Association between peroxisome proliferator-activated receptor-alpha, -delta and -gamma gene (*PPARA, PPARD, PPARG*) polymorphisms and overweight parameters in physically active men

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ABSTRACT: Peroxisome proliferator-activated receptors (PPARs) have unique functions in energy metabolism regulation but are also involved in regulation of the inflammatory process and obesity. The aim of this study was to analyse potential associations between polymorphisms of *PPARA* (rs1800206), *PPARD* (rs1053049; rs2267668) and *PPARG* (rs1801282) and overweight parameters. One hundred and sixty-six males, unrelated Caucasian military professionals, were recruited in the genetic case-control study conducted in the period 2016–2019. All the participants were aged 21–41 and had similar levels of physical activity. Body mass, height and body composition were measured. The participants were divided into two groups depending on their BMI (body mass index) and FMI (fat mass index). The control group consisted of people with BMI between 20.0 and 25.0 or FMI values ≤ 6, while the overweight group consisted of people with BMI of ≥ 25.0 or FMI values > 6. Genomic DNA was isolated from extracted buccal cells. All samples were genotyped using real-time polymerase chain reaction (real-time PCR). It was found that two polymorphisms rs2267668 and rs1053049 of the *PPARD* gene were significantly associated with BMI: SNP rs2267668 for the dominant (OR = 2.04, 95%CI 1.01–4.11, p-value = 0.04) model (A/G-G/G vs A/A). The likelihood of being overweight was over 2 times smaller for allele A. A relationship between the polymorphism of *PPARG* (rs1801282) and BMI was found for the overdominant (OR = 2.03, 95%CI 1.03–4.00, p-value = 0.04) model (C/G vs C/C-G/G). Significant associations were found in different models for *PPARD, PPARG* and *PPARA* genes with BMI. In SNP rs2267668 for the codominant genetic model (G/G vs A/A) (p-value = 0.04) and in SNP rs1053049 for the codominant (C/C vs T/T) (p-value = 0.01) and the recessive genetic model (C/C vs T/T–C/T) (p-value = 0.004) all polymorphisms were associated with BMI. In conclusion, it was found that three of the four polymorphisms (rs1053049, rs2267668, rs1801282) selected are associated with the risk of being overweight. Having said that, one has to bear in mind that DNA variants do not fully explain the reasons for being overweight. Therefore more research is needed to make a thorough assessment using the latest genomic methods in sequencing and genotyping, combined with epigenomics, proteomics, transcriptomics, and metabolomics.

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INTRODUCTION

The number of people suffering from obesity has almost tripled since 1975 according to the World Health Organization. This global problem affects all societies regardless of socioeconomic status. Overweight and obesity classified by body mass index (BMI) are defined as abnormal or excessive fat accumulation that may impair health. The fundamental cause of obesity and overweight

is an energy imbalance in total energy expenditure between consumed and expended calories. It results both from increased intake of foods rich in fat and sugars and from decreased physical activity due to the sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization, as well as irregular eating, overeating, too long or too short breaks between meals, and eating to relieve negative emotions. These lead to excessive fat accumulation in adipocytes and increased volume of lipid droplets in skeletal muscles. Moreover, lower rates of triglyceride turnover, decreased activity of oxidative enzyme and diminished lipid oxidation are also observed, which in turn result in adipose tissue retention [1].

Peroxisomes are cellular organelles playing an important role in fatty acid metabolism, with essential metabolic functions, e.g. shortening of very-long-chain fatty acids later to be degraded in the mitochondria or getting rid of toxic peroxides. They proliferate and increase in response to dietary lipids, hormones, hypolipidemic drugs, herbicides, and leukotriene antagonists, which bind to nuclear regulatory proteins called peroxisome proliferator-activated receptors (PPARs). PPARs are involved in the regulation of inflammation and energy homeostasis [2]. PPAR proteins are lipid-activated nuclear receptors belonging to nuclear hormone receptor superfamily 1 [3, 4]. Three PPAR isotypes – PPARα, PPARδ and PPARγ – have been identified in vertebrates and mammals [5]. In humans, each PPAR isoform is encoded by a separate gene: PPARα is encoded by the *PPARA* gene located on chromosome 22, PPARγ by the *PPARG* gene on chromosome 3, and PPARδ by the *PPARD* gene on chromosome 6 [6]. Three variants of PPAR proteins are distributed among various tissues. PPARα and PPARγ are predominant in liver and adipose tissue, respectively; PPARα is involved in fat metabolism and fatty acid oxidation, whereas PPARγ influences adipocyte differentiation and insulin action. PPARδ is abundantly expressed throughout the body but at low levels in the liver; however, its function is not yet fully understood. Consistent with their expression profiles, the PPARs have unique functions in regulation of energy metabolism [7] but also are involved in the regulation of inflammation.

