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Role of G1359A polymorphism of the cannabinoid receptor gene on weight loss and adipocytokines levels after two different hypocaloric diets☆

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Abstract

Background: A silent intragenic polymorphism (1359 G/A) of the cannabinoid receptor 1 gene resulting in the substitution of the G to A at nucleotide position 1359 in codon 435 (Thr) was reported as a common polymorphism in Caucasian populations. Intervention studies with this polymorphism have not been realized.
Objective: We decide to investigate the role of missense polymorphism (G1359A) of cannabinoid receptor 1 gene on adipocytokines response and weight loss secondary to a low-fat versus a low-carbohydrate diet in obese patients.
Design: A population of 249 patients was analyzed. A nutritional evaluation was performed at the beginning and at the end of a 3-month period in which subjects received one of two diets (diet I: low fat vs. diet II: low carbohydrate).
Results: One hundred forty three patients (57.4%) had the genotype *G1359G* (wild-type group), and 106 (42.6%) patients had *G1359A* (92 patients, or 36.9%) or *A1359A* (14 patients, or 5.6%; mutant-type group). With both diets in wild-type and mutant-type groups, body mass index (BMI), weight, fat mass, waist circumference and systolic blood pressure levels decreased. With both diets and in wild-type group, glucose, total cholesterol and insulin levels and homeostasis model assessment test score decreased. No metabolic effects were observed in mutant-type group. Leptin levels decreased significantly in the wild-type group with both diets (diet I: 10.8% vs. diet II: 28.9%; $P<.05$).
Conclusion: The novel finding of this study is the lack of metabolic improvement of the mutant-type groups G1359A and A1359A after weight loss with both diets. Decrease in leptin level was higher with low-carbohydrate diet than low-fat diet.

Keywords: Adipocytokines; Cannabinoid receptor gene; Low-fat diet; Low-carbohydrate diet; Obesity

1. Introduction

Hypocaloric diets are known to be an effective treatment for obese patients and patients with metabolic syndrome [1]. However, obesity has multiple causes and is determined by the interaction between genetic and environmental factors.

In this scenario, the important role played by endocannabinoid system is emerging: it controls food intake, energy balance and lipid and glucose metabolism through both central and peripheral effect stimulates lipogenesis and fat accumulation. For example, herbal *Cannabis sativa* (marijuana) has been known to have many psychoactive effects on humans including increases in body weight [2]. The endogenous cannabinoid system mediates and is considered both functionally and anatomically [3,4] to be an important modulator of normal human brain behavior. This system consists of endogenous ligands anandamide and 2-arachidonoylglycerol and two types of G-protein-coupled cannabinoid receptors: cannabinoid type-1 receptor (CNR1), located in several brain areas and in a variety of peripheral tissues including adipose tissue, and CB2, present in the immune system [5]. A greater insight into the endocannabinoid system has been derived from studies in animals with a genetic deletion of the CB1 receptor which have a lean phenotype and are resistant to diet-induced obesity and the associated insulin resistance induced by a high palatable high-fat diet [6].

The role of genetic variation has been mentioned previously. For example, a silent intragenic biallelic polymorphism (1359 G/A) of the *CB1* gene resulting in the substitution of the G to A at nucleotide position 1359 in codon 435 (Thr) was reported as a common polymorphism in the German population [7], reaching frequencies of 24%–32% for the allele (A).

Considering the evidence that endogenous cannabinoid system plays a role in the metabolic aspects of body weight and feeding

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behavior [8] and that long-term maintenance of body weight is determined by balance between energy intake and expenditure, we decide to investigate the role of missense polymorphism (G1359A) of CB1 receptor gene on adipocytokines response and weight loss secondary to a low-fat versus a low-carbohydrate diet in obese patients.

