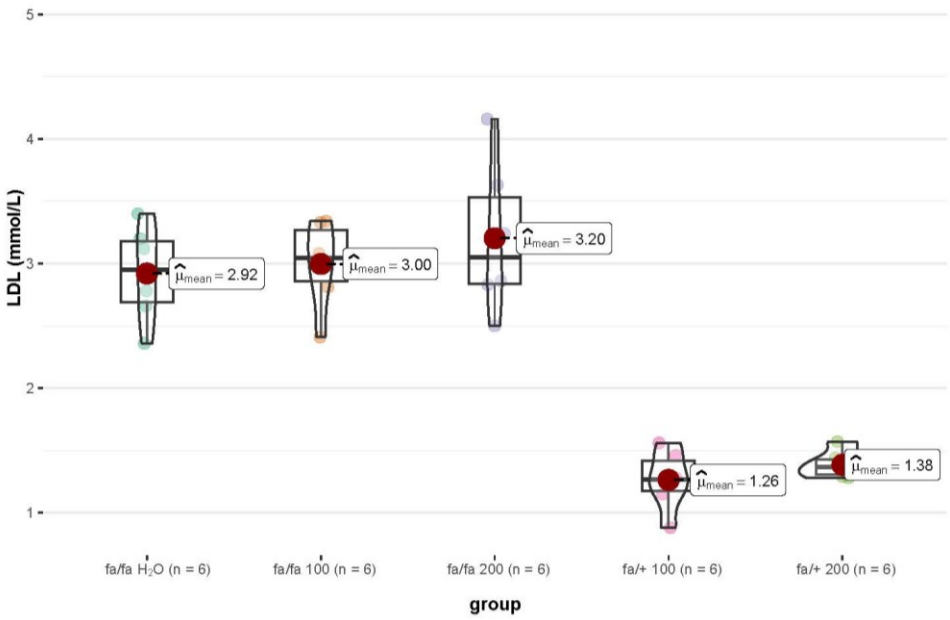


Fig. 8. Violin plots of lipid profiles by group after eight weeks of pomegranate peel supplementation. A – serum triglyceride concentration; B – serum total cholesterol concentration; C – serum high-density lipoprotein concentration; D – serum low-density lipoprotein concentration. fa/fa H_2O – control group of Zucker diabetic fatty (ZDF) rats with missense mutation in the *Lepr* leptin receptor gene administered only water; fa/fa 100 – experimental group of ZDF rats with this mutation administered 100 mg/kg body weight (b.w.) pomegranate peel extract (EPP); fa/fa 200 – experimental group of ZDF rats with this mutation administered 200 mg/kg b.w. EPP; fa/+ 100 – experimental group of ZDF rats without this mutation administered 100 mg/kg b.w. EPP; fa/+ 200 – experimental group of ZDF rats without this mutation administered 200 mg/kg b.w. EPP; $\hat{\mu}$ – predicted value of group means

Discussion

Pomegranate (*Punica granatum* L.) is a promising source of polyphenols that could help prevent or mitigate the course of MetS. The flesh excluded, pomegranates contain a substantial amount of active phenolic compounds. These compounds scavenge various reactive oxygen species (ROS) (14). For many years juice was believed to contain the most bioactive particles that could be utilised as supplements and nutraceuticals in miscellaneous diseases (2). However, studies investigating the health benefits of pomegranate peel showed that polyphenols in this material possess even higher antioxidant activity than the pulp itself (14, 24). Its nutritional value has been explored in many *in vivo* studies on animal models, and these proved that pomegranate peel extract reduces obesity (38), dyslipidaemia (34), hypertension (11), and hyperinsulinaemia and insulin resistance (49), and also ameliorates chronic inflammatory status (30). In the presented study, we aimed to evaluate the health benefits of pomegranate peel extract supplementation in a model of MetS and healthy control rats maintained on a high-calorie diet.

The role of pomegranate peel extract in preventing obesity has been previously reported (30). Our result consonantly indicates that phenolic compounds derived from pomegranate peel possess bioactivity that could be helpful in the prevention or mitigation of body weight gain. The ability of pomegranate peel to prevent obesity depends on the impact of polyphenol metabolites in the expression of four proteins: an adipose-formulation–related one such as adiponectin, peroxisome proliferator–activated receptor γ, glucose transporter type 4, and fatty acid–binding protein 4 (23). It has also been proposed that oral supplementation with polyphenols modulates gut microbiota, which elicits an obesity control effect (30, 38). *In vivo* studies have shown that phenolic compounds could promote beneficial bacterial species, such as *Bifidobacterium* spp. and *Lactobacillus* spp., as well as suppress potentially pathogenic bacteria (46). Targeting microbiota with the pomegranate by-product phenolic compounds which have probiotic properties encourages the proliferation of *Bacteroides* spp. and significantly reduces the abundance of *Firmicutes* spp. and *Proteobacteria* spp. (38). Inverse proportionality of these species of bacteria was reported to be a likely diagnostic biomarker of gut dysbiosis correlated with the risk of metabolic diseases (36). One benefit of phenolic compounds is that they promote the abundance of beneficial bacterial genera, and a separate positive effect is that the fermentation of polyphenols by bacteria contributes to the generation of metabolite products, especially urolithin A, that inhibit triglyceride accumulation and suppress the gene expression involved in adipogenesis (23, 41). The relationship between the consumption of polyphenols and gut microbiota is complex and certainly requires further investigation.

