Clinical Nutrition 38 (2019) 2632–2638

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Common polymorphism in the cannabinoid receptor gene type 2 (*CB2R*) rs3123554 are associated with metabolic changes after two different hypocaloric diets with different dietary fatty profiles

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ARTICLE INFO

Article history:
Received 5 May 2018
Accepted 18 November 2018

Keywords:
Cannabinoid receptor gene type 2
Lipid profile
Obesity
Hypocaloric diet
rs3123554

SUMMARY

Background: The role of *CB2R* gene variants on weight loss after a dietary intervention has been investigated in few studies.
Objective: We evaluate the effect of this genetic variant (rs3123554) of *CB2R* gene on cardiovascular risk factors and weight loss secondary to high monounsaturated fat vs a high polyunsaturated fat hypocaloric diets.
Design: A Caucasian population of 362 obese patients was enrolled. Patients were randomly allocated during 3 months to one of two diets (Diet P high polyunsaturated (PUFAs) fat hypocaloric diet vs, Diet M high monounsaturated (MUFAs) fat hypocaloric diet).
Results: In both genotype groups (GG vs GA+AA), body weight, body mass index (BMI), fat mass, waist circumference and systolic blood pressure decreased after diet P and M. Body weight, BMI, fat mass and waist circumference were higher in A allele carriers than non A allele carriers. The improvement of these parameters was higher in non A allele carriers than A allele carriers. In non A allele carriers with both diets, the decrease of total cholesterol, LDL-cholesterol, insulin and HOMA-IR was higher than A allele carriers after both diets. After diet P, triglyceride levels decrease in non A allele carriers.
Conclusion: Our data suggest that carriers of the minor allele of rs3123554 variant of *CB2R* gene lose less body weight during to different hypocaloric diets with different fatty acid. Moreover, non A-allele carriers showed a better response of LDL-cholesterol, HOMA-IR and insulin levels than A-carriers with both hypocaloric diets.

1. Introduction

In western countries, the prevalence of obesity and its related diseases such as metabolic syndrome, hypertension and dyslipidemia is growing and represent an important health-care problem [1]. This increase in the prevalence of obesity is due to environmental and genetic factors [2]. Environmental factors are the excessive calorie intake and diminished physical activity. In genetic factors topic, single nucleotide polymorphisms (SNPs) have been associated with obesity. These SNPs includes; cannabinoid receptors (CBRs), fat related obese gene (FTO), brain derived neutrophic factor (BDNF), melanocortin receptor subtype 4 (Mc4R) and so on [3].

In this scenario, the role of the endocannabinoid system is emerging as an important pathway. Cannabinoid receptors CB1R and CB2R belong to the family of G protein-coupled receptors, and bind exogenous ligands derived from *Cannabis Sativa* as well endogenous endocannabinoids. CB1R is mainly located in the brain: its role in eating behavior is well-established [4]. CB2R receptors are primarily expressed in peripheral tissues such as the immune system cells, and regulate the inflammatory response in various settings [5].

A single nucleotide polymorphism (SNP) rs1049353 of the *CB1R* gene resulting in the substitution of the G to A at nucleotide position 1359 in codon 435 (Thr), was reported as a common SNP in Caucasian population, with many metabolic implications [6]. Other common SNPs of *CB2R* gene has been described in *CB2R* gene, for

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https://doi.org/10.1016/j.clnu.2018.11.013
0261-5614/

example rs3123554. Ketterer et al. [7] have found that carriers of minor allele (A) of this SNP showed a lower body weight being accompanied by decreased body weight loss during lifestyle intervention. Moreover, the role of *CB2R* gene variants on weight loss after a dietary intervention has been investigated only in two studies [8,9]. In one study [8], non-A allele carriers had a better improvement in body weight, fat mass, insulin resistance and lipid profile than A allele carriers after weight loss secondary to a Mediterranean hypocaloric diet during 3 months. In a further study [9], the interaction of two hypocaloric diets (moderate in carbohydrate vs normal in carbohydrate levels) with the rs3123554 of *CB2R* gene have been evaluated in a randomized clinical trial. Carriers of the A allele lose less body weight during two different hypocaloric diets. The improvement of biochemical parameters were worse in A allele carriers than non-A allele carriers, too.