2. Subjects and methods

2.1. Subjects

A population of 249 obese patients was analyzed in a prospective way. These patients were studied in a Nutrition Clinic Unit and signed an informed consent form. Exclusion criteria included history of cardiovascular disease or stroke during the previous 12 months, total cholesterol >275 mg/dl, triglycerides >350 mg/dl, blood pressure >140/90 mm Hg, fasting plasma glucose >110 mg/dl as well as the use of sulfonylurea, dipeptidyl type IV inhibitors drugs, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Hospital Rio Hortega Committee number 2/2008. Written informed consent was obtained from all subjects/patients.

2.2. Procedure

Patients were randomly allocated to one of two diets for a period of 3 months. Diet I was low in fat and provided 1500 kcal/day (53% carbohydrates, 20% proteins and 27% fats). Diet II was low in carbohydrate and provided 1507 kcal/day (38% carbohydrates, 26% proteins and 36% fats). The exercise program consisted of an aerobic exercise at least three times per week (60 min each).

Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, insulin resistance (homeostasis model assessment, or HOMA), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides blood and adipocytokines [leptin, adiponectin, resistin, tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6)] levels were measured at basal time and at the end of the 3 months on hypocaloric diet. A tetrapolar bioimpedance, an indirect calorimetry and a prospective serial assessment of nutritional intake with 3-day written food records were performed. Genotype of CNR1 receptor gene polymorphism was studied.

3. Genotyping of CNR1 gene polymorphism

DNA extraction was performed with a lysate of blood cells (Geneall, Seoul, Korea). Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International, LA, CA, USA). Polymerase chain reaction was carried out with 50 ng of genomic DNA, 0.5 μl of each oligonucleotide primer (primer forward: 5′-TTC ACA GGG CCG CAG AAA G-3′ and reverse 5′-GAG GCA TCA GGC TCA CAG AG-3′) and 0.25 μl of each probes (wild probe: 5′-Fam-ATC AAG AGC ACG GTC AAG ATT GCC-BHQ-1-3′ and mutant probe: 5′-Texas red- ATC AAG AGC ACA GTC AAG ATT GCC -BHQ-1-3′) in a 25-μl final volume (Termociclador iCycler IQ; Bio-Rad, Hercules, CA, USA). DNA was denatured at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s and annealing at 59.3° for 45 s. Polymerase chain reactions were run in a 25-μl final volume containing 12.5 μl of IQTM Supermix (Bio-Rad) with hot start Taq DNA polymerase. Hardy–Weinberger equilibrium was assessed.

3.1. Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2; Beckman Instruments, Fullerton, CA, USA). Insulin was measured by radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA, USA), with a sensitivity of 0.5 mUI/l (normal range, 0.5–30 mUI/l) [8], and the HOMA for insulin sensitivity was calculated using these values [9]. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0–7 mg/dl and analytical sensitivity 0.5 mg/dl [10].

Serum total cholesterol and triglyceride concentrations were determined by an enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), whereas HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate–magnesium. LDL cholesterol was calculated using Friedewald formula.

3.2. Adipocytokines

Resistin was measured by enzyme-linked immunosorbent assay (ELISA; Biovendor Laboratory, Inc., Brno, Czech Republic), with a sensitivity of 0.2 ng/ml and normal range of 4–12 ng/ml [11]. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA), with a sensitivity of 0.05 ng/ml and a normal range of 10–100 ng/ml [12]. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN), with a sensitivity of 0.246 ng/ml and a normal range of 8.65–21.43 ng/ml [13]. IL-6 and TNF-α were measured by ELISA (R&D Systems, Inc.), with a sensitivity of 0.7 and 0.5 pg/ml, respectively. Normal values of IL-6 was 1.12–12.5 pg/ml and those of TNF-α was 0.5–15.6 pg/ml [14,15].

3.3. Indirect calorimetry

In order to measure resting energy expenditure, subjects were admitted to a metabolic ward. After a 12-h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20-min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture) fitted with a face mask (MedGem; Health Tech, Golden, USA), with a coefficient of variation of 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated [16].

