Nutrition 60 (2019) 1–5

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Role of the variant in adiponectin gene rs266729 on weight loss and cardiovascular risk factors after a hypocaloric diet with the Mediterranean pattern

Daniel Antonio de Luis M.D.P.H. *, David Primo, Olatz Izaola M.D.P.H., Emilia Gomez Hoyos M.D.P.H., Juan Jose Lopez Gomez M.D.P.H., Ana Ortola, Rocio Aller M.D.P.H.

Center of Investigation of Endocrinology and Nutrition, Medical School, Department of Endocrinology and Investigation, Hospital Clinico Universitario, University of Valladolid, Valladolid, Spain

ARTICLE INFO

Article History:
Received 22 March 2018
Received in revised form 17 August 2018
Accepted 22 August 2018

Keywords:
Adiponectin gene
Adipokines
Cardiovascular risk factors
Hypocaloric diet
rs266729

ABSTRACT

Objectives: The role of *ADIPOQ* gene variants on weight loss after a dietary intervention remain unclear. The aim of this study was to analyze the effects of *rs266729* of the *ADIPOQ* gene on cardiovascular risk factors and adiposity parameters after adherence to a Mediterranean-type hypocaloric diet.
Method: Eighty-three obese patients were studied before and after 12 wk on a Mediterranean-type hypocaloric diet. Anthropometric parameters and biochemical profiles were measured. The variant of *ADIPOQ* gene rs266729 was assessed at basal time by polymerase chain reaction at real time.
Results: Two genotype groups were realized (CC versus CG + GG). The final genotype distribution was 48 patients CC (57.8%), 30 patients CG (36.2%) and 5 patients GG (6%). After dietary intervention with a moderate calorie restriction and in both genotypes, body mass index (BMI), weight, fat mass, systolic blood pressure, and waist circumference decreased. After dietary intervention and in non-G allele carriers (CC versus CG+ GG), glucose (δ: −6.2 ± 1.1 versus −2.9 ± 1.2 mg/dL; $P = 0.02$), total cholesterol (δ:-15.2 ± 3.1 versus −3.4 ± 2 mg/dL; $P = 0.02$), low-density lipoprotein cholesterol (δ, −14.9 ± 3.1 versus −4.9 ± 1.2 mg/dL; $P = 0.01$), insulin levels (δ, −4± 0.6 versus 0.7 ± 0.3 UI/L;$P = 0.01$), homeostasis model assessment for insulin resistance (δ, −1.6 ± 0.4 versus −0.2 ± 0.4 units; $P = 0.01$), and adiponectin (δ, −10.4 ± 3.1 versus −1.3 ± 1.0 ng/dL; $P = 0.01$) improved.
Conclusion: After weight loss, the CC genotype of *ADIPOQ* gene variant (rs266729) is associated with increases in adiponectin levels and decreases of low-density lipoprotein cholesterol, insulin and homeostasis model assessment for insulin resistance after weight loss.

Introduction

Adiponectin is an adipose-derived protein that is known to regulate insulin sensitivity and glucose metabolism. This adipokine has been linked with metabolic syndrome (MetS) and obesity [1]. Adiponectin is encoded by the adiponectin C1 Q and collagen domain-containing (*ADIPOQ*) gene, which is located on chromosome 3 q27. A common single nucleotide polymorphism (SNP), rs266729 (−11,377 C>G), in the proximal promoter region of the *ADIPOQ* gene has drawn much attention. Data in the literature indicates that *ADIPOQ* rs266729 polymorphism functionally regulates adiponectin promoter activity and its protein levels [2,3]. Moreover, *ADIPOQ* rs266729 has been found to be correlated with circulating adiponectin levels in obesity and diabetes [4,5]. This *ADIPOQ* variant has been identified to be associated with high body mass index, insulin resistance, and diabetic nephropathy [6–8].