In this study, we evaluate the effect of this genetic variant (rs3123554) of *CB2R* gene on cardiovascular risk factors and weight loss secondary to high monounsaturated fat vs a high polyunsaturated fat hypocaloric diets.

2. Subjects and methods

2.1. Subjects and experimental design

We enrolled a total of 362 Caucasians subjects aged 25–65 years with a body mass index $\geq$30 kg/m^2. The recruitment of subjects was a consecutive method of sampling among patients sent from Primary Care Physicians. This study was realized according to the guidelines laid down in the Declaration of Helsinki. All participants signed informed consent to a protocol approved by the local ethical review boards.

Major exclusion criteria were: a diet during the 9 months prior to the study, unstable cardiovascular or cerebrovascular diseases, insufficient motivation as well as the use of any of these drugs; metformin, dipeptidyl type IV inhibitors drugs, thiazolidinedione, GLP-1 analogs, sGLT2 inhibitors, insulin, glucocorticoids, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs. The inclusion criteria were the following; body mass index $\geq$30 kg/m^2 and an adult age ranged from 25 to 65 years.

Venous blood specimens (15 ml) were collected in EDTA-treated tubes after a 10-h fast period. Basal glucose, insulin, insulin resistance as homeostasis model assessment (HOMA-IR), total cholesterol, LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration, serum adipokines (leptin, adiponectin and resistin) and inflammatory markers (IL-6, TNF-alpha and c-reactive protein (CRP) were measured within the start of the trial and repeated after 3 months of both hypocaloric diets. Anthropometric parameters (weight, height, waist circumference and fat mass by bioimpedance) and blood pressure were measured in both times, too. These measures were realized at same time of the day (morning). Genotype of *CB2R* receptor gene polymorphism was determined, too.

2.2. Dietary intervention

Patients were randomly allocated to one of two diets for a period of twelve weeks. The randomization was performed with a table of random numbers by blocks. The target percentage of energy derived from carbohydrate, fat and protein in the two diets were; Diet P (high polyunsaturated (PUFAs) fat hypocaloric diet, 45.7% of carbohydrates, 34.4% of lipids and 19.9% of proteins) and Diet M (high monounsaturated fat hypocaloric diet; 46.6% of carbohydrates, 34.1% of lipids and 19.2% of proteins). The distribution of fats in Diet P was; 21.8% of saturated fats, 55.5% of monounsaturated fats and 22.7% of polyunsaturated fats (7 g per day of w-6 fatty acids, 2 g per day of w-3 fatty acids and a ratio w6/w3 of 3.5). The distribution of fats in Diet M was; 21.7% of saturated fats, 67.5% of monounsaturated fats and 10.8% of polyunsaturated fats. The exercise program consisted of an aerobic exercise at least 3 times per week (60 min each) and the patient with a self-reported questionnaire recorded it. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day and all patients were screened for potential causes of noncompliance. A dietitian assessed this adherence each 14 days with a phone call in order to improve compliment of the calorie restriction and macronutrient distribution. Records were analysed with a computer-based data evaluation system. National composition food tables were used as reference [10].

2.3. Measurements

Body weight and waist circumference were measured in the morning before breakfast, at baseline and 3 months of follow-up. Height was measured at baseline. Body mass index was calculated as body weight in kilograms/(height ^{2} in meters). Waist circumference was measured in the narrowest diameter between xiphoid process and iliac crest. Electrical bioimpedance was used to measure body composition with an accuracy of 50 g [11]. Blood pressure was measured twice after a 10 min rest with a random zero mercury sphygmomanometer, and averaged (Omrom, LA,CA).

Insulin was determined by radio-immunoanalysis (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5mUI/L (normal range 0.5–30 mUI/L) [20], plasma glucose levels were measured by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California) and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values [12]. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA). HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula (LDL cholesterol = total cholesterol-HDL cholesterol-triglycerides/5) [13].