3.4. Anthropometric measurements

Body weight was measured to an accuracy of 0.5 kg, and body mass index (BMI) was computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g [17]. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer and was averaged.

3.5. Dietary intake and habits

Patients received prospective serial assessment of nutritional intake with 3-day written food records. All enrolled subjects received instruction to record their daily dietary intake for 3 days including a

Table 1
Changes in anthropometric variables (mean±S.D.)

Characteristics	Diet I				Diet II			
	G1359G		G1359A or A1359A		G1359G		G1359A or A1359A	
	0 time at 3 months		0 time at 3 months		0 time at 3 months		0 time at 3 months	
BMI (kg/m^2)	35.1±5.3	33.7±5.5*	35.4±6.5	33.9±6.1*	36.2±7.8	34.5±7.6*	35.7±5.3	34.4±5.4*
Weight (kg)	92.4±16.5	88.8±16.7*	92.1±17	88.3±16.6*	94.4±21	90.3±20*	90.7±16.3	86.4±15.7*
FFM (kg)	53.6±15.1	51.9±14.6	52.3±11	51.2±12	52.1±15.6	50.7±14.9	50.4±12.8	49.8±9.3
Fat mass (kg)	38.2±10.9	36.1±11.3*	38.2±10.1	37.1±12.1*	40.1±10.1	36.9±11.7*	40.8±8.7	38.6±9.3*
WC (cm)	107.1±14	102.4±14.1*	106.8±16	104.1±16*	108.8±15	103.1±15*	108.7±13	103.6±12.9*
WHR	0.90±0.07	0.88±0.06	0.89±0.1	0.89±0.1	0.9±0.08	0.88±0.09	0.92±0.1	0.90±0.1
SBP (mm Hg)	137.2±18	123±12.9*	137.2±14	125.3±12*	143.7±19	126.4±16*	144.3±18	118.1±12.5*
DBP (mm Hg)	84.5±9.1	81.4±6.8	83.1±6.5	83.4±14.8	81.2±11	81.7±18.4	82.9±6.8	77.2±24.4
RMR (kcal/day)	2201±723	2375±528	2225±422	2405±514	2184±358	2074±439	2270±422	2392±656
VO_2 c. (ml/min)	325.1±63	363.8±60	324.9±68	350.6±71	309.5±81	295.5±61	299.7±57.3	343.4±80

DBP, diastolic blood pressure; FFM, fat-free mass; RMR, resting metabolic rate; SBP, systolic blood pressure; VO_2 c., oxygen consumption; WHR, waist-to-hip ratio; WC, waist circumference. No statistical differences between genotypes in each diet or in different diet groups were found.

* $P < .05$, in each genotype group with basal values.

weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference [18]. Regular aerobic physical activity (walking was allowed, no other exercises) was maintained during the period study for at least three times per week (60 min each).

3.6. Statistical analysis

Sample size was calculated to detect differences of 2 kg in body weight, with 90% power and 5% significance (n=90, in each diet group). The results were expressed as average±S.D. The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed Student's t test. Analysis of variance or analysis of covariance test to compare data for response between diets or between genotypes has been used. Nonparametric variables were analyzed with the Wilcoxon test. Qualitative variables were analyzed using the χ^2 test, with Yates correction as necessary, and Fisher's test. The statistical analysis was performed for the combined *G1359A* and *A1359A* as a group and wild-type G1359G as second group, with a dominant model. P value <.05 was considered statistically significative.

4. Results

Two hundred ninety-four patients gave informed consent and were enrolled in the study. The mean age was 45.9±14.8 years, and the mean BMI was 34.8±4.1 kg/m^2, with 56 men (22.5%) and 193 women (77.5%).

