ADIPOQ polymorphisms are associated with changes in obesity-related traits in response to aerobic training programme in women

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ABSTRACT: Among genetic variants of the *ADIPOQ* gene +276 G>T (rs1501299) and –11377 G>C (rs266729) are the most frequently investigated polymorphisms which were described in the context of genetic conditioning for a predisposition to obesity. However, the information of polymorphisms' potential modifying effect on obesity-related traits achieved through training procedures are still unknown. DNA was extracted from buccal cells donated by the 201 participants and genotyping was carried out using real-time PCR. The genotype distribution was examined in a group of women measured for chosen traits before and after the completion of a 12-week training programme. Our results suggest that the *ADIPOQ* genotypes analyzed individually or in combination can modulate training-induced body mass measurements changes: after the training programme, carriers of rs1501299 T allele and rs266729 C allele were characterized by a greater reduction in fat mass percentage (FM), fat mass, and body mass. Moreover, the *ADIPOQ* polymorphisms were associated with changes in lipid profile in response to training. Additionally, we showed three main effects of genotypes for the FM, LDL-C (rs266729), and TBW (rs1501299). Our study indicate that the both polymorphisms are associated with changes in obesity-related traits in response to 12-week aerobic training programme in Caucasian women. From this evidence, it could be concluded that rs1501299 G and rs266728 G variants may be considered as disadvantageous factor in the context of training-induced effects on body mass traits.

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INTRODUCTION

Adipose tissue plays various significant roles in body weight regulation and energy homeostasis, including production and secretion of numerous cytokines, chemokines, and hormone-like factors known as adipokines [1]. One of the most frequently investigated adipose tissue-derived hormones is an adiponectin. The adiponectin is a 247-amino acid peptide with a collagenous domain at the n-terminus and a globular domain that shares substantial homology with the subunits of complement factor C1q [2]. Data from human studies indicate that it is an important anti-inflammatory and insulin-sensitizing hormone, which promotes lipid oxidation in tissues such as skeletal muscle and liver [3,4]. The hormone also directs antiatherosclerotic properties, as it strongly inhibits expression of adhesion molecules and growth factors [5].

Decreased circulating levels of the adiponectin are inversely correlated with obesity, type 2 diabetes mellitus (T2DM), and atherosclerosis [6,7,8]. By contrast, the increased adiponectin levels are associated with reduced body weight and improved insulin sensitivity [8]. The plasma levels of the adiponectin are partly influenced by genetic factors, the heritability ranges from 40% to even 70% [9]. In humans, the adiponectin is encoded by *ADIPOQ* gene which is located on chromosome 3q27. The gene is 15.8 kb long and contains three exons and two introns [10]. A total of 42 single nucleotide polymorphisms (SNPs) in the gene and its regulatory region with a minor allele frequency of >1.5% have been described [11]. On the other hand, circulating adiponectin levels are also modulated by exercise training [12] and diet [13,14] associated with weight loss.

The effect of exercise on adiponectin levels varies among individuals, what may be connected with interaction between environmental and genetic factors [15].

Nowadays, among genetic variants of the *ADIPOQ* gene which were described in the context of genetic conditioning for a predisposition to obesity in some ethnic populations, +276 G>T SNP (rs1501299) and –11377 G>C SNP (rs266729) are the most frequently investigated polymorphisms associated with serum levels of adiponectin. Moreover, the SNPs can influence metabolic traits, including total cholesterol (Chol), triglycerides (TGL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as well as waist-hip ratio [16]. The first SNP is located at intron 2 and acts as an enhancer. The G allele of rs1501299 is primarily associated with lower insulin sensitivity and increased T2DM risk, lower adiponectin levels, and increased blood lipids. Conversely, many carriers of T allele have higher adiponectin levels and as a result a lower BMI (body mass index) [17, 18]. The second polymorphism is located at the 5'-flanking region and also has an influence on transcription level of the *ADIPO*Q [17]. The studies have shown that the G allele of rs266729 is associated with various detrimental conditions, including lower adiponectin levels, risk for developing hypertension, and, in some cases, risk for developing colorectal cancer [19, 20,21]. On the other hand, the presence of the C allele has also been associated with higher BMI and obesity risk, increased fasting glucose levels and T2DM [22, 23, 24].

Few studies have investigated whether the SNPs in *ADIPOQ* gene influence the effect of lifestyle intervention on obesity-related traits. Nevertheless, the studies have reported inconsistent results in terms of population, gender, age, the degree of metabolic risk levels, and gene x physical activity interactions [13,16,25]. Consequently, more diverse intervention studies have been suggested as necessary for identifying the independent effects of each *ADIPOQ* genotype on obesity-related traits. In the present study, we examined whether +276 G>T (rs1501299) and –11377 G>C (rs266729) polymorphisms in the *ADIPOQ* gene, analyzed individually or in combination, would differentially influence the effect of training programme. Therefore, we studied the allele and genotype distribution in young Polish women measured for selected body mass and body composition, as well as metabolic traits before and after cessation of a 12-week training programme to find out if there is an interaction between genotype and training.