In humans, weight reduction increases serum adiponectin levels [9], and in animal models, administration of recombinant adiponectin produces an increase of fatty acid oxidation and weight loss [10]. The Finnish Diabetes Prevention Study, conducted with white adults, showed that the genetic variant of 82 *ADIPOQ* gene rs266729 contributes to variations in body weight and serum adiponectin concentrations [11] and modifies the risk for developing type 2 diabetes mellitus. Hsiao et al. [12] showed that this genetic variant may contribute to weight reduction in response to

DAL and RA designed the study and conducted the statistical analysis. OI, EG, and JJL were responsible for the anthropometric evaluation and control of dietary intake. DP was responsible for the biochemical evaluation and genotyping. The authors have no conflicts of interest to declare.

* Corresponding author: Tel.: +34 983 42 0000; Fax: +34 983 25 7511.
E-mail address: Spaindadluis@yahoo.es (D.A. de Luis).

https://doi.org/10.1016/j.nut.2018.08.018
0899-9007/

sibutramine therapy in obese patients. A recent study [13] demonstrated that the C allele of this genetic variant is a predictor of better lipid profiles after bariatric surgery in morbid obese patients.

As far as we know, no study has yet evaluated the effect of this genetic variant on weight loss in an intervention with a hypocaloric diet. The aim of the present study was to analyze the effects of rs266729 of the *ADIPOQ* gene on cardiovascular risk factors, serum adipokine levels, and adiposity parameters after a hypocaloric Mediterranean-type diet in obese individuals.

Participants and methods

Participants and clinical investigation

For this trial, 83 unrelated obese patients were recruited from primary care physician offices from an urban area of Castilla y Leon (northwest Spain). Data of these patients were collected at the beginning and after 12 wk of dietary treatment and all participants provided written informed consent. The Ethics Committee (HCUVA Committee 04/2017) approved the study and the study was in accordance with the guidelines laid down in the Declaration of Helsinki. All recruited patients fulfilled the following inclusion criteria: between 20 and 70 y of age and had a body mass index (BMI) $\geq$30 kg/m^2. Individuals with a history of cardiovascular disease, thyroid disease, renal or hepatic disorders, alcoholism, or malignant tumor and who were receiving medications known to influence lipid levels (hormonal therapy, glucocorticoids, and anti-inflammatory drugs) within the 6 mo before the study were excluded.

Data on anthropometric parameters (weight, height, BMI, waist circumference [WC], fat mass [FM] by impedance) and blood pressure were recorded. Blood samples were collected in EDTA-treated and plain tubes after a 12-h overnight fast for analysis of insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TGs), and serum adipokine levels (leptin, total adiponectin, and resistin). The variant of *ADIPOQ* gene was assessed at basal time by real-time polymerase chain reaction. The first endpoint was body weight loss after 12 wk versus baseline. The second endpoints were cardiovascular risk factors and serum adipokine changes.

Dietary intervention

The participants in this interventional study received individualized counseling on diet and exercise. The dietary intervention was a hypocaloric Mediterranean-type diet of fruits, vegetables, fish, and whole grains, that limited unhealthy fats (restricting 500 daily calories to the usual intake) over a 12-wk period. The percentage of macronutrients was 52% carbohydrates, 25% lipids, and 23% proteins. Percentage of fats was 50.6% monounsaturated fats, 37.5% saturated fats, and 11.9% polyunsaturated fats. All participants had two individual sessions (60 min with diet sheets and sample menu plans) with the dietitian at the start of the trial to explain the diet and resolve any questions. The dietitian assessed adherence to the diet every 7 d with a phone call. All enrolled participants received instruction to record their daily dietary intake for 4 d including a weekend day. Records were analyzed with a computer-based data evaluation system (Dietosource, Geneve, Swiss). National composition food tables were used as reference [14]. The exercise program consisted of an aerobic exercise at least three times per week (60 min each) and was recorded by the patient with a self-reported questionnaire.