All patients completed the 3-month follow-up, with an average weight loss of 3.3±2.8 kg (3.5%). One hundred forty-three patients (57.4%) had the genotype *G1359G* (wild-type group), and 106 (42.6%) patients had *G1359A* (92 patients, or 36.9%) or *A1359A* (14 patients, or 5.6%) (mutant-type group). Age was similar in both groups (wild-type group: 43.95±15.4 years vs. mutant-type group: 43.8±15.1 years; *ns*). Sex distribution was similar in both groups, male (23.8% vs. 20.8%) and female (76.2% vs. 79.2%).

The 122 subjects (68 wild type and 54 mutant type) treated with diet I basal assessment of nutritional intake with a 3-day written food record showed a calorie intake of 1870±701.6 kcal/day, a carbohydrate intake of 183.9±68.9 g/day (40% of calories), a fat intake of 90.2 ±37.9 g/day (39.9% of calories) and a protein intake of 95.9±22.6 g/day (20.1% of calories). During the intervention, these subjects reached the recommendations of diet I (low fat: 1628 kcal/day, with a 25.3% of the total calorie intakes as fats).

The 127 subjects (75 wild type and 52 mutant type) treated with diet II basal assessment of nutritional intake with a 3-day written food record showed a calorie intake of 1908±862.1 kcal/day, a carbohydrate intake of 195±58 g/day (41.3% of calories), a fat intake of 83.1±37.2 g/day (39.1% of calories) and a protein intake of 94.3±28.7 g/day (19.6% of calories). During the intervention, these patients reached the recommendations of diet II (low carbohydrate: 1578 kcal/day, with a 30.8% of total calorie intakes as carbohydrates).

Table 1 shows the differences in anthropometric variables. With the diet type I (low fat) and in wild-type and mutant-type groups, BMI, weight, fat mass, waist circumference and systolic blood pressure levels decreased. With the diet type II (low carbohydrate) and in both genotypes, BMI, weight, fat mass, waist circumference and systolic blood pressure levels decreased. There were no significant differences between the effects (on weight, BMI, waist circumference, fat mass and systolic blood pressure) of the different diets in either group. No differences were detected among basal and posttreatment values of anthropometric variables between both genotypes. The most relevant results were the improvement in anthropometric and blood pressure detected with both diets.

Table 2 shows the classic cardiovascular risk factors. With the diet type I (low fat) and in wild-type group, glucose, total cholesterol, triglyceride, insulin and HOMA score decreased. In the mutant-type group, no metabolic changes were observed after the treatment with the diet I.

With the diet type II (low carbohydrate) and in wild-type genotype, glucose, total cholesterol, LDL cholesterol and insulin levels and HOMA score decreased. There were no significant differences between the effects (on glucose, total cholesterol, insulin and HOMA) of the different diets in either group. No differences were detected among basal and posttreatment values of biochemical variables between both genotypes. The most relevant results were the lack of effect on biochemical parameters in mutant-type group with both diets. The biochemical improvement in wild-type group was similar with both diets.

Table 3 shows levels of adipocytokines. In the wild-type group on diet I (low fat), TNF-α and leptin levels decreased. In the wild-type group on diet II (low carbohydrate), leptin level decreased, too. In the mutant-type group, on diet I or II, values of all adipocytokines remained unchanged. There were significant differences between the effects on leptin levels of the different diets in either group. Leptin levels decreased significantly in the wild-type group with both diets (diet I 10.8% vs. diet II 28.9%; P<.05). No differences were detected

Table 2
Classical cardiovascular risk factors (mean±S.D.)