MATERIALS AND METHODS

Ethics Statement

All the procedures followed in the study were approved by the Ethics Committee of the Regional Medical Chamber in Szczecin (Approval number 09/KB/IV/2011) and were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. Furthermore, the experimental procedures were conducted in accordance with the set of guiding principles for reporting the results of genetic association studies defined by the Strengthening the Reporting of Genetic Association studies (STREGA) Statement. All participants were given a consent form and a written information sheet concerning the study, providing all pertinent information (purpose, procedures, risks, and benefits of participation). After ensuring that the participant had understood the information, every participant gave written informed consent (signed consent form) to genotyping with the understanding that it was anonymous and that the obtained results would be confidential.

Participants

Two hundred and one Polish Caucasian women aged 21 ± 1 years (range 19–24) met the inclusion criteria and were included in the study. None of these individuals had engaged in regular physical activity in the previous 6 months. They had no history of any metabolic or cardiovascular diseases. Participants were nonsmokers and refrained from taking any medications or supplements known to affect metabolism. Prior to the start of the intervention, participants were included into a dietary program and on the basis of an individual dietary plan, they were asked to keep a balanced diet of approximately 2000 kcal/day. The participants were asked to keep a food diary every day. Weekly consultations were held on which the quality and quantity of meals were analyzed and, if necessary, minor adjustments were made.

Physical exercise training protocol

Before the training program, maximum heart rate (HRmax) of each participant was evaluated on the basis of a continuous graded exercise test on an electronically braked cycle ergometer (Oxycon Pro, Erich JAEGER GmbH, Hoechberg, Germany) according to previously described protocol [26]. Individual heart rate (HR) of all participants was also monitored with HR monitors to control the intensity during the class. All participants were instructed to maintain an intensity during the class, based on previously indicated ranges of HR or relative value of HRmax. The training stage was preceded by a week-long familiarization stage, when the examined women exercised 3 times a week for 30 minutes at an intensity of about 50% of their HRmax. After the week-long familiarization stage, the proper training started. Each training unit consisted of a warm-up routine (10 minutes), the main aerobic routine (43 minutes), and cool-down phase (stretching and breathing exercise for 7 minutes). The main aerobic routine was a combination of two alternating styles – low and high impact. Low impact style is comprised of movements with at least one foot on the floor at all times, whereas high impact styles include running, hopping, and jumping with a variety of flight phases. Music of variable rhythm intensity (tempo) was incorporated into both styles. A 12-week program of low-high impact aerobics was divided as follows: (i) 3 weeks (9 training units), 60 minutes each, at about 50–60% of HRmax, tempo 135–140 BPM, (ii) 3 weeks (9 training units), 60 minutes each, at 60–70% of HRmax, tempo 140–152 BPM, (iii) 3 weeks (9 training units), 60 minutes with

the intensity of 65%–75% of HRmax, tempo 145–158 BPM, and (iv) 3 weeks (9 training units), 60 minutes with an intensity of 65%–80% of HRmax, tempo 145–160 BPM. All 36 training units were administered and supervised by the same instructor.

Body Composition Measurements

All participants were measured for selected body mass and body composition variables before and after the completion of a 12-week training period. Body mass and body composition were assessed with the bioimpedance method (body's inherent resistance to an electrical current) using a "Tanita TBF 300M" electronic scale (Horton Health Initiatives, USA). The device was plugged in and calibrated to account for the weight of clothing (0.2 kg). Afterwards, data regarding age, body height, and sex of the subject was inserted. Then, the subjects stood on the scale with their bare feet on the marked places. Body mass and body composition measurements taken with the use of the "Tanita" electronic scale are as follows: total body mass (kg), fat free mass (FFM, kg), fat mass (kg), fat mass percentage (FM, %), BMI (kg/m^2), and total body water (TBW, kg).