PPARα is expressed in metabolically active tissues e.g. liver, brown adipose tissue, muscle, and heart. PPARα is produced in cells involved in immune responses including monocytes, macrophages, and lymphocytes [8]. PPARα, among other functions, regulates the adaptive response to fasting, meaning lipid homeostasis, hepatic glucose homeostasis, and hepatic amino acid metabolism during fasting. It regulates biological processes by altering the expression of the hundreds of genes, but the regulation depends on whether PPARα is activated pharmacologically (several PPARα agonists have been introduced into therapy, such as fibrates (e.g. fenofibrate, ciprofibrate)), physiologically (eicosanoids), or nutritionally (n-3 fatty acids) [9].

PPARδ is regarded as a key regulator of energy metabolism due to its ability to enhance fatty acid catabolism, energy uncoupling, and insulin sensitivity in adipose tissue and skeletal muscle [10]. According to Seedorf and Aberle [11] PPARδ activation in the liver appears to decrease hepatic glucose output, thereby contributing to improved glucose tolerance and insulin sensitivity.

PPARγ is considered to be the master regulator of adipogenesis. Target genes of PPARγ are involved in adipocyte differentiation, glucose metabolism and lipid storage [12].

A number of polymorphisms have been described in *PPARA*, *PPARD* and *PPARG* genes in the context of their association with obesity. The presence of polymorphisms in the studied genes seems to be related also to hypertriglyceridemia, decreased serum HDL level and disturbed carbohydrate metabolism, which make up the metabolic syndrome. We hypothesized that the polymorphisms of *PPARA* (rs1800206), *PPARD* (rs1053049; rs2267668) and *PPARG* (rs1801282) may be associated with obesity. Thus the presence of single nucleotide polymorphisms (SNPs) in genes encoding PPAR correlated with selected anthropometric indicators may be a predictive and informative genetic marker of the risk of selected diet-dependent metabolic diseases. The aim of this study was to investigate the distribution of aforementioned genetic polymorphisms of *PPAR* genes in healthy normal-weigh (normal body mass index – BMI and fat mass index – FMI) and overweight people (high BMI and FMI on the verge of obesity).

MATERIALS AND METHODS ■

Participants and Procedure

166 male, unrelated, Caucasian military professionals (volunteers) were recruited to the genetic case-control study conducted in the years 2016–2019. All the participants were ancestrally fitted (all the volunteers were Polish, living in Eastern Europe for 3 generations), aged 21–41 and with similar levels of physical effort exposure.

Procedures used in this research were conducted in accordance with the World Medical Association's Declaration of Helsinki and the research protocol was approved by the Ethics Committee of the Military Institute of Hygiene and Epidemiology – resolution number 1/XXI /2016. All the participants were given an information sheet regarding the research details, aim of the study and procedures applied, as well as potential risks and benefits associated with their participation. All volunteers gave written, informed consent for the genotyping, provided that it would be anonymous and the results would be confidential.

Anthropometry, Body Composition

Height was measured using a portable stadiometer (without shoes) (TANITA HR-001, Tanita Corporation, Japan). Body composition (including fat %) and body weight were measured using bioelectrical impedance analysis (BIA) using the TANITA MC-780 machine (Tanita Corporation, Japan) with accuracy of 0.1 kg according to the procedure specified in the instruction manual (lightly dressed, without shoes). The assessment of BMI values was made in accordance with the criteria set out by the WHO [13]. All measurements were performed according to the procedure specified in the instruction manual and without any metal objects.

TABLE 1. Anthropometry and body composition of the participants

Group	CON_BMI (n = 77)	OVER_BMI (n = 89)	*p*-value	CON_FMI (n = 124)	OVER_FMI (n = 42)	*p*-value
BMI [kg/m2]	23.4±1.3	28.8 ±3.6	<0.001	24.6±1.9	31.2±4.0	<0.001
Age [years]	29.6±7.2	34.8±7.6	<0.001	31.3±7.3	35.4±8.6	<0.001
Height [cm]	180.2±7.4	180.5±6.4	0.77	180.0±6.8	181.3±6.8	0.30
Weight [kg]	76.1±7.6	93.8±13.8	<0.001	79.9±8.4	102.5±15.1	<0.001
FMI [kg/m2]	20.1±7.7	37.4±12.4	<0.001	23.5±8.7	47.3±9.3	<0.001
Visceral tissue index [-]	3.2±1.8	8.2±3.6	<0.001	4.2±2.2	10.9±3.5	<0.001
Fat [%]	14.2±3.6	22.2±5.1	<0.001	15.8±3.8	26.5±3.7	<0.001

Note: BMI — body mass index, FMI — fat mass index, CON — control group, OVER — overweight group.