Characteristics	Diet I				Diet II			
	G1359G		G1359A or A1359A		G1359G		G1359A or A1359A	
	0 time at 3 months		0 time at 3 months		0 time at 3 months		0 time at 3 month	
Glucose (mg/dl)	100.6±16.3	94.5±12.7*	100.2±18	97.2±11.6	100.3±21	95±13.6*	102.3±12	99.8±10.6
Total ch. (mg/dl)	207.4±29	194.8±34*	201±29	194.7±36	196.7±38	186.4±36*	204.4±46.4	192.7±40.4
LDL-ch. (mg/dl)	125±34	118.7±46	124±38	118.7±30	119.7±39	104.1±38*	123.8±34	118.7±22
HDL-ch. (mg/dl)	49.4±14.6	48.3±12.5	55.4±16	52.5±12.8	56.3±13.1	56.2±19	57.5±18	55.3±12.6
TG (mg/dl)	154.1±70	124.4±42*	121.4±48	113±39	111.8±53	106.5±40	108.1±58	113.3±56
Insulin (mUI/l)	22.2±10.9	14.8±7.5*	16.4±11.8	13.3±5.9	19.5±14	13.1±6.6*	13.8±8.6	12.1±7.1
HOMA	6.2±8.1	3.4±1.7*	4.4±4.3	3.3±1.9	5.3±5.1	3.1±1.2*	3.1±2.1	2.8±1.6
CRP (mg/dl)	4.6±3.5	4.2±3.4	5.1±5.4	5.2±3.1	4.9±4.8	4.4±4.2	5.1±5.1	3.9±3.6

Chol, cholesterol; TG, triglycerides; HOMA, homeostasis model assessment. No statistical differences between genotypes in each diet or in different diet groups were found.
* $P < .05$, in each group with basal values.

among basal and posttreatment values of adipocytokines between both genotypes.

5. Discussion

The finding of this study is the association of the G1359A and A1359A CNR1 genotypes with a lack of improvement on glucose, total cholesterol and insulin, HOMA and leptin levels with both diets after weight loss.

We do not know how the A1359 allele may be associated with a lack of metabolic improvement after weight loss with two different hypocaloric diets. However, the literature supports the notion that endocannabinoid system is positioned for regulation of endocannabinoid levels that could influence craving and reward behaviors through the relevant neuronal circuitry and metabolic parameters [19]. This provide a link between the consequences of this polymorphism and the present epidemiological study indicating that the CB1 receptor G1359A polymorphism may be one risk factor for susceptibility to obesity and metabolic abnormalities. Novel peripheral mechanisms of action have been attributed to the endocannabinoid system through its ability to directly target adipocytes [20], skeletal muscle [21] and gastrointestinal tract [22]. Also, a marked down-regulation of the fatty acid amide hydrolase gene expression was found in the adipose tissue, suggesting that adipose tissue may be an important contributor to endocannabinoid metabolism, with unknown relations between this system and adipocytokines [23].

In our study, the prevalence of GA genotype was (36.9%), similar to that other studies: 43.5% [24], 19.6% [25] and 33.1% [26]. However, a lack of association between body mass index and this polymorphism is present in our results; this fact is in contrast with the association detected by Gazerro et al. [24] with single nucleotide polymorphism (SNP) G1359A of CB1 receptor, A3813A and A4895A SNPs of CNR1 receptor [27] and SNP G1422A of CNR1 receptor [28]. Perhaps, these different results could be explained by bias secondary to an unmeasured dietary intake. These previous studies would require composition analysis of the diet to determine whether dietary components could be responsible for the lipid profile modifications. In our study, dietary intake did not show basal statistical differences between groups, and two different hypocaloric diets have been used as an intervention therapy, and it was controlled during the protocol.

Ravinet et al. [6] found that CNR1 gene-deficient mice were lean and resistant to diet-induced obesity and showed reduced plasma insulin and leptin levels. In our patients, leptin and insulin levels with both diets and IL-6 with low-fat diet decreased secondary to weight loss in obese patients carrying the wild-type CB1 allele. This metabolic relationship between the polymorphism and metabolic parameters has been detected by other study. Alberle et al. [25] have shown that carriers of at least one A allele in CB1 lost more weight and reduced LDL cholesterol than wild-type patients. However, this study did not measure adipocytokines to explore these data, and the lipid changes were contradictory with ours. There are no clear reasons why we have not seen in the carriers of the mutations a better amelioration of biochemical parameters. Perhaps, during pathological states, as obesity, the levels of endocannabinoids in tissues change, and their effects vary from those of protective endogenous compounds to those of dysregulated signals. When moving to the clinic, the pleiotropic nature of endocannabinoid functions will require careful interpretation in the stage of the disorder and in the choice of patients for future treatments.