Blood Analyses

Fasting blood samples were obtained in the morning from the elbow vein. Blood samples from each participant were collected in two tubes. For biochemical analyses, a 4.9 mL S-Monovette tube with ethylenediaminetetraacetic acid (K 3 EDTA; 1.6 mg EDTA/mL blood) and separating gel (SARSTEDT AG & Co., Nümbrecht, Germany) were used. For complete blood count, a 2.6 mL S-Monovette tube with K 3 EDTA (1.6 mg EDTA/mL blood) (SARSTEDT AG & Co., Nümbrecht, Germany) was used. Blood samples for biochemical analyses were centrifuged 300 × g for 15 minutes at room temperature in order to receive blood plasma. Biochemical and hematological analyses were performed before the start of the aerobic fitness training programme and repeated at the 12th week of this

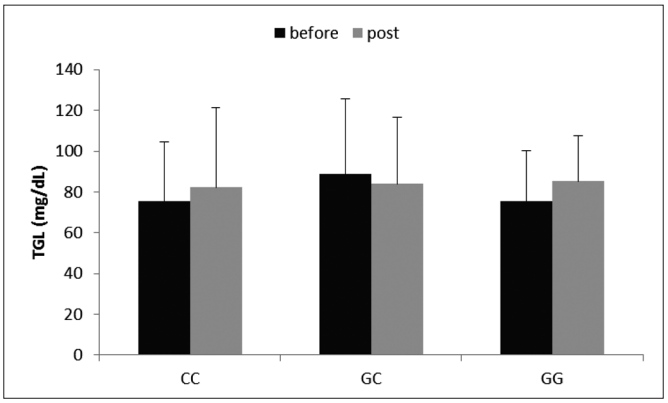

FIG. 2. Change in the TGL with respect to the *ADIPOQ* rs266729.

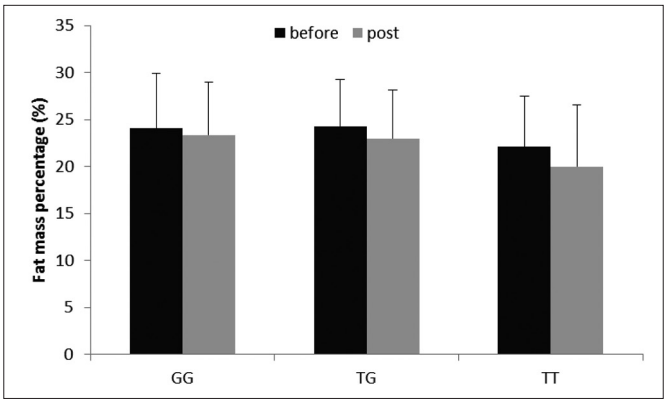

FIG. 3. The change in fat mass percentage with respect to the *ADIPOQ* rs1501299.

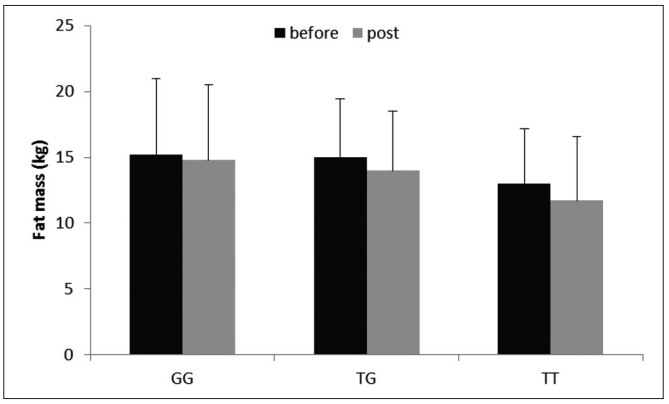

FIG. 4. The change in fat mass with respect to the *ADIPOQ* rs1501299.

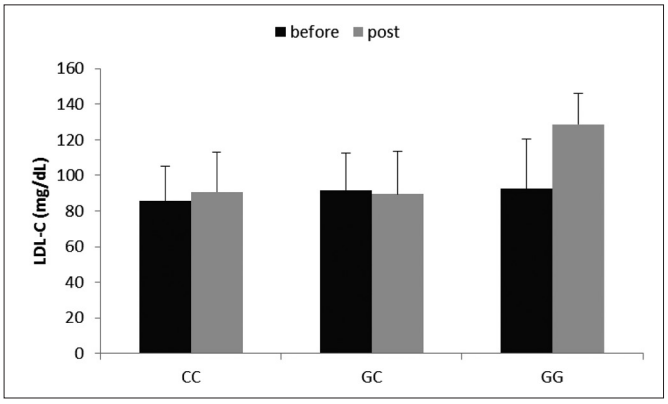

FIG. 1. Change in the LDL-C with respect to the *ADIPOQ* rs266729.