The subjects (n = 166) were divided into two groups depending on their BMI. The following standard formula was used to calculate the BMI: BMI = body weight/height^2 (kg/m^2). The control group (CON_BMI, n = 77) consisted of people with BMI between 20.0 and 25.0, while the overweight group (OVER_BMI, n = 89) was made up of people with BMI of ≥ 25.0 [14].

The subjects (n = 166) were also divided into two groups depending on their fat mass index (FMI) value. The following formula was used to calculate the FMI: FMI = fat mass/height^2 (kg/m^2). The FMI classification scale was developed by Kelly et al. [14]. FMI values between 3 and 6 were classified as normal fat mass, FMI lower than 3 was considered as a fat deficit and FMI higher than 6 was considered as excess fat. Participants whose FMI values were 6 and lower were classified into the CON_FMI group (n = 124), while those whose FMI values were higher than 6 were grouped into the OVER_FMI group (n = 42). Detailed characteristics of experimental groups are given in Table 1.

Genetic analyses

The buccal cells donated by the subjects were collected using two Copan FLOQSwabs (Interpath, Australia) according to the standard procedure. Genomic DNA was extracted from the buccal cells using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany). The extraction was performed according to the manufacturer's instructions. DNA samples of good quality and quantity were stored at -20°C for further analysis. The exclusion criteria were: failure in DNA extraction; DNA degradation; abnormal gene detect results; incomplete basic information.

All samples were genotyped in duplicate, using TaqMan Pre-Designed SNP Genotyping Assays for *PPARA* (rs1800206) C___8817670_20, *PPARD* (rs1053049) C___8851955_60, *PPARD* (rs2267668) C__15872729_10 and *PPARG* (rs1801282) C___1129864_10 (Applied Biosystems, USA) on a CFX Connect Real-Time PCR Detection System (Bio-Rad, USA) following the manufacturer's recommendations. The PCR conditions were as follows: 5 min of initial denaturation (95°C), then 40 cycles of denaturation (15 s, 95°C) and annealing/extension (60 s, 60°C).

Statistical analyses

All statistical analysis was made using the program R [15]. Anthropometric data are shown as mean values ± standard deviation and differences among experimental groups were analysed with Student's t test. They are considered statistically significant when p < 0.05. Single locus analysis including SNP × SNP interaction was performed with the SNPassoc package considering four genetic models (co-dominant, dominant, recessive and overdominant). The models were constructed with respect to the minor allele. The p value for SNP × SNP (epistasis) were calculated using the log-likelihood ratio test with the SNPassoc package. Multifactor dimensionality reduction (MDR) was used to detect the influence of the common effect of gene × gene interactions on FMI; it was calculated with MDR3.0.2 (http://sourceforge.net/projects/mdr/), for checking statistical significance of the model the chi-square test was used. The association of single alleles with BMI was calculated with Pearson's chi-squared test using the STAT.package. Haplotype analysis was performed using the haplo.stats package. The haplo.score function was used to test the association between allele combinations with BMI under the different models of inheritance (additive, dominant and recessive). The magnitude and direction of the haplotype-based association were determined with haplo.score statistics. The level of statistical significance was set at the level of p < 0.05. Genotype frequencies were analysed using Fisher's exact test.

RESULTS

Mean BMI of subjects classified to OVER_BMI was significantly higher than in CON_BMI. Values of other parameters (weight, fat %, visceral tissue index and FMI) were significantly higher for OVER_BMI. No differences were observed only for height of subjects from both groups. Moreover, subjects of OVER_BMI were on average 5 years older than subjects from CON_BMI, which may confirm that overweight may increase with age. The mean FMI value for OVER_FMI was more than twice as high as in CON_FMI. Significant differences between OVER_BMI and CON_BMI were observed for most of the investigated parameters, except height. OVER_BMI was characterized by almost twice as high fat content, which accumulated mainly as visceral fat. Such

TABLE 2. The probabilities that the genotype frequencies do not differ from Hardy-Weinberg expectations and minor allele frequencies (MAF)