Leptin was determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) (Diagnostic Systems Laboratories, Inc., Texas, USA) with a CV% 3.5% [14]. Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a CV% 3.2% [15]. Adiponectin was measured by ELISA (R&D systems, Inc., Mineapolis, USA) (DRP300) with a CV% 3.8% [16].

Interleukin 6 and TNF alpha were measured by ELISA (R&D systems, Inc., Mineapolis, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12–12.5 pg/ml) and TNF-alpha (0.5–15.6 pg/ml) [17,18]. Finally, CRP was determined by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a CV% 2.8%

2.4. Genotyping CB2R gene

Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International ®, LA, CA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5′- ACGTTGGATGATTGTACCGAGGAGGGAACT-3′ and reverse 5′- ACGTTGGATGGAGACACGTATTCTAGTCCC-3′ in a 2 uL final volume (Termociclador Life Tecnologies, LA, CA). DNA was denaturated at 95 °C for 3 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 59.3 °C for 45 s). The PCR were run in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-

Rad®, Hercules, CA) with hot start Taq DNA polymerase Hardy Weimberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed counts. The variant was in Hardy Weimberg equilibrium ($p = 0.37$).

2.5. Statistical analysis

Sample size was calculated to detect differences over 3 kg in body weight loss with 90% power and 5% significance ($n = 170$, in each diet group). The Kolmogorov–Smirnov test was used to determine variable distribution. The results were expressed as average+/- standard deviation. Other variables were analyzed with ANOVA test (for normally distributed variable) or Kruskal–Wallis test (for non-normally-distributed variable. The covariates used was age, sex and BMI). A two-way repeated-measures ANOVA was used in order to test the effects of genotypes, time and interaction genotypes x time. The statistical analysis was performed for a dominant model and multiple testing correction was used (Bonferroni correction).Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A Chi square test was used to evaluate the Hardy–Weinberg equilibrium. A p-value <0.05 was considered significant.

3. Results

A total of 105 patients (29.0%) had the genotype *GG* and 247 (71.0%) subjects had the next genotypes; *GA* (174 patients, 46.9%) or *AA* (87 study subjects, 24.1%) (second group). The average age of the total group ($n = 362$) was 49.1 ± 8.1 years. Age was similar in both genotype groups (wild type GG vs. mutant type (GA+AA)) (48.8 ± 7.2 years vs 49.3 ± 6.9 years: ns). Gender distribution in the all group was 27.9% males and 72.1% females. Sex distribution was similar in both genotype groups, males (28.0% vs 27.3%: ns) and females (72.0% vs 72.7%:ns). Mean body mass index (BMI) was 36.4 ± 4.7 kg/m^2, BMI was higher in A allele carriers (36.1 ± 4.1 kg/m^2 vs 36.8 ± 4.7 kg/m^2:$p = 0.01$). All patients completed the 3-month follow-up period without drop-outs.

In the group of (Diet M) 177 subjects (50 GG genotype and 127 A allele carriers), basal evaluation of nutritional intake with a 3 days written food record showed a calorie intake of 2010.9 ± 316.3 kcal/day, a carbohydrate intake of 200.8 ± 30.1 g/day (43.5% of calories), a fat intake of 63.1 ± 11.2 g/day (33.8% of calories) and a protein intake of 77.0 ± 19.1 g/day (23.8% of calories). During the dietary intervention, these patients reached the recommendations of the diet M; 1456.8 calories per day (45.1% of carbohydrates, 33.9% of lipids and 21.0% of proteins). The distribution of dietary fats was; 20.5% of saturated fats, 67.6% of monounsaturated fats and 11.9% of polyunsaturated fats. Finally, physical activity was similar in both genotype groups (66.5 ± 44.1 min/week vs 70.5 ± 31.3 min/week:$p = 0.51$).