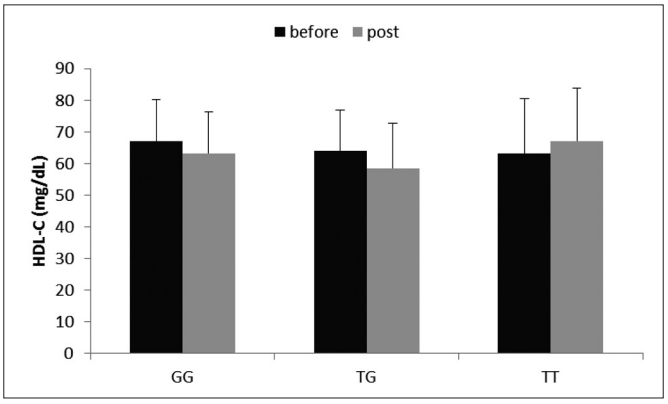

FIG. 5. The change in HDL-C with respect to the *ADIPOQ* rs1501299.

training programme (after the 36th training unit). The analyses were performed immediately after the blood collection. Complete blood count, including white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and total platelet level (PLT) were obtained using Sysmex K-4500 Haematology Analyzer (TOA SYSMEX, Kobe, Japan). All biochemical analyses were conducted using Random Access Automatic Biochemical Analyzer for Clinical Chemistry and Turbidimetry A15 (BIO- SYSTEMS S.A., Barcelona, Spain). Blood plasma was used to determine lipid profile: TGL, Chol, HDL-C, and LDL-C concentrations. Plasma TGL and Chol concentrations were determined using diagnostic colorimetric enzymatic method according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). Manufacturer's declared intra-assay coefficients of variation (CV) of the method were < 2.5% and < 1.5% for the TGL and Chol determinations, respectively. HDL-C plasma concentration was determined using human anti-ß-lipoprotein antibody and colorimetric enzymatic method according to the manufacturer's protocol (BioMaxima S.A., Lublin, Poland). The manufacturer's declared intra-assay CV of the method was < 1.5%. Plasma concentrations of LDL-C were determined using a direct method according to the manufacturer's protocol (PZ Cormay S.A., Lomianki, Poland). The manufacturer's declared intra-assay CV of the method was 4.97%. All analysis procedures were verified with the use of multiparameteric control serum (BIOLABO S.A.S, Maizy, France), as well as control serum of normal level (BioNormL) and high level (BioPathL) lipid profiles (BioMaxima S.A., Lublin, Poland).

Genetic Analyses

The buccal cells donated by the subjects were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, Germany) with the use of sterile foam-tipped applicators (Puritan, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's protocol. All samples were genotyped in duplicate using an allelic discrimination assay on a C1000 Touch Thermal Cycler (Bio-Rad, Germany) instrument with TaqMan® probes. To discriminate *ADIPOQ* rs1501299 and rs266729 alleles, TaqMan® Pre-Designed SNP Genotyping Assays were used

TABLE 1. The *ADIPOQ* rs266729 genotypes and response to training (two-way mixed ANOVA).

Parameter	CC (n=105)		GC (n=71)		GG (n=25)		Genotype	Training	Genotype x Training	Genotype x Training GG+GC vs. CC	Genotype x Training CC+GC vs. GG
	Before training	After training	Before training	After training	Before training	After training					
Body mass (kg)	60.67 ±7.16	59.78 ±6.88	61.87 ±8.10	61.23 ±8.37	58.45 ±7.27	58.38 ±7.19	p=0.177	p<0.001*	p=0.063	p=0.076	p=0.035*
BMI (kg x m^{-2})	21.64 ±2.48	21.41 ±2.41	21.86 ±2.62	21.66 ±2.67	21.34 ±2.09	21.29 ±2.09	p=0.694	p<0.001*	p=0.244	p=0.295	p=0.105
FM (%)	23.76 ±5.29	22.54 ±5.45	25.10 ±5.35	23.92 ±5.67	21.73 ±5.91	21.26 ±5.66	p=0.042*	p<0.001*	p=0.323	p=0.483	p=0.134
Fat mass (kg)	14.73 ±4.83	13.82 ±4.87	15.88 ±5.28	15.05 ±5.58	13.06 ±5.12	12.82 ±5.01	p=0.075	p<0.001*	p=0.182	p=0.301	p=0.070
FFM (kg)	45.74 ±2.96	46.14 ±3.08	45.95 ±3.36	46.20 ±3.25	45.38 ±2.96	45.70 ±2.99	p=0.757	p=0.002*	p=0.761	p=0.481	p=0.944
TBW (kg)	33.41 ±2.39	33.51 ±3.72	33.63 ±2.47	33.87 ±2.46	33.67 ±2.93	33.50 ±2.22	p=0.767	p=0.770	p=0.749	p=0.889	p=0.526
Chol (mg/dl)	168.41 ±23.56	168.57 ±25.68	173.80 ±25.89	168.15 ±29.10	169.88 ±31.71	172.12 ±28.49	p=0.769	p=0.528	p=0.111	p=0.198	p=0.315
TGL (mg/dl)	75.59 ±28.93	82.24 ±38.92	88.70 ±36.90	83.92 ±32.68	75.40 ±24.93	85.24 ±22.37	p=0.253	p=0.151	p=0.038*	p=0.097	p=0.263
HDL-C (mg/dl)	66.29 ±11.79	61.99 ±14.22	64.30 ±15.62	61.35 ±14.79	64.42 ±14.13	60.35 ±14.22	p=0.721	p<0.001*	p=0.715	p=0.492	p=0.890
LDL-C (mg/dl)	85.74 ±19.60	90.67 ±22.58	91.78 ±20.65	89.55 ±24.17	92.54 ±27.85	128.64 ±17.63	p=0.023*	p=0.015*	p=0.033*	p=0.754	p=0.012*
Glucose (mg/dl)	78.48 ±10.41	75.74 ±10.30	77.81 ±11.10	76.08 ±10.48	74.92 ±10.55	72.84 ±11.77	p=0.296	p=0.006*	p=0.779	p=0.491	p=0.899