	MAF (%)	ALL	CON$_{BMI}$	OVER$_{BMI}$	CON$_{FMI}$	OVER$_{FMI}$
PPARA (rs1800206)	allele G (6.02)	0.10	0.22	0.28	0.06	1.00
PPARD (rs1053049)	allele C (21.39)	0.49	0.11	0.09	1.00	0.48
PPARD (rs2267668)	allele G (15.36)	1.00	1.00	0.74	1.00	0.69
PPARG (rs1801282)	allele G (18.67)	0.88	0.21	0.11	0.51	0.42

Note: MAF — minor allele frequency, BMI — body mass index, FMI — fat mass index, CON — control group, OVER — overweight group

TABLE 3. Association analysis of the *PPARD* rs1053049 polymorphism with BMI

		OVER$_{BMI}$ (n = 89)	%	CON$_{BMI}$ (n = 77)	%	OR	95% CI		p-value	AIC
Codominant	T/T	53	59.6	51	66.2	1.00			**0.01**	223.6
	C/T	27	30.3	26	33.8	1.00	0.52	1.94		
	C/C	9	10.1	0	0.0		0.00			
Dominant	T/T	53	59.6	51	66.2	1.00			0.37	232.5
	C/T-C/C	36	40.4	26	33.8	1.33	0.71	2.51		
Recessive	T/T-C/T	80	89.9	77	100	1.00			**0.004**	221.6
	C/C	9	10.1	0	0.0		0.00			
Overdominant	T/T-C/C	62	69.7	51	66.2	1.00			0.64	233.0
	C/T	27	30.3	26	33.8	0.85	0.44	1.64		
Alleles	C	45	25.3	26	16.9	1.66	0.94	2.99	0.08	
	T	133	74.7	128	83.1					

Note: OR — odds ratio, 95% CI — confidence interval, AIC — Akaike information criterion; NA — not applicable, BMI — body mass index, OVER — overweight group, CON — control group.

TABLE 4. Association analysis of the *PPARG* rs1801282 polymorphism with BMI

		OVER$_{BMI}$ (n = 89)	%	CON$_{BMI}$ (n = 77)	%	OR	95% CI		p-value	AIC
Codominant	C/C	54	60.7	55	71.4	1.00			0.05	229.2
	C/G	34	38.2	18	23.4	1.92	0.97	3.81		
	G/G	1	1.10	4	5.20	0.25	0.03	2.35		
Dominant	C/C	54	60.7	55	71.4	1.00			0.14	231.1
	C/G-G/G	35	39.3	22	28.6	1.62	0.84	3.11		
Recessive	C/C-C/G	88	98.9	73	94.8	1.00			0.12	230.8
	G/G	1	1.10	4	5.20	0.21	0.02	1.90		
Overdominant	C/C-G/G	55	61.8	59	76.6	1.00			**0.04**	229.0
	C/G	34	38.2	18	23.4	2.03	1.03	4.00		
Alleles	C	142	79.8	128	83.1	0.80	0.44	1.45	0.52	
	G	36	20.2	26	16.9					

Note: OR — odds ratio, 95% CI — confidence intervals, AIC — Akaike information criterion; NA — not applicable, BMI — body mass index, OVER — overweight group, CON — control group.

TABLE 5. Association analysis of the *PPARD* rs2267668 polymorphism with BMI

		OVER_{BMI} (n = 89)	%	CON_{BMI} (n = 77)	%	OR	95% CI		*p*-value	AIC
Codominant	A/A	58	65.2	61	79.2	1.00			**0.04**	227.7
	A/G	27	30.3	16	20.8	1.77	0.87	3.63		
	G/G	4	4.50	0	0.00	0.00				
Dominant	A/A	58	65.2	61	79.2	1.00			**0.04**	229.2
	A/G-G/G	31	34.8	16	20.8	2.04	1.01	4.11		
Recessive	A/A-A/G	85	95.5	77	100.0	1.00			0.12	228.2
	G/G	4	4.50	0	0.00	0.00				
Overdominant	A/A-G/G	62	69.7	61	79.2	1.00			0.16	231.3
	A/G	27	30.3	16	20.8	1.66	0.81	3.38		
Alleles	A	143	80.3	138	89.6	0.47	0.23	0.93	**0.03**	
	G	35	19.7	16	10.4					

Note: OR — odds ratio, 95% CI — confidence intervals, AIC — Akaike information criterion; NA — not applicable, BMI — body mass index, OVER — overweight group, CON — control group.

a tendency was age-related as subjects from OVER_{FMI} were significantly older that CON_{FMI} participants.