In the group of (Diet P) 185 subjects (55 GG genotype and 130 A allele carriers), basal evaluation of nutritional intake with a 3 days written food record showed a calorie intake of 1991.8 ± 413.8 kcal/day, a carbohydrate intake of 206.0 ± 48.9 g/day (43.7% of calories), a fat intake of 82.0 ± 17.3 g/day (36.1% of calories) and a protein intake of 88.1 ± 28.2 g/day (20.2% of calories). During the intervention, these subjects reached the recommendations of diet P; 1450.3 calories per day (45.1% of carbohydrates, 34.3% of lipids and 20.8% of proteins). The distribution of fats was; 20.5% of saturated fats, a 53.8% of monounsaturated fats and a 23.7% of polyunsaturated fats (6.9 g per day of w-6 fatty acids, 2.0 g per day of w-3 fatty acids and a ratio w6/w3 of 3.6). Physical activity was similar in both genotype groups (70.3 ± 51.3 min/week vs 69.9 ± 39.1 min/week:$p = 0.37$).

Adiposity parameters and blood pressure levels of obese subjects at baseline and at 12 weeks of intervention are reported in Table 1. In both genotype groups and with both hypocaloric diets, body weight, body mass index (BMI), fat mass, waist circumference and systolic blood pressure decreased. Before and after dietary intervention, body weight, BMI, fat mass and waist circumference were higher in A allele carriers than non A allele carriers.

Table 1 shows that after a dietary intervention with a high monounsaturated fat hypocaloric diet (Diet M), non A allele carriers showed a higher decrease in body weight −5.0 ± 1.2 kg (A allele group −3.9 ± 1.1 kg:$p = 0.02$), BMI -1.6 ± 0.2 kg/m^2 (A allele group −1.4 ± 0.5 kg/m^2:$p = 0.03$), fat mass −3.7 ± 0.9 kg (A allele group −2.3 ± 0.8 kg:$p = 0.02$) and waist circumference −3.5 ± 1.0 cm (A allele group −1,4 ± 1.1 cm:$p = 0.01$) than A allele carriers. In non A allele carriers, the decrease in systolic blood pressure was similar −7.0 ± 3.1 mmHg to A allele carriers −6.1 ± 2.4 mmHg (n.s).

After dietary intervention with high polyunsaturated fat hypocaloric diet, non A allele carriers showed a higher decrease in body weight −4.9 ± 1.1 kg (A allele group −3.2 ± 1.2 kg:$p = 0.02$), BMI -1.6 ± 0.2 kg/m^2 (A allele group −1.3 ± 0.1 kg/m^2:$p = 0.02$), fat mass −3.5 ± 1.0 kg (A allele group −2.1 ± 0.7 kg:$p = 0.01$) and waist circumference −3.3 ± 1.1 cm (A allele group −1.9 ± 0.8 cm:$p = 0.01$) than A allele carriers. In non A allele carriers, the decrease in systolic blood pressure were similar −6.8 ± 2.9 mmHg to A allele carriers −6.1 ± 2.2 mmHg (n.s).

No differences were detected among baseline and post-treatment values of metabolic parameters between both genotypes (Table 2). After dietary intervention with both diets, non A allele carriers showed a significant decrease of total cholesterol, LDL cholesterol, insulin and HOMA-IR.

In non A allele carriers after both diets, the decrease in total cholesterol levels was higher −12.1 ± 2.3 mg/dl (A allele carriers −1.1 ± 1.3 mg/dl:$p = 0.01$ after Diet M) and −7.1 ± 1.3 mg/dl (A allele carriers −0.3 ± 1.1 mg/dl:$p = 0.004$ after Diet P) than A allele carriers. Changes in HDL-cholesterol did not show statistical differences. Moreover, the decrease in LDL-cholesterol levels was higher −12.1 ± 2.1 mg/dl (A allele carriers −1.2 ± 1.0 mg/dl:$p = 0.01$ after Diet M) and −7.3 ± 1.2 mg/dl (A allele carriers −3.9 ± 1.3 mg/dl:$p = 0.005$ after Diet P) than A allele carriers. Similarly, the decrease of insulin levels in non-A allele carriers after both diets was higher −2.3 ± 1.1 mUI/L (in A allele −0.8 ± 0.9 mUI/L:$p = 0.02$ after Diet M) and −3.1 ± 1.0 mUI/L (A allele carriers −0.2 ± 0.7 mUI/L:$p = 0.01$ after Diet P) than A allele carriers. Finally, the decrease of HOMA-IR levels was higher in non A allele carriers −0.9 ± 0.1 units (A allele group −0.1 ± 0.2:$p = 0.01$ after Diet I) and −1.2 ± 0.8 units (A allele carriers −0.2 ± 0.5 mg/dl:$p = 0.03$ after Diet II) than A allele carriers, too. After diet P, triglyceride levels decrease in non A allele carriers.

