Journal of Diabetes and Its Complications 39 (2025) 109062

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: www.elsevier.com/locate/jdiacomp

Role of rs490683 variant in the promoter region of the ghrelin receptor gene on body weight and metabolic syndrome after a partial meal replacement hypocaloric diet

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ARTICLE INFO

Keywords:
rs490683
Partial meal replacement diet
GHSR gene
Body weight

ABSTRACT

Background and aims: Few studies have evaluated the effect of rs490683 on weight loss. The objective of our study was to evaluate the role of this variant of *GHSR* gene on body weight loss and cardiovascular risk factors secondary to a partial meal replacement (pMR) hypocaloric diet.

Methods: 96 individuals with a body mass index (BMI > 35 kg/m^2) were enrolled. Participants consumed a normocaloric, hyperproteic formula twice daily (12-w). Measurements were taken for body weight, BMI, fat mass, waist circumference, blood pressure, lipid profile, fasting insulin levels and HOMA-IR.

Results: The genotype was 70 patients (72.9 %) CC genotype, 19 patients(19.8 %) CG genotype, and 7 patients (7.3 %) GG genotype. The intake of calories, grams of carbohydrates, fats and proteins was higher at 12w in patients carrying the G allele. BMI (−3.5 ± 0.4 kg/m^2 vs −1.0 ± 0.2 kg/m^2 ($p = 0.01$)), body weight (−8.5 ± 1.0 kg vs −2.6 ± 1.1 kg ($p = 0.01$)), fat mass (−7.7 ± 0.3 kg vs −2.6 ± 0.2 kg ($p = 0.01$)), waist circumference (−7.2 ± 0.3 cm vs −2.9 ± 0.1 cm ($p = 0.01$)), glucose levels (−12.1 ± 1.4 mg/dl vs −3.1 ± 1.8 mg/dl, $p = 0.01$), insulin (−10.8 ± 1.2 UI/L vs −3.9 ± 1.1 UI/L, $p = 0.01$), HOMA-IR (−2.1 ± 1.0 units vs −0.58 ± 0.2 units, $p = 0.01$), CRP (−1.2 ± 0.1 mg/dl vs −0.7 ± 0.2 mg/dl, $p = 0.01$), triglycerides (−22.1 ± 4.1 mg/dl vs −5.1 ± 3.2 mg/dl, $p = 0.01$), total-cholesterol (−22.2 ± 1.3 mg/dl vs −8.8 ± 1.9 mg/dl, $p = 0.01$), LDL-cholesterol (−15.2 ± 1.1 mg/dl vs −4.7 ± 1.2 mg/dl, p = 0.01), and HDL-cholesterol (6.2 ± 0.4 mg/dl vs −2.9 ± 1.2 mg/dl, p = 0.01) modifications were better in non-G allele carriers. After intervention, the odds ratio (OR) of MS in non-carrier of G allele improved OR 0.48 (95%CI: 0.31–0.73; $p = 0.02$).

Conclusions: G allele of rs490683 have a deleterious effect on dietary restrictions, body weight and metabolic response after a pMR diet.

1. Introduction

The rs490683 polymorphism is located in the promoter region of the ghrelin receptor type 1a (*GHSR*) gene, which encodes the ghrelin receptor. Ghrelin, often referred to as the "hunger hormone," is a peptide hormone that plays a crucial role in regulating appetite, energy balance, and body weight.[1] The GHSR is a critical component in this regulatory pathway, influencing food intake and metabolic processes. Genetic variations in the promoter region of the *GHSR* gene, such as the rs490683 polymorphism, can alter the expression and functionality of the ghrelin receptor,[2,3] potentially impacting individual responses to nutritional interventions and weight management strategies.

The foundation of all therapeutic strategies for obesity encompasses a low-calorie diet combined with physical exercise, aiming to achieve a clinically meaningful weight reduction of at least 5–10 %. Achieving this level of body weight loss can mitigate the risk of metabolic syndrome (MS) and other cardiovascular risk factors. Evidence supports the efficacy of low-calorie diets with partial meal replacement (pMR) in facilitating weight loss. A noteworthy meta-analysis has demonstrated that pMR hypocaloric diets lead to a 7 % reduction in body weight, compared to a 3 % reduction achieved with conventional hypocaloric diets.[4] Our research team has evidenced the effectiveness of pMR diets in promoting weight loss[5] and enhancing certain cardiovascular risk factors.[6] Additionally, we have observed positive impacts on other health parameters,