The measured genotype frequencies did not significantly differ from the Hardy-Weinberg equilibrium expectations in the CON_{BMI} (p range 0.21 to 1), OVER_{BMI} (p range 0.09 to 0.74), CON_{FMI} (p range 0.06 to 1.00), OVER_{FMI} (p range 0.42 to 1), as well as the case-control group (p range 0.10 to 1.0) (Table 2).

Tables 3–5 summarize the results of the association analysis between SNPs within the *PPARD* and *PPARG* genes and BMI values. It was found that the polymorphism of the *PPARD* gene (rs1053049) was significantly associated with BMI exceeding 25 (Table 3). An association was found for the codominant model and also for the recessive genetic model (T/T–C/T vs C/C). Another significant association was found between the polymorphism of *PPARG* (rs1801282) with BMI exceeding 25 for the overdominant model (C/G vs C/C-G/G) (Table 4). It was also found that the polymorphism of the *PPARD* gene (rs2267668) was significantly associated with BMI. An association was demonstrated for the codominant (G/G vs A/A and A/G vs A/A), and dominant model (A/G-G/G vs A/A) (Table 5). Moreover, the genotypic frequencies of *PPARD* rs2267668 polymorphisms were significantly different between OVER_{BMI} and CON_{BMI} groups (Table 5).

The chance of being OVER for the combination C/T-C/C was 1.33 times greater than for the remaining combinations (dominant); every person with the combination C/C was OVER_{BMI} (codominant) (Table 3).

The chance of being OVER_{BMI} for the combination C/G was over 2 times greater than for the combination C/C-G/G in the overdominant model (Table 4).

The chance of being OVER for the combination A/G-G/G was over 2 times greater than for the combination A/A in the dominant model. The chance of being OVER was over 2 times smaller for allele A (Table 5).

Gene-gene interactions (only pairwise interactions were considered) were investigated for the same genetic models as for single gene analysis (except the overdominant model). A significant association was found for the interaction *PPARD* rs2267668 × *PPARG* rs1801282 with FMI, exceeding 6 in the recessive, codominant and dominant models (Table 8). Gene-gene interactions' influence on FMI division was calculated with the MDR function. For the best fit model the p-value for the cross-validation statistic was 0.01. Only when genotypes AA × CC (*PPARD rs2267668* × *PPARG rs1801282*) appear does the model sort the observations to join the CON_{FMI} group with higher probability than to join the CASE_{FMI} group. For genotypes AA × CG, AA × GG, AG × CC, AG × CG, AG × GG, GG × CC, GG × CG (*PPARD rs2267668* × *PPARG rs1801282*) the model sorts the observations to join the CASE_{FMI} group.

For *PPARD* (rs1053049) × *PPARD* (rs2267668) haplotype analysis was made. Only haplotypes with frequency over 5% were considered. The most common haplotype was TA (77%, *PPARD* (rs1053049) T > C, *PPARD* (rs2267668) A > G). A statistically significant association with BMI was found for TA haplotype in the additive model (p = 0.04) and the dominant model (p = 0.004) and also for CG haplotype in the additive model (p = 0.03). For haplotype TA (rs1053049 × rs2267668), there was a greater chance of being in the CON group than in the OVER group. For CG (rs1053049 × rs2267668), there was a higher chance of being OVER than CON. Therefore, the rare allele haplotype of the *PPARD* gene can cause being overweight (Table 6). No significant association was found for haplotypes and FMI.

The only significant association with FMI exceeding 6 was found for *PPARG* (rs1801282) polymorphism. The chance of being OVER_{FMI} was over two times higher for C/G-G/G than for C/C (dominant, Fisher's p = 0.04). The chance of being OVER_{FMI} was also over two

TABLE 6. Haplotype-based association of *PPARD* rs1053049 and *PPARD* rs2267668 with BMI

Haplotype (*PPARD* rs1053049, *PPARD* rs2267668)		Frequency [%]			additive (score = 5.66, p-value = 0.13)		dominant (score = 9.68, p-value = 0.02)		recessive (score = 1.32, p-value = 0.25)	
		OVER_BMI + CON_BMI	OVER_BMI	CON_BMI	score	p-value	score	p-value	score	p-value
T	A	77.0	73.0	82.0	-2,03	**0.04**	-2,88	**0.004**	-1,15	0.25
C	A	7.00	7.00	72.0	0.95	0.95	-0,14	0.88	NA	NA
C	G	14.0	18.0	10.0	2.11	**0.03**	1.77	**0.08**	NA	NA

NA – not applicable.