Table 3 shows levels of adipokines and inflammatory markers. Adipokines and inflammatory levels did not show statistical between both genotypes. Leptin levels decrease in both genotypes after diet M and P (Table 3). Changes of IL6, TNF-alpha, resistin and adiponectin were not statistical differences.

4. Discussion

In our study, we were able to show an association of the A allele of SNP rs3123554 within the *CNR2* gene with higher body mass index and other anthropometric parameters. In addition, non A-allele carriers showed a better response of LDL-cholesterol, HOMA-IR and insulin levels than A-carriers with both hypocaloric diets.

As obesity is a major health problem and a major risk factor, one main target of lifestyle interventions is loss of body weight. Aiming to realize personalized medicine, gene-environment interactions

Table 1
Adiposity parameters and blood pressure (mean ± SD).

Characteristics	Rs3123554											
	Diet M n = 177						Diet P n = 185					
	GG			GA+AA			GG			GA+AA		
	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time
BMI	37.3 ± 2.1	35.7 ± 5.0*	P = 0.007 P = 0.42 P = 0.03	37.7 ± 5.0[a]	36.0 ± 4.1*,[a]	P = 0.008 P = 0.41 P = 0.01	37.0 ± 4.0	35.4 ± 4.1*	P = 0.008 P = 0.36 P = 0.02	38.0 ± 5.2[a]	36.7 ± 4.1*,[a]	P = 0.01 P = 0.39 P = 0.03
Weight (kg)	95.6 ± 11.2	90.6 ± 10.1$	P = 0.005 P = 0.41 P = 0.003	97.5 ± 9.0[a]	93.3 ± 8.1$,[a]	P = 0.004 P = 0.38 P = 0.002	95.7 ± 10.1	90.8 ± 8.1$	P = 0.01 P = 0.43 P = 0.04	97.4 ± 7.0[a]	94.1 ± 7.0$,[a]	P = 0.008 P = 0.32 P = 0.02
Fat mass (kg)	40.5 ± 9.1	36.8 ± 9.0#	P = 0.009 P = 0.52 P = 0.003	41.7 ± 8.2[a]	39.4 ± 8.0#,[a]	P = 0.008 P = 0.41 P = 0.006	40.5 ± 6.2	37.0 ± 7.0#	P = 0.01 P = 0.56 P = 0.02	41.9 ± 7.0[a]	39.8 ± 6.1#,[a]	P = 0.01 P = 0.52 P = 0.03
WC (cm)	111.8 ± 6.0	108.3 ± 5.5&	P = 0.01 P = 0.35 P = 0.02	116.1 ± 6.0[a]	114.7 ± 8.2&,[a]	P = 0.02 P = 0.31 P = 0.03	112.9 ± 7.0	109.6 ± 7.1&	P = 0.01 P = 0.53 P = 0.02	117.7 ± 7.1[a]	115.9 ± 5.8&,[a]	P = 0.01 P = 0.49 P = 0.03
SBP (mmHg)	129.7 ± 4.2	122.8 ± 4.1**	P = 0.03 P = 0.37 P = 0.04	128.4 ± 7.1	122.3 ± 6.1**	P = 0.02 P = 0.41 P = 0.04	129.9 ± 7.0	123.1 ± 7.1**	P = 0.04 P = 0.61 P = 0.03	130.8 ± 5.1	124.4 ± 7.2**	P = 0.02 P = 0.60 P = 0.03
DBP (mmHg)	82.3 ± 7.1	78.9 ± 4.2	P = 0.45 P = 0.43 P = 0.60	80.2 ± 6.0	79.3 ± 7.4	P = 0.46 P = 0.41 P = 0.58	80.3 ± 5.9	79.8 ± 4.8	P = 0.61 P = 0.72 P = 0.23	81.1 ± 5.0	80.2 ± 4.1	P = 0.49 P = 0.57 P = 0.22