Mean±standard deviation; p values for main effects (genotype and training) and genotype x training interaction; BMI – body mass index; FM – fat mass percentage; FFM – fat free mass; TBW – total body water; Chol – cholesterol; TGL – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

(Applied Biosystems, USA) (assay ID: C___7497299_10 and C___2412786_10, respectively), including primers and fluorescently labelled (FAM and VIC) MGBTM probes to detect alleles. Genotypes were assigned using all of the data from the study simultaneously.

Statistical Analyses

Allele frequencies were determined by gene counting. A chi-square test was used to test the Hardy-Weinberg equilibrium. To test the influence of the +276 G>T (rs1501299) and –11377 G>C (rs266729) polymorphisms in the *ADIPOQ* gene on training response, the 2 × 2 mixed-design ANOVA test was used followed by Tukey's post-hoc and analysis of contrasts (STATISTICA, StatSoft, Inc. (2014). STATISTICA (data analysis software system), version 12. www.statsoft.com). Haplotype analysis was conducted with R (https://cran-r.project.org, version 3.1.0) using haplo.stats package and haplo.glm regression function. Percentage change over training was used as the dependent variable, while the *ADIPOQ* haplotypes were used as the independent variables. The level of statistical significance was set at $p < 0.05$.

RESULTS

Single locus analysis

Changes in the parameters over an 12-week training with respect to the *ADIPOQ* rs266729 and rs1501299 genotypes are presented in Table 1 and Table 2, respectively. The significant main effect of training was evident for body mass, BMI, tissue impedance, FM, FFM, and glucose (Tables 1, 2). We also found three main effects of genotype for the FM, LDL-C (rs266729), and TBW (rs1501299). In addition, there were 5 genotype x training interactions. As opposed to rs266729 GG homozygotes (92.54±27.85 vs 128.64±17.63, p=0.048), LDL-C did not change significantly after the training in the carriers of the C allele (p=0.967, p=0.999, for the CC and GC, respectively, Figure 1). Although there were no rs266729 genotype-dependent significant differences in the TGL before and after training (Figure 2), analysis of contrast showed a significant opposite effect of training for the CC (75.59±28.93 vs 82.24±38.92) and GC genotypes (88.70±36.90 vs 83.92±32.68, p=0.022), but not for the GG and GC genotypes (p=0.052). The FM decreased by 9.6% (22.12±5.39 vs 20.00±6.57, p=0.0005) in the TT homozygotes compared with 5.4% in the TG (24.29±5.01 vs 22.98±5.16,

TABLE 2. The *ADIPOQ* rs1501299 genotypes and response to training (two-way mixed ANOVA).