TABLE 7. Association analysis of the *PPARG* rs1801282 polymorphism with FMI

		OVER_FMI (n = 42)	%	CON_FMI (n = 124)	%	OR	95% CI		p-value	AIC
Codominant	C/C	22	52.4	87	70.2	1.00			0.09	188.9
	C/G	19	45.2	33	26.6	2.28	1.09	4.74		
	G/G	1	2.4	4	3.2	0.99	0.11	9.29		
Dominant	C/C	22	52.4	87	70.2	1.00			**0.04**	187.5
	C/G-G/G	20	47.6	37	29.8	2.14	1.04	4.38		
Recessive	C/C-C/G	41	97.6	120	96.8	1.00			0.78	191.7
	G/G	1	2.4	4	3.2	0.73	0.08	6.74		
Overdominant	C/C-G/G	23	54.8	91	73.4	1.00			**0.03**	186.9
	C/G	19	45.2	33	26.6	2.28	1.10	4.71		
Alleles	C	63	75	207	83.5	0.60	0.32	1.14	0.12	
	G	21	25	41	16.5					

OR – odds ratio, 95% CI – confidence intervals; AIC – Akaike information criterion; NA – not applicable.

TABLE 8. Association analysis of the *PPARD* rs2267668 x *PPARG* rs1801282 interaction with FMI (recessive/codominant/dominant model)

		PPARG rs1801282														p-value
		C/C				C/G				G/G						
		OVER_FMI	CON_FMI	OR	95% CI	OVER_FMI	CON_FMI	OR	95% CI		OVER_FMI	CON_FMI	OR	95% CI		
PPARG rs2267668	**A/A**	12	68	1.00	NA NA	15	20	4.25	1.71 10.54		1	1.04	1.89	0.18 19.71		
	A/G	9	19	2.68	0.98 7.32	4	10	2.27	0.61 8.42		0	0	1	0.00 0.00		0.01/
	G/G	1	0	NA	0 NA	0	3	0	0 NA		0	0	0	NA NA		0.02/
	A/G-G/G	10	19	2.98	1.12 7.96	4	13	1.74	0.49 6.26		0	1	0.00	0.00 NA		0.04
	A/A-A/G	21	87	1.00	NA NA	19	30	2.62	1.24 5.54		1	4	1.04	0.11 9.75		

OR – odds ratio, 95% CI – confidence intervals; NA – not applicable.

times higher for C/G than for C/C-G/G (overdominant, Fisher's p = 0.03) (Table 7).

For the combination A/A-A/G × C/G the chance of being OVER was over 2.5 times greater than for A/A-A/G × C/C (*PPARD × PPARG*, Fisher's p = 0.01). The chance of being OVER was over 4 times higher for A/A × C/G than for A/A × C/C (Fisher's p = 0.02) (Table 8).

DISCUSSION

The occurrence of obesity is accompanied by lipid metabolism disorders. Fatty acids are stored in lipid droplets within adipocytes as triglycerides. Their excess accumulation results in an increase in mature adipocyte size and hypertrophic adipocytes tend to be insulin resistant. This results in greater lipolysis with less lipogenesis. Thus, increased fatty acid efflux from adipose tissue represents

a key feature in pathophysiology of metabolic complications in obese individuals such as insulin resistance, diabetes, hypertriglyceridemia and metabolic syndrome. Metabolic syndrome occurrence is positively correlated with both microcirculation disturbance and the risk of damage of the cardiovascular system, which may consequently lead to death. The prevalence of metabolic syndrome and its serious complications are a significant burden for patients and the health care system, prompting researchers to look for methods of prevention, diagnosis and effective therapy of metabolic disorders co-occurring in metabolic syndrome. Counteracting its occurrence is particularly important in military professionals, who, due to the occurrence of metabolic diseases, may be at risk of early dismissal from service.

It has been found that obesity gives rise to a heightened state of inflammation. Obesity is associated with a low-grade inflammatory process in white adipose tissue (WAT) [16]. Increased mass of adipose tissue activates the inflammatory process in WAT, in the liver and the immune system. This leads to an increase of circulating levels of proinflammatory cytokines, hormone-like molecules and other inflammatory markers [17]. The strong association between obesity and inflammation, especially due to PPAR involvement, caused that the subjects in the present study – young men, genetically healthy, without inflammatory disorders, were diverse in relation to BMI (normal or high BMI, which may lead to obesity in future).