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference.
Statistical differences in Diet M (* BMI, $ Weight, #fat mass, & WC, **SBP) and diet P (* BMI, $ Weight, #fat mass, & WC, **SBP).
[a] Statistical differences between (GG vs GA+AA) genotype groups.

have become of more interest, and in this topic area, SNPs associated with obesity risk and/or metabolic parameters have been identified to influence the weight loss during dietary intervention [19] or bariatric intervention [20]. This prompted us to investigate the effects of this SNP within *CNR2* gene on weight loss and metabolic changes after a dietary intervention. Surprisingly, we found that minor allele of rs3123554 that was associated with higher body weight led to a significantly reduced loss of body weight and improvement in metabolic parameters during dietary intervention.

In the last years, the endocannabinoid system is well recognized for its relationship with inflammatory pathways [21] and increasing data has been reported highlighting the role of inflammation in the pathogenesis of cardiovascular risk in obese subjects [22,23]. As above-mention, CB2R has long been referred to as the peripheral cannabinoid receptor isoform. Moreover, there is now evidence of CB2R expression in different areas of the brain [24]. This presence at the central level of this receptor can explain the findings of our study relating this polymorphism with parameters of obesity. Firstly, CB2R activation in humans influences eating behavior [25], and secondly, CB2R is been positioned for regulation of endocannabinoid levels that could influence craving and reward behaviors through the relevant neuronal circuitry [26]. We could speculate that the different weight loss during dietary interventions is due to altered cerebral activity involved in self-directed learning and modification of the reward system.

On the other hand, the different metabolic response between the two genotypes deserves special attention. Surprisingly, we found that the A allele of rs31235554 variant led to a significantly reduced loss of body weight, fat mass, parameters related with carbohydrate metabolism (insulin levels and HOMA-IR) and lipid profile (LDL-cholesterol) after 3 months with two different hypocaloric diets. To explain this association, we can have several hypotheses, firstly a central hypothesis; Ketterer et al. [7] have found that carriers of A allele showed lower cerebral insulin sensitivity and it is necessary to bear in mind that cerebral insulin sensitivity rather facilities body weight loss during caloric restriction [23]. Taking to account that brain insulin sensitivity determines effectiveness of dietary intervention in terms of weight loss [27], we could postulate that the diminished cerebral insulin sensitivity in A allele carriers may be related for their inability to improve as much weight and metabolic parameters as non A allele carriers. Unfortunately, the determination in the clinical practice of insulin sensitivity is difficult, being able to use magnetoencephalography techniques in which a relationship between the decrease in Theta activity and insulin sensitivity has been demonstrated [28].

A second hypothesis could be called a peripheral hypothesis, since CB2R has been isolated in some target organs related with the control of metabolism like liver, adipose tissue, and skeletal muscle [29]. In obese subjects, all these tissues have been implied in insulin resistance. The peripheral effect of CB2R has been reported in two different studies. Two examples of these peripheral effects are the role of CB2R rs35761398 polymorphism on earlier age of menarche in subjects carrying the Q63 allele [30] and the role of rs3003336, rs2501431, rs2502992, rs2501432 SNPs of *CB2R* genes in the etiology of osteoporosis and suggest that CB2R may play an important role on bone density and osteoporosis in postmenopausal women [31].

Finally, the relationship between this SNP and weight loss through a dietetic intervention introduces us to personalized medicine, gene-environment, interactions have become of more interest, and in this context our findings must be taking to account during intervention programs of obesity [32].