Biochemical and adipokine assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was determined by RIA (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5 mUI/L (normal range 0.5–30 mUI/L) [15] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values [16]. Serum TC and TG concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), whereas HDL-C was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate magnesium. LDL-C was calculated using Friedewald formula [17]. Resistin was measured by enzyme-linked immunosorbent assay (ELISA; Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/mL (normal range 4–12 ng/mL) [18]. Adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng/mL (normal range 8.65–21.43 ng/mL) [19]. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) with a sensitivity of 0.05 ng/mL (normal range 10–100 ng/mL) [20].

Genotyping the ADIPOQ *gene*

Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International, Los Angeles, CA, USA). The polymerase chain reaction was carried out with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'- CGTTGGATGATGTGTGGCTTGCAAGAACC-3' and reverse 5'-ACGTTGGATGCAACATTCAACACCTTGGAC-3' in a 2 uL final volume (Termociclador Life Tecnologies, Los Angeles, CA, USA). DNA was denaturated at 90°C for 2 min and followed by 50 cycles of denaturation at 90°C for 20s, and annealing at 56.1°C for 50s. The polymerase chain reaction was run in a 25 uL final volume containing 10.5 uL of IQTM Supermix (Bio-Rad, Hercules, CA, USA) with hot start Taq DNA polymerase Hardy–Weinberg equilibrium, which was assessed with a statistical test (χ^2) to compare the expected and observed counts. The variant was in Hardy–Weinberg equilibrium ($P = 0.36$).

Blood pressure determination and adiposity parameters

Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer (Omrom, Los Angeles, CA, USA) and averaged. Weight and height were measured with an electrical scale (Omrom) and a telescopic height measuring instrument (Omrom). Body weight was measured in the morning with participants minimally unclothed and not wearing shoes. BMI was calculated as body weight (in kg) divided by height (in m^2). WC was measured with a flexible nonstretchable measuring tape (Type SECA, SECA, Birmingham, UK). Bioimpedance was used to determine body composition with an accuracy of 5 g (EFG, Akern, Italy) [21].

Statistical analysis

All data were analyzed using SPSS version 19 software package (SPSS Inc. Chicago, IL, USA). Sample size was calculated to detect differences >2 kg in body weight loss with 90% power and 5% significance (n = 80). The Kolmogorov–Smirnov test was used to determine variable distribution. The results were expressed as average ± SD. Numerical variables with normal distribution were analyzed with a two-tailed Student's *t* test. Non-parametric

variables were analyzed with the Mann–Whitney's U test. Categorical variables were analyzed with the χ^2 test, with Yates correction as necessary, and Fisher's test. The statistical analysis to evaluate the gene–diet interaction was a univariate analysis of covariance. Multiple regression analysis (stepwise method) was used to analyze relationship of this gene variant as the independent variable and the changes in adiposity and cardiovascular risk markers as the dependent variable. A χ^2 test was used to evaluate the Hardy–Weinberg equilibrium. All analysis were performed under a dominant genetic model with rs266729 G allele as the risk allele (GG + GC versus CC). $P < 0.05$ was considered significant.

Results

Eighty-three obese individuals were recruited. The mean age was 46.2 ± 5.1 y (range 26–68 y) and the average BMI 34.1 ± 3.9 kg/m^2 (range 31.2–39.3 kg/m^2). There were 63 women (75.9%) and 20 men (24.1%). The final genotype distribution was 48 patients CC (57.8%), 30 patients CG (36.2%), and 5 patient GG (6%). Sex distribution was similar in all genotype groups (CC: 26.1% men versus 73.9% women; CG: 33.3% men versus 66.7% women; and GG: 20% men versus 80% women). The average age was similar in the three genotype groups (CC 46.4 ± 4 y; CG 45.9 ± 5.8 y; GG 46.2 ± 4.3 y; not significant).