It was found that the polymorphism of the *PPARA* gene (rs1800206, L162V) was not significantly associated with the BMI exceeding 25.

Goni et al. used least-angle regression as a regression model selection technique, where the dependent variable was BMI and the independent variables were age, sex, energy intake, physical activity level, and 16 polymorphisms previously related to obesity and lipid metabolism. The four polymorphisms *FTO* (rs9939609), *APOE* (rs429358), *PPARG* (rs1801282), and *PPARA* (rs1800206) were selected; however, they accounted for 0.5% of BMI variability [18].

On the other hand, Bosse et al. [19] found that subjects carrying the V162 allele had lower values compared with L162 homozygotes of BMI, percentage body fat, waist circumference, and total computed tomography abdominal fat areas. L162V SNP was not associated with the prevalence or the age of onset of type 2 diabetes; nor was the age of first insulin treatment affected by this genotype. In subjects without diabetes this polymorphisms was not associated with fasting of glucose or insulin, insulin sensitivity or first phase insulin secretion [20]. Additionally, the PPARα gene is associated with lower body mass index in patients with non-insulin-dependent diabetes mellitus [21].

The STOP-NIDDM trial was a double-blind, placebo-controlled study that randomized 1,429 subjects with impaired glucose tolerance (IGT) to either acarbose or placebo groups [22]. DNA was available from 767 subjects from seven countries and a significant interaction between rs1800206 and the treatment group

was observed in effects on diabetes relapse. In the placebo group, carriers of the rare G (162V) allele of rs1800206 diabetes relapsed to diabetes more often than carriers of the common CC genotype (P ≤ 0.044). In the acarbose group, the conversion to diabetes was higher in carriers of the rare G allele of rs4253776 (P ≤ 0.044). The presence of the G (162V) allele of rs1800206 increased the risk of developing diabetes in the placebo group.

Tai et al. [23] studied the association among lipids, lipoproteins, and apolipoproteins and the presence of the L162V polymorphism in 2373 participants of the Framingham Offspring Study. The V162 allele was associated with increased serum concentrations of total and LDL cholesterol in men and apolipoprotein B in men and women. In the authors' opinion lipoprotein metabolism may be involved in the generation of the increase LDL cholesterol observed with the L162V *PPARA* polymorphism.

Aberle et al. [24] researched the relationship between genetic variations in *PPARA* (L162V) and indices of obesity and metabolism in subjects who attended the medical/obesity outpatient clinic of the University of Hamburg Hospital. *PPARA* V allele carriers had a slightly higher mean total cholesterol and LDL and a lower mean body weight and BMI (not statistically significant).

Examining the correlation between the presence of polymorphisms of the *PPARD* gene (rs1053049, rs2267668) and the risk of obesity in young and genetically healthy men it was found that the polymorphism of the *PPARD* gene (rs1053049) was significantly associated with the BMI exceeding 25. Associations were found for the codominant model with p = 0.01 and also for the recessive genetic model (T/T–C/T vs C/C) with p = 0.004. The odds of being OVER$_{BMI}$ for the C/T-C/C combination were 1.33 times higher than for the other combinations (dominant), all those who had a C/C genotype were OVER$_{BMI}$ (codominant). Also, a significant association between the polymorphism of the *PPARD* gene (rs2267668) and BMI was proved. A relationship was demonstrated for codominant (G/G vs A/A and A/G vs A/A) with OR = 1.77, (95% CI 0.87–3.63) p = 0.04 and the dominant model (A/G-G/G vs A/A) with OR = 2.04 (95% CI 1.01–4.11) p = 0.04 (Table 5). Moreover, the genotypic frequencies of *PPARD* rs2267668 polymorphisms were significantly different between OVER and CON group with OR = 0.47 and p = 0.03. The chance of being OVER$_{BMI}$ for the A/G-G/G combination was more than twice as high as for A/A in the dominant model. The odds of being OVER were more than twice as low for the A allele.

Results of Vänttinen et al. [25] using the hyperinsulinaemic-euglycaemic clamp technique combined with fluorine-18-labelled fluorodeoxyglucose and positron emission tomography (PET) proved the effects of *PPARD* SNPs on whole body, skeletal muscle, and subcutaneous adipose tissue glucose uptake in 129 healthy individuals. Three of six SNPs of *PPARD* and their haplogenotypes were significantly associated with whole-body insulin sensitivity. 18F-FDG-PET scanning indicated that *PPARD* SNPs primarily affected insulin sensitivity by modifying glucose uptake in skeletal muscle

but not in adipose tissue. Their results give evidence that *PPARD* SNPs regulate insulin sensitivity particularly in skeletal muscle.