Parameter	GG (n=93)		TG (n=89)		TT (n=19)		Genotype	Training	Genotype x Training	Genotype x Training GG+GT vs. TT	Genotype x Training TT+GT vs. GG
	Before training	After training	Before training	After training	Before training	After training					
Body mass (kg)	61.40 ±8.63	60.88 ±8.54	60.89 ±6.48	60.00 ±6.41	57.61 ±5.94	56.91 ±6.03	p=0.120	p<0.001*	p=0.268	p=0.988	p=0.120
BMI (kg x m^{-2})	21.78 ±2.63	21.64 ±2.60	21.75 ±2.37	21.49 ±2.37	20.88 ±2.21	20.68 ±2.20	p=0.319	p<0.001*	p=0.332	p=0.981	p=0.154
FM (%)	24.06 ±5.87	23.35 ±5.67	24.29 ±5.01	22.98 ±5.16	22.12 ±5.39	20.00 ±6.57	p=0.135	p<0.001*	p=0.025*	p=0.041*	p=0.020*
Fat mass (kg)	15.23 ±5.76	14.77 ±5.72	15.02 ±4.42	13.98 ±4.50	13.03 ±4.17	11.71 ±4.83	p=0.119	p<0.001*	p=0.020*	p=0.138	p=0.006*
FFM (kg)	46.08 ±3.37	46.26 ±3.47	45.70 ±2.90	46.12 ±2.86	44.57 ±2.34	45.26 ±2.40	p=0.262	p<0.001*	p=0.195	p=0.198	p=0.110
TBW (kg)	33.84 ±2.65	33.88 ±2.56	33.37 ±2.40	33.76 ±2.16	32.64 ±1.72	31.86 ±7.11	p=0.046*	p=0.576	p=0.132	p=0.080	p=0.671
Chol (mg/dl)	171.98 ±26.59	173.24 ±30.47	170.17 ±23.23	166.02 ±23.73	164.73 ±30.43	160.73 ±22.27	p=0.192	p=0.219	p=0.180	p=0.600	p=0.064
TGL (mg/dl)	79.66 ±31.87	86.22 ±38.27	81.39 ±32.37	79.81 ±32.77	77.21 ±32.63	84.37 ±27.20	p=0.858	p=0.173	p=0.205	p=0.560	p=0.153
HDL-C (mg/dl)	67.10 ±13.24	63.25 ±13.18	63.96 ±12.87	58.60 ±14.04	63.32 ±17.14	67.18 ±16.70	p=0.096	p=0.065	p=0.003*	p=0.001*	p=0.939
LDL-C (mg/dl)	89.35 ±22.93	101.96 ±93.80	89.31 ±19.54	91.42 ±21.70	82.79 ±20.67	77.65 ±15.44	p=0.224	p=0.583	p=0.388	p=0.414	p=0.193
Glucose (mg/dl)	78.45 ±11.00	75.48 ±10.70	77.40 ±10.08	75.32 ±10.04	76.52 ±12.13	76.42 ±12.54	p=0.910	p=0.047*	p=0.464	p=0.287	p=0.355

Mean±standard deviation; p values for main effects (genotype and training) and genotype x training interaction; BMI – body mass index; FM – fat mass percentage; FFM – fat free mass; TBW – total body water; Chol – cholesterol; TGL – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

p=0.00002) and 3.1% in GG (24.06±5.87 vs 23.35±5.67, p=0.027) (Figure 3). Corresponding reductions in the fat mass were 10.1% (13.03±4.17 vs 11.71±4.83, p=0.004), 6.9% (15.02±4.42 vs 13.98±4.50, p=0.00002) and 3.0% (15.23±5.76 vs 14.77±5.72, p=0.057) (Figure 4). In addition, HDL-C decreased significantly over training period, but only in the GG (67.10±13.24 vs 63.25±13.18, p=0.006) and TG genotypes (63.96±12.87 vs 58.60±14.04, p=0.00005) (Figure 5).

Haplotype-based analysis

The estimated the *ADIPOQ* rs266729 and rs1501299 haplotype frequencies were 41.31%, 28.65%, 27.15% and 2.94% for the [C;G], [C;T], [G;G] and [G;T], respectively. Haplotype-based association analysis is presented in Table 3. Carriers of the [G;G] haplotypes had significantly smaller percentage decrease in body mass (by 0.8% per copy, p=0.013) compared with individuals homozygous for the baseline [C;G] haplotype. Carriers of the [G;T] haplotypes, in turn, had significantly smaller percentage decrease in HDL-C and glucose, by 15.9% per copy, p=0.011, and 10.7% per copy, p=0.011, respectively, compared with individuals homozygous for the baseline [C;G] haplotype. Conversely, the mean percentage decrease in FM and fat mass were greater, by 4.4% per copy, p=0.002, and by 4.8% per copy, p=0.004, respectively in the carriers of the [C;T] haplotype.

DISCUSSION

Numerous previous studies reported that aerobic training showed variable effects on changes in the plasma levels of adiponectin [12,25,27]. Some results indicated that physical activity could increase adiponectin concentrations during weight loss [27]. However, not all studies have demonstrated this association [28]. The differences in these results might be due to the *ADIPOQ* variants [15].

The present study investigates the difference in the influence of +276 G>T (rs1501299) and –11377 G>C (rs266729) polymorphisms in the *ADIPOQ* gene, which are well known to be associated with serum levels of adiponectin, and as a result obesity-related traits, on effect of 12-week training programme in young healthy women. Our results showed three main effects of genotypes for the FM, LDL-C, and TBW. The carriers of the GC genotype (rs266729) had higher FM, carriers of CC genotype (rs266729) had lower LDL-C level, and the carriers of GG genotype (rs1501299) had higher TBW. In addition, there were five genotype x physical activity interactions. However, it should be noted that when a stringent correction for multiple testing was applied (alpha value 0.05 divided by the number of hypotheses for each polymorphism, i.e. 11) only one significant interaction remained (for the HDL-C). After training programme, the greatest reduction in the FM and fat mass was found in rs1501299 TT genotypes compared with TG, and GG. Moreover, the *ADIPOQ* polymorphisms were associated with changes in lipid profile in response to training. Haplotype analyze revealed that the carriers of the [G;G] haplotypes had significantly smaller percentage decrease in body mass compared with individuals homozygous for the baseline [C;G] haplotype. Carriers of the [G;T] haplotypes, in turn, had significantly smaller percentage decrease in HDL-C and glucose, compared with individuals with [C;G] haplotype. Conversely, the mean percentage decrease in FM and fat mass were greater in the carriers of the [C;T] haplotype.