Obesity is associated with many pathological conditions, such as insulin resistance, atherosclerosis

and lipid metabolism disorders (18). Pomegranate peel extract also improves the glycaemic and lipid profiles. It has been proved that EPP lowers the serum concentration of triglycerides, total cholesterol and LDL-cholesterol in obese hamsters and an obese mice model (25, 30, 38). The mechanisms lying behind this propitious impact on lipid metabolism were partially revealed. The outcomes elucidated how phenolic compounds derived from pomegranate peel upregulated liver X receptor α, peroxisome proliferator-activated receptor α, peroxisome proliferator-activated receptor γ and gene ATP-binding cassette transporter A1, downregulated fatty acid synthase through inhibition of the keto-acetyl synthase and acetyl/malonyl transferase domains, and supported cholesterol removal by enhancing faecal bile acid (25, 26). Interestingly, in the study on hamsters, the effect of pomegranate ellagic acid extracted from peel on lipid metabolism was stronger than that of simvastatin alone in raising HDL and lowering LDL (25). Contrary to these findings, our research indicated no change in lipid profile in individuals treated with pomegranate peel extract. However, some other *in vivo* studies with results consistent with ours also reported no difference in serum levels of triglycerides and cholesterol fractions between the control group and pomegranate-treated groups on a high-fat, high-sucrose diet (16).

Metabolic syndrome also leads to improper insulin utilisation and production (47). As evidenced by recent studies, many natural plant derivatives classified as flavonoids, alkaloids, terpenoids and phenolics display antidiabetic properties (35). Phenolic components from pomegranate peels, of which punicalagin, gallic acid and ellagic acid are examples, have been proved to exhibit antidiabetic, antihyperglycemic and antiglycation effects (28, 29). Pomegranate peel diminishes fasting blood glucose concentration and improves insulin sensitivity through various mechanisms. Research findings highlighted the crucial involvement of pomegranate phenolics in carbohydrate regulation (44). The principal mechanisms are the inhibition of α-glucosidases and α-amylases (20), inhibition of advanced glycation end-product formation (8), and mitigation of hyperglycaemic-induced oxidative stress (10). Pomegranate seed oil extract improved insulin tolerance and reduced serum fasting blood glucose in diet-induced obese mice (16). However, in the same study, extracts from pomegranate flowers and peel did not invigorate carbohydrate metabolism and in this were inferior to rosiglitazone; the extracts did nevertheless decrease the plasma level of proinflammatory cytokines IL-6 and TNF-α, which also protects against dysregulation of glucose metabolism (10, 16). Furthermore, anthocyanins extracted from pomegranate peel favourably altered the insulin signalling pathway. These findings implied that the supplementation with polyphenols from pomegranate peel might alleviate insulin resistance (38). In the present study, EPP did not lower the glucose level in the serum. The data from other research on animal models are also at some variance on this point.

The cardiovascular consequences of MetS also comprise an alteration in cardiac function associated with obesity. This cardiac failure in MetS was described as “cardiomyopathy of obesity” (43). It is characterised in humans by the development of concentric left ventricular hypertrophy and mild diastolic or systolic dysfunction with normal or elevated EF (3). Impairment in myocardial contractility also has been reported in animal models of obesity (27). Furthermore, changes occurring in MetS disrupt the equilibrium between coronary blood flow and myocardial metabolism, significantly increasing the risk of myocardial infarction and mortality (42). The pleiotropic effect of phenolic compounds on cardiovascular diseases comprises vasodilatative activity and anti-inflammatory, antithrombotic and antiatherogenic effects (32). Cardiovascular protection is also provided with polyphenols from pomegranate peel. A study in a spontaneously hypertensive rat model found the consumption of EPP to reduce systolic blood pressure, coronary angiotensin-converting enzyme activity and oxidative stress level, and prevent vascular remodelling (11). Evidence suggests that phenolics from peel may find application in mitigating coronary heart disease by attenuation of electrocardiographic changes, myocardial tissue damage and heart weight increase. Notably, the phenolics’ role as a cardioprotective agent arises from their upregulating of endothelial nitric oxide synthase expression, leading to intensification of antioxidant mechanisms and inhibition of apoptosis (15). In addition, punicalagin suppresses cardiac fat accumulation by stimulating the cardiac adenosine monophosphate–activated protein kinase signalling pathway. Concomitantly, it prevents mitochondrial loss by enhancing mitochondrial biogenesis and ameliorating oxidative stress (6). In our study, we also noted a cardioprotective effect of the largest EPP supplementation in the relative decrease in heart rate. Likewise, EPP improves mFS, representing myocardial contractility.

Blood morphological and haematological indices represent the general state of health of individuals. The assessment of these parameters could indicate metabolic abnormalities or the noxious effect of xenobiotics (31). Changes in blood morphology and haematology may also suggest pathological conditions such as anaemia, infection, thrombotic state and bone marrow impairment. In this context, red blood cell morphology is a very sensitive marker of exposure to ROS (17). Polyphenols are characterised by low toxicity and, in recent years, have gained attention as natural radioprotective and cytoprotective agents (40). Our findings demonstrated that no toxicity levels manifested by haematological changes were observed in individuals treated with EPP. In the blood smears, the only alterations were the increased number of acanthocytes and schistocytes in rats without genetically programmed MetS, which may have resulted from diet-induced

obesity and its consequences. No blast cells nor anaemic state were observed.

The present study suggests that phenolic compounds from pomegranate peel have a potentially beneficial effect in dietary intervention in metabolic syndrome. Together these results confirm the promise of pomegranate peel as a nutrient, especially in restricting body weight gain. However, studies on humans and animals suffering from MetS are needed in order to determine the bioaccessibility of bioactive constituents and metabolites and to indicate their actual effectiveness in ameliorating individual abnormalities involved in the pathogenesis of MetS. Polyphenols incorporated into the diet of humans and animals could help maintain health and counteract metabolic syndrome.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The experiment was conducted in accordance with the local Ethical Committee rules and regulations.

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