Applying an analysis with a dominant model, no statistical association was found between rs266729 G- allele for either blood pressure or anthropometric parameters (BMI, weight, FM, and WC; Table 1). After dietary intervention with a moderate calorie restriction and in patients with both genotypes (CC versus CG + GG), BMI (δ, −0.5 ± 0.2 versus −0.4 ± 0.1 kg/m^2; $P = 0.12$), weight (δ, −3.4 ± 2.2 versus −3.5 ± 2.1 kg; $P = 0.23$), FM (δ, −1.6 ± 1.1 versus −2 ± 1 kg; $P = 0.36$) and WC (δ, −5.4 ±1.6 versus −5.1 ± 1.9 cm; $P = 0.39$) decreased. The improvement of all anthropometric parameters was statistically significant in both genotype groups without differences between them. Systolic blood pressure showed a statistical decrease in both genotype groups after dietary intervention (δ, −5.7 ± 2.1 versus −5.1 ± 1.4 mm Hg; $P = 0.31$). This improvement was similar in both genotype groups. Biochemical characteristics according to genotype are shown in Table 2.

After dietary intervention and in non-G allele carriers (CC versus CG + GG), glucose (δ,: −6.2 ± 1.1 versus −2.9 ± 1.2 mg/dL; $P = 0.02$), TC (δ, −15.2 ± 3.1 versus −3.4 ± 2 mg/dL; $P = 0.02$), LDL-C (δ, −14.9 ± 3.1 versus −4.9 ± 1.2 mg/dL; $P = 0.01$), insulin levels (δ, −4 ± 0.6 versus 0.7 ± 0.3 UI/L; $P = 0.01$), and HOMA-IR (δ: −1.6 ± 0.4 versus −0.2 ± 0.4 units; $P = 0.01$) improved. Moreover, these parameters remained unchanged in G allele carriers.

Table 3 shows changes of serum adipokines. Basal and post-treatment adiponectin levels were higher in non-G allele carriers than in G allele carriers. After dietary intervention and in non-G allele carriers (CC versus CG + GG), serum adiponectin (δ, 10.4 ± 3.1 ng/dL versus −1.3 ± 10 ng/dL; $P = 0.01$) increased. In addition, patients with both genotypes (CC versus CG + GG) showed a significand decrease in leptin levels (δ, −17.2 ± 6.1 versus −2 ± 7.1 ng/dL; $P = 0.18$). The improvement of leptin was similar in both genotype groups. Resistin levels remained unchanged after dietary intervention.

In the multiple regression analysis adjusted by age and sex, the rs266729 genotype remained in each model with the following dependent variables: glucose levels (β, 2.3 mg/dL; 95% confidence interval [CI], 1.9–3.5), insulin levels (β, 4.2 UI/L; 95% CI, 1.8 to −6.9), TC mg/dL (β, 4.4 mg/dL; 95% CI, 1.9–7.1), LDL-C (β, 3 mg/dL; 95% CI, 1.2–6.6), HOMA-IR (β, 7.1 units; 95% CI, 2.1–13.1), and adiponectin levels (β, 7.1 ng/dL; 95% CI, 2.8–18.1).

Discussion

To our knowledge this is the first study to evaluate whether the *ADIPOQ* variant rs266729 is significantly associated with weight decrease, modification of cardiovascular risk factors, and adiponectin levels after moderate calorie restriction with a Mediterranean-type diet among obese individuals. Non-G allele carriers showed a better response in glucose, HOMA-IR, insulin, TC, LDL-C, and adiponectin levels than G-allele carriers after intervention. In addition, non-G alelle carriers had higher adiponectin levels than G allele carriers.

Table 1
Anthropometric parameters and blood pressure (mean ± SD)