TABLE 3. Haplotype-based association analysis.

Parameter	[C;T] 28,65%			[G;G] 27,15%			[G;T] 2,942%		
	coef	t	p	coef	t	p	coef	t	p
Body mass (kg) [-0.016]	0.002	0.498	0.619	0.008	2.509	0.013*	-0.011	-1.157	0.249
BMI (kg x m^{-2}) [-0.011]	0.0001	0.031	0.975	0.004	1.390	0.166	-0.007	-0.706	0.481
FM (%) [-0.019]	-0.044	-3.194	0.002*	-0.002	-0.192	0.848	-0.006	-0.166	0.869
Fat mass (kg) [-0.028]	-0.048	-2.906	0.004*	0.004	0.271	0.786	-0.032	-0.725	0.469
FFM (kg) [0.004]	0.006	1.411	0.160	0.001	0.154	0.878	0.007	0.629	0.530
TBW (kg) [0.013]	-0.010	-0.978	0.329	-0.006	-0.656	0.513	0.011	0.444	0.658
Chol (mg/dl) [0.020]	-0.025	-1.511	0.132	-0.014	-0.920	0.359	-0.032	-0.733	0.464
TGL (mg/dl) [0.087]	0.022	0.415	0.679	0.010	0.221	0.825	-0.175	-1.413	0.159
HDL-C (mg/dl) [-0.069]	0.014	0.578	0.564	0.009	0.418	0.677	0.159	2.582	0.011*
LDL-C (mg/dl) [0.0704]	-0.049	-0.534	0.594	0.090	1.097	0.274	-0.104	-0.480	0.632
Glucose (mg/dl) [-0.027]	-0.001	-0.076	0.939	-0.003	-0.172	0.864	0.107	2.562	0.011*

BMI – body mass index; FM – fat mass percentage; FFM – fat free mass; TBW – total body water; Chol – cholesterol; TGL – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

Our results of individual and haplotype analyzes clearly indicate that rs1501299 T allele may improve the training-induced positive effects on obesity-related traits such as FM and fat mass. Such hypothesis may be supported by the findings of previous studies showing the role of T allele in maintaining proper body mass measurements [18;29,30]. On the other hand, the results of rs266729 are inconsistent. We revealed the effect of genotype, which suggested that the C allele is connected with higher FM. However, the haplotype analyze showed that the C allele may improve the training-induced favorable effect on body mass, FM, and fat mass. The obesity-related traits are undoubtedly determined by numerous genes, and studies concerning the assessment of the influence of individual genetic factors on the obesity-related traits are very complex. Complicated interactions between various genes affecting a studied features in humans as well as many possible epigenetic and environmental factors may modify the function of genetic factors. Considering that the ADIPOQ protein is very important anti-inflammatory and insulin-sensitizing hormone, which promotes lipid oxidation, it may be assumed that the *ADIPOQ* gene is one of such genetic factors. In consequence, numerous studies have attempted to find an association between the gene and obesity-related traits. However, little is known about the *ADIPOQ* gene x lifestyle interactions, and obtained results are inconsistent. Authors usually indicated that the G allele of rs1501299 is primarily associated with lower insulin sensitivity and adiponectin levels, higher blood lipids and BMI. As a result, the G allele is known as a risk allele. Conversely, carriers of T allele have higher adiponectin levels and lower BMI [18,29,30]. These findings are confirmed by our results, which clearly indicate that the T allele is associated with the training-induced positive effects on obesity-related traits. Only two notable exceptions to this trend are described by Beede-Dimmer et al. [31] and Bouatia-Naji et al. [22]. In the mentioned studied, the presence of T allele corresponded with obesity-related traits. The first was a study of African American, and the second of French Caucasians. However, some studies did not show associations between the *ADIPOQ* polymorphisms and various risk factors usually in the normal weight participants [32, 33]. It is more difficult to draw conclusions about rs266729, because current findings are inconsistent. The C allele was described in context of higher risk of obesity, increased fasting glucose levels and T2DM [17,21,22,23]. On the other hand, the G allele was associated with various detrimental conditions, including lower adiponectin levels, risk for developing hypertension, and, in some cases, risk for developing colorectal cancer [19,20,21]. In other studies none of the associations between rs266729 and various risk factors were described [34, 35]. Our study also did not completely explain the role of each allele on maintaining proper body mass measurements. The obtained results suggest that the G variant of rs266728 may be considered as disadvantageous factor in the context of training-induced effects on body mass traits, however, continuing researches are necessary.