Limitations of our study include the recruitment of our obese subjects from Primary Care without established cardiovascular disease. Second, there are many uncontrolled factors that could influence our results (epigenetic, hormonal status, other unknown environmental factors,…..). Third, the lack of a control group

Table 2
Biochemical parameters (mean ± SD).

Characteristics	Rs3123554											
	DietMI n = 177						Diet P n = 185					
	GG			GA+AA			GG			GA+AA		
	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time
Glucose (mg/dl)	101.5 ± 8.1	99.3 ± 8.0	P = 0.12 P = 0.71 P = 0.13	100.6 ± 9.0	99.7 ± 7.2	P = 0.13 P = 0.53 P = 0.21	102.1 ± 8.0	97.3 ± 7.1	P = 0.13 P = 0.39 P = 0.14	104.4 ± 9.2	100.4 ± 8.1	P = 0.18 P = 0.56 P = 0.23
Total cholesterol (mg/dl)	212.5 ± 10.1	200.7 ± 9.2 #	P = 0.01 P = 0.25 P = 0.02	207.2 ± 11.7	206.3 ± 9.2	P = 0.48 P = 0.59 P = 0.19	196.7 ± 7.1	189.1 ± 4.9#	P = 0.03 P = 0.31 P = 0.02	204.7 ± 8.1	195.4 ± 13.1	P = 0.44 P = 0.70 P = 0.21
LDL-cholesterol (mg/dl)	134.1 ± 10.3	122.0 ± 8.9*	P = 0.01 P = 0.32 P = 0.02	126.1 ± 20.1	127.7 ± 17.2	P = 0.68 P = 0.79 P = 0.48	121.4 ± 12.1	114.7 ± 10.1*	P = 0.03 P = 0.47 P = 0.04	128.3 ± 18.1	122.1 ± 12.1	P = 0.21 P = 0.62 P = 0.35
HDL-cholesterol (mg/dl)	53.4 ± 8.0	52.4 ± 5.1	P = 0.45 P = 0.72 P = 0.63	54.5 ± 6.0	50.8 ± 6.1	P = 0.30 P = 0.69 P = 0.62	49.9 ± 7.1	50.6 ± 8.2	P = 0.60 P = 0.81 P = 0.21	50.5 ± 7.0	50.3 ± 6.2	P = 0.71 P = 0.90 P = 0.21
Triglycerides (mg/dl)	118.6 ± 17.1	113.35 ± 16.2	P = 0.28 P = 0.68 P = 0.30	126.2 ± 13.2	127.5 ± 23.1	P = 0.21 P = 0.64 P = 0.21	129.8 ± 9.1	110.7 ± 10.0**	P = 0.03 P = 0.28 P = 0.04	137.7 ± 12.9	125.6 ± 13.1	P = 0.53 P = 0.64 P = 0.41
CRP (ng/dl)	6.1 ± 3.0	6.2 ± 2.9	P = 0.52 P = 0.64 P = 0.31	5.6 ± 3.1	5.8 ± 3.9	P = 0.50 P = 0.61 P = 0.32	5.3 ± 3.1	5.2 ± 2.9	P = 0.69 P = 0.78 P = 0.22	5.9 ± 3.2	5.8 ± 3.1	P = 0.63 P = 0.78 P = 0.29
Insulin (mUI/l)	12.2 ± 7.0	9.9 ± 5.0$	P = 0.007 P = 0.16 P = 0.01	13.6 ± 6.2	12.8 ± 5.1	P = 0.23 P = 0.44 P = 0.32	13.2 ± 7.0	10.1 ± 5.8$	P = 0.01 P = 0.23 P = 0.02	13.2 ± 6.1	13.1 ± 4.9	P = 0.17 P = 0.31 P = 0.17
HOMA-IR	2.9 ± 2.0	2.0 ± 1.2&	P = 0.02 P = 0.36 P = 0.03	3.4 ± 2.0	3.3 ± 2.3	P = 0.18 P = 0.41 P = 0.35	3.3 ± 1.3	2.1 ± 1.2&	P = 0.009 P = 0.19 P = 0.02	3.4 ± 1.9	3.2 ± 2.0	P = 0.16 P = 0.29 P = 0.21

CRP: C reactive protein. HOMA-IR (homeostasis model assessment). No statistical differences between genotype groups.
Statistical differences in Diet M (#total cholesterol *LDL-cholesterol, $ Insulin, &HOMA IR) and diet P #total cholesterol *LDL-cholesterol, $ Insulin, &HOMA IR, **triglycerides).