Parameters	N = 83					
	CC (n = 48)			CG + GG (n = 35)		
	Basal	3 mo	*P*-value time genotype genotype × time	Basal	3 mo	*P*-value time genotype genotype × time
BMI	34.2 ± 2.1	33.7 ± 4.2*	0.005 0.42 0.01	34 ± 5.3	33.6 ± 4.9*	0.004 0.37 0.01
Weight (kg)	86.7 ± 9.6	83.3 ± 9*	0.004 0.41 0.001	86.9 ± 9.1	83.4 ± 6.1*	0.006 0.39 0.002
Fat mass (kg)	37.6 ± 11.2	35.3 ± 8.1*	0.005 0.36 0.001	37.8 ± 8.0	35.8 ± 7.1*	0.005 0.46 0.002
WC (cm)	106.5 ± 15.1	101.1 ± 8.3*	0.02 0.39 0.02	108.2 ± 6.1	103.1 ± 8*	0.03 0.54 0.02
SBP (mm Hg)	129.8 ± 8.2	124.1 ± 4.1*	0.02 0.21 0.04	128.9 ± 7.1	123.9 ±6.2*	0.03 0.34 *P* = 0.04
DBP (mm Hg)	80.1 ±7.1	82.4 ± 3.1	0.49 0.56 0.63	79.9 ± 6.1	80.9 ± 7.1	0.50 0.61 0.68

BMI, body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.
No statistical differences between genotype groups.
*Statistical differences $P < 0.05$, in each genotype group (BMI, weight, fat mass, WC, SBP).

Table 2
Biochemical parameters (mean ± SD)

	Diet I (N = 83)					
	CC (n = 48)			CG + GG (n = 35)		
	Basal	3 mo	*P*-value time genotype genotype × time	Basal	3 mo	*P*-value time genotype genotype × time
Glucose (mg/dL)	101.3 ± 10.1	95.1 ± 7.1*	0.02 0.53 0.03	98.9 ± 9	95.9 ± 7	0.15 0.56 0.27
Total cholesterol (mg/dL)	202.9 ± 8.7	187.7 ± 11.2*	0.01 0.33 0.02	199.9 ± 8.3	195.5 ± 9.2	0.44 0.51 0.12
LDL cholesterol (mg/dL)	133.1 ± 9.3	118.3 ± 7.9*	0.04 0.36 0.03	130.3 ± 8.1	125.9 ± 9.9	0.61 0.89 0.35
HDL cholesterol (mg/dL)	50.1 ± 4.1	49.9 ± 5	0.44 0.70 0.67	51.2 ± 5	52 ± 5.1	0.21 0.67 0.55
Triacylglycerols (mg/dL)	116.6 ± 18.1	115.2 ± 13.2	0.28 0.61 0.32	117.1 ± 13.2	16.5 ± 23.2	0.22 0.61 0.21
Insulin (mUI/L)	15.3 ± 7	11.3 ± 5*	0.009 0.11 0.02	15.9 ± 6	15.2 ± 6.1	0.18 0.39 0.31
HOMA-IR	4 ± 2	2.4 ± 1.8*	0.03 0.13 0.04	4.1 ± 2.1	3.9 ± 2.4	0.15 0.36 0.37

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.
No statistical differences between genotype groups.
*Statistical differences $P < 0.05$, in each genotype group (glucose, total cholesterol, LDL cholesterol, insulin, [|]HOMA-IR).

Some meta-analysis have evaluated the association between this SNP on the *ADIPOQ* gene and diabetes mellitus, obesity, and MetS [22,23]; however, there is a lack of information about the influence of weight loss on this association in interventional designs. In a recent analysis of the Finnish Diabetes Prevention Program [11], a randomized controlled multicenter study with a dietary intervention of reduction in the intake of total fat <30%, saturated fat to<10% of daily energy, and an increase of dietary fiber to ≥15 g/1000 kcal, the authors showed that the rs266729 G allele was associated with higher weight after a 4-y follow-up and C allele was associated with an increased risk for developing type 2 diabetes mellitus. It appears that the G allele, associated with higher posttreatment body weight and lower diabetes mellitus, of this *ADIPOQ* variant on metabolic parameters is independent of its effect on body weight, as our findings show.