It may be suggested that the *ADIPOQ* polymorphisms exert differential effects on BMI probably due to the modifying impact of other genetic components and/or environmental factors especially diet and physical activity. However, little is known about the *ADIPOQ* gene x lifestyle interactions. In a study performed on 90 Korean women with uncomplicated obesity exercising for 3 months, Lee et al. [25] revealed results contrary to ours and described that aerobic training affects adiponectin levels regardless of weight loss, but this effect would not be influenced by SNPs in the *ADIPOQ* gene. The rs2241766, rs1501299, and rs266729 were also examined in 363 subjects with impaired fasting glucose or newly diagnosed type 2 diabetes following a dietary intervention and regular physical activity for 12 weeks. After this lifestyle intervention, fasting glucose levels decreased in all three rs1501299 genotype groups. The rs1501299 showed its effect on adiponectin concentrations in overweight-obese participants only in haplotype analyzes [13]. In another study, obese Japanese women within the rs1501299 were placed on a low-calorie diet for 8 weeks. At the study conclusion, those with GT or TT genotype had a greater decrease in waist circumference, what is in accordance with our results. In the same population, the participants with CG and GG genotypes at rs266729 enjoyed a greater decrease in systolic blood pressure and fasting plasma glucose than those with CC [14].

Several studies have reported contradictory results in terms of a geographic difference, gender, BMI, and age, what may also explain contradictory findings [16, 17]. A large-scale meta-analysis of 54 studies revealed the association between rs266729 and excess of human body weight only in Asian ethnicity, but not in Caucasian ethnicity. Conversely, rs1501299 was linked with obesity risk only in Caucasian ethnicity, not in participants of Asian ethnicity [17]. However, other meta-analysis conducted by Enns et al. [16] did not show similar associations. Polymorphism rs266729 was also described in context its impact on body weight excess in Caucasian ethnicity e.g. French and Swedish populations [22,23]. Whereas, positive correlation between rs1501299 and obesity-related traits was showed not only in mentioned Caucasian ethnicity, but also in Asian and American populations [22,31,32,36]. Our result showed that both the *ADIPOQ* SNPs are connected with obesity-related traits in Caucasian population, but continuing researches are necessary.

Considering the aforementioned facts, it is more reasonable to compare results in a homogeneity population. To date, there have been some studies about the *ADIPOQ* gene involving participants from Poland. However, none of them described the potential impact of the *ADIPOQ* gene on the extent and nature of the response to training in healthy individuals. Additionally, the existing results obtained during studies of homogeneity Polish population are also inconsistent. In a study performed on obese children, adolescents and non-obese adults, Cieslak et al. [34,35] found no consistent evidence for association between obesity and rs266729. By contrast, among five analyzed polymorphisms in a group of 101 obese and 67 normal-weight children, Gajewska et al. [37] established that only rs266729

were associated with a higher risk of obesity during the prepubertal period. The obtained results indicate that adipokine abnormalities coexisting with the lack of relations between BMI and dietary intake may predict a higher risk of future obesity-related disorders in obese children carrying the GG genotype than in those with other genotypes. The second rs1501299 was analyzed in the context its impact on endometrial cancer risk in Polish obese females. The study revealed that allele G in obese women with endometrial cancer is significantly more frequent, than in lean controls. Consequently, rs1501299 may be considered to be a risk factor of endometrial cancer [38].

In conclusion, the results of our experiment suggest that the *ADIPOQ* rs266729 is associated with FM, LDL-C level, and the *ADIPOQ* rs1501299 with TBW. Moreover, the both polymorphisms, analyzed individually or in combination, are associated with changes in obesity-related traits such as body mass, FM, fat mass, and lipid profile, in response to 12-week aerobic training programme in Caucasian women. The individual and haplotype analyzes of the SNPs clearly indicate that rs1501299 T allele is associated with the greatest reduction in the FM and fat mass achieved through training procedures. From this evidence, it could be concluded that the G variant of rs1501299 may be considered as disadvantageous factor in the context of training-induced effects on body mass traits. Unfortunately, the results of rs266729 are inconsistent. We revealed the effect of genotype, which suggested that the C allele is connected with higher FM. However, the haplotype analyze showed that the C allele may improve the training-induced favorable effect on body mass, FM, and fat mass, so continuing researches are necessary.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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