The theoretical explanation of this complex relationship with adipocytokines and metabolic parameters could be due by the own adipose tissue metabolism. Cannabinoids modulate the expression of several cellular target genes via the CB1 receptor-dependent pathway. In brown adipose tissue, cannabinoid antagonist treatment is able to stimulate the expression of genes favoring energy dissipation through mitochondrial heat production [29]. Other evidence of this interaction, which is the increase expression of adiponectin induced by CB1 antagonists, in vitro, in 3T3 F442A adipocytes [30] and in vivo obese mice [31], suggests a close

Table 3
Circulating adipocytokines (mean±S.D.)

	Diet I				Diet II			
	G1359G		G1359A or A1359A		G1359G		G1359A or A1359A	
	0 time at 3 months		0 time at 3 months		0 time at 3 months		0 time at 3 months	
IL-6 (pg/ml)	1.9±2.3	2.1±2.8	2.0±2.3	2.1±3.4	2.1±2.9	2.8±3.9	2.1±2.2	2.4±2.6
TNF-α (pg/ml)	6.6±4.4	5.9±3.7*	6.7±4.7	7.5±5.4	6.2±3.9	6.7±4.8	5.2±4.3	5.3±4.6
Adiponectin (ng/ml)	31.4±26.9	28.2±29.3	44.8±36	38.8±41	50.8±43.2	43.8±21.2	37.9±25	39±13.3
Resistin (ng/ml)	3.6±2.2	3.7±1.8	3.9±2.2	4.1±1.4	3.6±1.5	3.6±1.6	3.3±1.6	3.0±1.2
Leptin (ng/ml)	83.1±75	74.5±57*	89.5±90	75±70	96.9±64	68.1±60*	111±76	86.5±80

No statistical differences between genotypes in each diet or in different diet groups were found.
* $P< .05$, in each group with basal values.

relationship between CB1 receptor blockade and the production of this adipocyte-derived protein.

As we have shown, the beneficial effects on body fat reduction were different in subjects with wild type and in those with mutant type. The alterations of insulin sensitivity on response to weight loss observed with the A1354 variant could be explained with a hypothetical change in the lipolytic response of visceral fat and by adipocytokines, Leptin is yet another adipocytokine that has been implicated in glucose homeostasis. It is thought to have some role in regulating insulin sensitivity [32], and this could explain the different metabolic response in mutant-type and wild-type groups.

Recently, other relevant associations that could explain or results have been published. For example, Thanos et al. [33] have demonstrated that leptin receptor deficiency is associated with up-regulation of cannabinoid 1 receptors in limbic brain regions. In other animal model [34], a novel hormonal crosstalk between leptin and glucocorticoids that rapidly modulates synaptic excitation via endocannabinoid release in hypothalamus and provides a nutritional state-sensitive mechanism has been described. However, in this neural system, a lot of new findings appear constantly; for example, Monteleone et al. [35] suggest that the CNR1 1359 G/A and the fatty acid amide hydrolase cDNA 385C to A gene variants may contribute to the susceptibility to mood disorders.

In conclusion, the novel finding of this study is the lack of metabolic improvement of the mutant-type groups G1359A and A1359A after weight loss with both diets. Decrease in leptin level was higher with low-carbohydrate diet than low-fat diet. The presence of certain genotype may then be beneficial for obesity treatment. Further studies will be designed to elucidate this controversial area and to clarify the role of this polymorphism in metabolic response secondary to weight loss in obese patients and the relationship among adipocytokines, cardiovascular risk factors and G1359A polymorphism of the endocannabinoid system.

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