Thamer et al. [26] confirmed the impact of SNPs rs1053049 and rs2267668 in *PPARD* in overall adiposity, hepatic fat storage, and relative muscle mass, and in consequence the involvement of these genetic variations in the development of insulin resistance and type 2 diabetes.

Villegas et al. [27] obtained different results – the relationship between polymorphisms of the *PPARD* gene (rs1053049, rs2267668) and type 2 diabetes in middle age Chinese women was not confirmed. However, according to Thamer et al. (2008) [26], SNP rs1053049 influenced changes in body composition during a lifestyle intervention, including overall adiposity, hepatic fat storage and relative muscle mass. Additionally, Shin et. al. [28] observed a relationship between this polymorphism and fasting glucose levels in a Korean study.

Leońska-Duniec et al. [29] examined the genotype distribution of the *PPARD* rs2267668, rs2016520, and rs1053049 alleles in a group of women, before and after the completion of a 12-week training programme. After the end of the programme a decrease of total cholesterol and a significant increase of triglyceride in rs2267668 homozygotes, a decrease in total cholesterol and triglyceride in rs2016520 *PPARD* C allele carriers, and an increase of triglyceride in *PPARD* rs1053049 TT homozygotes were noted.

A significant association found in our research was a relationship between a polymorphism of *PPARG* (rs1801282) with BMI exceeding 25 for the overdominant model (C/C-G/G vs C/G) OR = 2.03 (95% CI 1.03–4.00) with p = 0.04. The odds of being OVER$_{BMI}$ for the C/G combination was more than 2 times higher than for the C/C-G/G combination.

Bordoni et al. [30] studied a group of 306 healthy Italian children and adolescents (7–18 years old); the participants were recruited during a sport medicine check-up for the assessment of their health status to practice physical activity. Significant direct associations of MC4R rs17782313 and *PPARG* rs1801282 with body composition were detected; that is, minor allele homozygotes showed significantly higher BMI (rs17782313, ß = 1.258, p = 0.031; rs1801282, ß = 6.689, p = 1.2×10^{-4}) and waist-to-height ratio (WHtR) (rs17782313, ß = 0.021, p = 0.005; rs1801282, ß = 0.069, p = 0.003) values.

A genetic study of 150 severely obese patients (body mass index (BMI) $\geq$ 35 kg/m^2) from Central Brazil on the SNP *PPARG*2 Pro12Ala showed that all carriers of the Pro12Ala polymorphism had higher adiposity measures and systolic blood pressure compared to Pro homozygotes [31].

Chan et al. [32] investigated the association of *PPARG* gene variants with type 2 diabetes risk in the multiethnic Women's Health Initiative (WHI). They found a borderline significant association between the Pro12Ala (rs1801282) variant and T2D risk in WHI-OS.

Similar results were obtained by Hasan et al. [33], who confirmed the significant association between the *PPARG* Pro12Ala polymorphism and obesity in Egyptian patients with coronary artery disease and type 2 diabetes mellitus.

The effect of SNPs of the PPAR-γ gene (rs1801282) on obesity indexes in subjects with type 2 diabetes mellitus was also confirmed by Kruzliak et al. (2015) [34].

Due to the fact of confirming the association of the PPAR-γ gene (rs1801282) with the phenomenon of obesity, Rocha et al. [35] examined this polymorphism as a candidate gene for determining the risk of metabolic syndrome, but this polymorphism was not correlated with higher predisposition.

Contradictory results of genetic tests were obtained by Tellechea et al. [36], who stated that healthy men, in particular non-smokers, carrying the Ala12 allele of the *PPARG* rs1801282 polymorphism have a high risk for metabolic syndrome and surrogate measures of insulin resistance.

However, it should be added that the phenomenon of obesity and its heritability are not entirely explained by the DNA variants. For this reason, more studies using the latest methods in sequencing and genotyping with epigenomics, proteomics, transcriptomics and metabolomics should be conducted [37].

The main limitations of this study were the small group and lack of severely obese individuals. However, these are a result of recruitment of a homogeneous group in order to avoid the possible influence of environmental factors. For that reason, we only recruited participants who ate similarly and had similar levels of physical activity. Due to the fact that BMI is a general, insufficient weight-height parameter, we also took into account the FMI parameter.

CONCLUSIONS

In conclusion, three of the four polymorphisms (rs1053049, rs2267668, rs1801282) selected by us based on the literature are associated with the risk of being overweight.

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