An interventional study [12] with sibutramine resulted in significantly greater reduction of body weight for *ADIPOQ* rs266729 CC genotype compared with the placebo group during a 12-wk intervention trial. This study included non-Caucasian patients—44 in the placebo group and 87 in the sibutramine group with 10 mg daily. The potential mechanisms to explain the dependency of the sibutramine effect between this genetic variant and body weight remain unknown. *ADIPOQ* encodes adiponectin expressed exclusively in both brown and fat adipose tissues [24]. Recent findings have indicated that G allele alters the sequence for one transcriptional stimulatory protein binding site and reduces adiponectin promoter activity [3]. One study has demonstrated that there was an association between adiponectin and adipose tissue mass and have suggested that adiponectin secretion and circulating levels were inversely proportional to body fat [25]. It is possible that an

Table 3
Serum adipokine levels (mean ± SD)

	Diet I (N = 83)					
	CC (n = 48)			CG + GG (n = 45)		
	Basal	3 mo	*P*-value time genotype genotype × time	Basal	3 mo	*P*-value time genotype genotype × timee
Resistin (ng/dL)	4 ± 1.9	4.2 ± 3	0.75 0.89 0.26	3.9 ± 2	3.7 ± 1.8	0.55 0.69 0.19
Adiponectin (ng/dL)	28.7 ± 5.1	39.1 ± 4$*	0.001 0.16 0.03	18.5 ± 5[†]	17.2 ± 4.1[†]	0.61 0.80 0.23
Leptin (ng/dL)	92.3 ± 20.6	75.1 ± 10.5*	0.03 0.18 0.01	84.1 ± 13.1	64.1 ± 9*	0.02 0.21 0.03

*Statistical differences $P < 0.05$, in each genotype group (adiponectin, leptin).
[†]Statistical differences $P < 0.05$ between genotype group (adiponectin).

unknown molecular mechanism relates this genetic variant of the *ADIPOQ* gene with adipogenesis.

One interventional study of 60 extremely obese patients who were evaluated 32 mo after bariatric surgery (Roux Y gastroenterostomy) [13] demonstrated that individuals with C allele were more prone to show a reduction in LDL-C levels after surgery (−43%) than G allele carriers (−18%). The decrease in LDL-C in this study was higher than our findings, possibly owing to greater weight loss after bariatric surgery (35%) than in our work with nutritional intervention (4%). However, the result was in the same direction as patients carrying the G allele did not demonstrate decreases in LDL-C levels. Weight loss was similar in both genotypes after bariatric surgery, as in our study, and similarly there were no genotype differences in the modifications of resistin and leptin levels.

Another hypothesis that might explain this association is a potential gene–nutrient interaction. Fergusson et al. [26] demonstrated that G allele for this SNP was identified as having degrees of insulin resistance, as measured by HOMA-IR, and was highly responsive to differences in plasma saturated fatty acids. In another study [27], a gene–nutrient interaction was found between the rs266729 variant of the *ADIPOQ* gene and the percentage of energy derived from fat in the diet for the development of obesity. With previous findings, we can speculate that it is indeed likely that fatty acids modulate the involvement of the adiponectin gene and its receptors in downstream pathways. In the present study, we manipulated dietary fatty acids with a Mediterranean-type diet, which might explain our relevant results.

The strength of the present study was in its design as an interventional controlled trial with high adherence and a practical relevance to the general population. Limitations included the recruitment of the obese individuals without established cardiovascular disease. We only analysed one SNP of the *ADIPOQ* gene, so other genetic variants might be related to metabolic parameters. Further, there were many uncontrolled factors that could influence the results (epigenetic, hormonal status, and level of physical activity). Also, the lack of a control group without diet might be a bias. Finally, the self-reported dietary intake was not reliable and might include bias of under- or overreporting.

Conclusion

The CC genotype of *ADIPOQ* gene variant (rs266729) is associated with increases in adiponectin levels and decreases of LDL-C, insulin, and HOMA-IR after weight loss. In addition G allele carriers demonstrated lower levels of adiponectin than non-G allele carriers.

References

[1] Yang WS, Chuang LM. Human genetics of adiponectin in the metabolic syndrome. J Mol Med 2006;84:112–21.