Table 3
Adipokines and cytokine levels (mean ± SD).

Characteristics	Rs3123554											
	Diet M n 177						Diet P n 185					
	GG			GA+AA			GG			GA+AA		
	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time	Basal	3 months	P Time Genotype Genotype x time
Resistin (ng/dl)	6.1 ± 2.1	5.5 ± 3.1	P = 0.61 P = 0.78 P = 0.22	6.8 ± 2.1	6.6 ± 3.0	P = 0.54 P = 0.68 P = 0.19	5.7 ± 1.9	5.6 ± 2.3	P = 0.70 P = 0.79 P = 0.24	5.4 ± 1.9	5.1 ± 2.2	P = 0.64 P = 0.78 P = 0.22
Adiponectin (ng/dl)	20.7 ± 8.1	25.5 ± 6.1	P = 0.67 P = 0.80 P = 0.24	18.5 ± 5.0	23.2 ± 6.1	P = 0.60 P = 0.81 P = 0.24	27.9 ± 6.9	30.9 ± 5.0	P = 0.65 P = 0.83 P = 0.38	29.5 ± 8.1	31.7 ± 6.2	P = 0.70 P = 0.81 P = 0.31
Leptin (ng/dl)	71.1 ± 17.6	56.3 ± 9.5*	P = 0.03 P = 0.20 P = 0.02	84.2 ± 10.1	65.7 ± 9.1*	P = 0.02 P = 0.20 P = 0.03	67.6 ± 13.1	56.8 ± 10.1*	P = 0.02 P = 0.21 P = 0.03	97.8 ± 13.8	63.1 ± 11.0*	P = 0.02 P = 0.20 P = 0.034
IL6 (ng/dl)	1.9 ± 1.3	2.1 ± 1.4	P = 0.16 P = 0.19 P = 0.13	2.1 ± 1.1	2.0 ± 1.0	P = 0.31 P = 0.46 P = 0.12	2.1 ± 2.0	1.9 ± 1.7	P = 0.31 P = 0.46 P = 0.12	2.2 ± 1.8	2.1 ± 1.1	P = 0.65 P = 0.71 P = 0.30
TNFalpha (ng/dl)	13.2 ± 4.1	12.3 ± .4.4	P = 0.31 P = 0.57 P = 0.46	8.7 ± 3.3	9.8 ± 1.3	P = 0.64 P = 0.71 P = 0.29	10.6 ± 3.9	11.2 ± 4.5	P = 0.50 P = 0.66 P = 0.31	9.5 ± 1.7	10.2 ± 1.8	P = 0.70 P = 0.80 P = 0.32

Statistical differences in Diet M(* leptin) and diet P (* leptin).

without a dietary intervention might be a bias. Finally, the self-reported dietary intake is not reliable and patients could under- or over-estimate dietary intake.

In conclusion, our data suggest that carriers of the minor allele of rs3123554 variant of *CB2R* gene lose less body weight during to different hypocaloric diets with different fatty acid. Moreover, non A-allele carriers showed a better response of LDL-cholesterol, HOMA-IR and insulin levels than A-carriers with both hypocaloric diets.

DA de Luis and R Aller designed the study and realized statistical analysis.O Izaola realized anthropometric evaluation and control f dietary intake. D Primo realized biochemical evaluation and genotype.

Compliance with ethical standards

No funding.

Conflict of interest

DA de Luis declares that he has no conflict of interest.
D Primo declares that he has no conflict of interest.
R Aller declares that she has no conflict of interest.
O Izaola declares that she has no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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