[2] Takahashi M, Arita Y, Yamagata K, et al. Genomic structure and mutations in adipose-specific gene, adiponectin. Int J Obes Relat Metab Disord 2000;24:861–8.

[3] Gu HF. Biomarkers of adiponectin: plasma protein variation and genomic DNA polymorphisms. Biomark Insights 2009;4:123–33.

[4] Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes 2006;55:375–84.

[5] Bouatia-Naji N, Meyre D, Lobbens S, Séron K, Fumeron F, Balkau B, et al. ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. Diabetes 2006;55:545–50.

[6] Yang M, Qiu CC, Chen W, Xu LL, Yu M, Xiang HD. Identification of a regulatory single nucleotide polymorphism in the adiponectin (APM1) gene associated with type 2 diabetes in Han nationality. Biomed Environ Sci 2008;21:454–9.

[7] Rasmussen-Torvik LJ, Pankow JS, Jr Jacobs DR, Steinberger J, Moran A, Sinaiko AR. The association of SNPs in ADIPOQ, ADIPOR1, and ADIPOR2 with insulin sensitivity in a cohort of adolescents and their parents. Hum Genet 2009;125:21–8.

[8] Wu LS, Hsieh CH, Pei D, Hung YJ, Kuo SW, Lin E. Association and interaction analyses of genetic variants in ADIPOQ, ENPP1, GHSR, PPARgamma and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes. Nephrol Dial Transplant 2009;24:3360–6.

[9] Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001;86:3815–9.

[10] Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–10.

[11] Siitonen N, Pulkkinen L, Lindström J, Kolehmainen M, Eriksson JG, Venojärvi M, et al. Association of ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin concentrations: the Finnish Diabetes Prevention Study. BMC Med Genet 2011;12:5.

[12] Hsiao TJ, Wu LS, Huang SY, Lin E. A common variant in the adiponectin gene on weight loss and body composition under sibutramine therapy in obesity. Clin Pharmacol 2010;2:105–10.

[13] Gasparotto AS, Borges DO, Zandoná MR, Ramos MJ, Meihnardt NG, Mattevi VS. Adiponectin promoter polymorphisms are predictors of lipid profile improvement after bariatric surgery. Genet Mol Biol 2017;40:736–42.

[14] Mataix J, Mañas M. Food composition tables. Granada, Spain: Editorial University of Granada; 2003.

[15] Duart MJ, Arroyo CO, Moreno JL. Validation of an insulin model for the reactions in RIA. Clin Chem Lab Med 2002;40:1161–7.

[16] Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–4.

[17] Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

[18] Pfutzner A, Langefeld M, Kunt T, Lobig M. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin Lab 2003;49:571–6.

[19] Suominen P. evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem 2004;50:219–21.

[20] Khan SS, Smith MS, Reda D, Suffredini AF, Mc Coy JP. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufactures. Cytometry B Clin Cytom 2004;61:35–9.

[21] Lukaski H, Johson PE. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985;41:810–7.

[22] Sun P, Liu L, Chen J, Chen Y, Shi L, Imam MU, et al. The polymorphism of rs266729 in adiponectin gene and type 2 diabetes mellitus: a meta-analysis. Medicine 2017;96:e8745.

[23] Zhou JM, Zhang M, Wang S, Wang BY, Han CY, Ren YC, et al. Association of the ADIPOQ rs2241766 and rs266729 polymorphisms with metabolic syndrome in the Chinese population: a meta-analysis. Biomed Environ Sci 2016;29:505–15.

[24] Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–92.

[25] Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin – a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2006;8:264–80.

[26] Ferguson JF, Phillips CM, Tierney AC, Pérez-Martínez P, Defoort C, Helal O, et al. Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. Am J Clin Nutr 2010;91:794–801.

[27] Santos JL, Boutin P, Verdich C, Holst C, Larsen LH, Toubro S, et al. Genotype-by-nutrient interactions assessed in European obese women. A case-only study. Eur J Nutr 2006;45:454–62.