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https://doi.org/10.1016/j.jdiacomp.2025.109062
Received 19 December 2024; Received in revised form 25 March 2025; Accepted 27 April 2025
Available online 29 April 2025

including bone mass and non-alcoholic fatty liver disease.[7]

Genetic variations within the ghrelin and ghrelin receptor genes have been extensively studied and linked to multiple aspects of metabolic health, including obesity, eating behaviour, appetite regulation, blood triglyceride concentrations, fasting insulin levels, and insulin resistance.[8,9] Of particular interest is one single nucleotide polymorphisms (SNPs) rs490683 located in the promoter region of the ghrelin receptor gene. This SNP has shown significant correlations with body mass index (BMI), the amount of weight loss achieved during dietary interventions, and insulin resistance.[8] Experimental research, utilizing luciferase assays to measure promoter activity in vitro, has reported differential activity of these ghrelin receptor promoters.[9] Such variation in promoter activity may result in reduced ghrelin signalling, potentially impacting the physiological regulation of hunger and metabolic processes. In other bariatric interventional study with Roux-en Y gastric bypass (RYGB),[10] weight loss patterns exhibited substantial variation when analyzed using an additive model for both ghrelin SNPs. Notably, individuals homozygous for the rs490683 CC genotype experienced the greatest degree of weight reduction. This suggests that the genetic makeup of ghrelin SNPs can significantly influence the effectiveness of weight loss interventions, with specific genotypes such as rs490683 CC playing a critical role in the extent of weight reduction achieved by patients.[9,10] These previous findings underscore the importance of considering genetic factors when evaluating weight loss outcomes and tailoring personalized treatment plans.

Considering the lack of evidence, the objective of our study was to evaluate the role of rs490683 genetic variant of *GHSR* gene on body weight loss and cardiovascular risk factors secondary to a pMR hypocaloric diet in Caucasian patients with obesity.

2. Methods and procedures

2.1. Patients and design

A total of 100 subjects with obesity stage II or higher, aged 30–60 years, volunteered to participate in this study. These individuals were referred by their primary care physicians to our Clinical Nutrition Unit with the goal of losing weight. Initially, 100 patients were referred, and 96 agreed to participate. The participants demonstrated stable body weight over the past 6 months, with a body mass index (BMI) $\geq$ 35 kg/m^2. They were selected without significant weight loss in the previous 6 months (<1 kg), as determined by their electronic medical records. A hypocaloric diet supplemented with a normocaloric hyperproteic formula was prescribed twice per day to these subjects, during 12 weeks (Table 1). Exclusion criteria included a history of cardiovascular events (heart attack or stroke), severe renal or hepatic dysfunction, active alcoholism, malignant tumours, pregnancy, and use of medications for hyperlipidemia, hyperuricemia, hypertension, and diabetes mellitus. All participants provided written informed consent, and the study protocol adhered to the Declaration of Helsinki and local institutional guidelines. The Ethics Committee approved the study (registration code HVUVA committee 2/2018).

Table 1
Quantities of energy and macronutrients in the partial meal replacement diet (comprising four intakes of natural food and two intakes of a normocaloric hyperproteic formula (Vegestart Complete®)).

Data	Oral diet plus formula	Diet alone	Normocaloric hyperproteic formula (200 ml per carton)
Calories(kcal)	1003.5	803.5	200
Dietary Fiber (g)	15.9	11.7	4.2
Proteins (g (% TCV))	64.4 (25 %)	59.0 (29.3 %)	15.4(31 %)
Fats (g(%TCV))	19.1 (16 %)	13.9 (14.6 %)	5.2(23 %)
Carbohydrates (g (%TCV))	151.6 (59 %)	130.6 (55.1 %)	21(42 %)

Normocaloric hyperproteic formula, VEGESTART complete® (%TCV: % Total Caloric Value), was included in the pMR diet at a rate of two bricks per day. The column labelled 'oral diet plus formula' represents the combined intake of the oral diet and the formula, while the column labelled 'normocaloric hyperproteic formula' indicates the intake of only one brick."

2.2. Nutritional intervention

This study was designed as a single-branch 12-week controlled intervention. Participants consumed natural foods at home, which they purchased themselves. The partial meal-replacement hypocaloric diet (pMR) was structured into six meals: breakfast, morning snack, lunch, afternoon snack, dinner, and an after-dinner snack. For lunch and dinner, participants replaced natural foods with a normocaloric hyperproteic formula (VEGESTART Complete®), provided by the researchers (Table 1). The rest of the 4 meals (breakfast, morning snack, afternoon snack and after-dinner snack) were made with natural foods. Throughout the study, a dietitian provided reinforcement via phone calls twice weekly to enhance adherence to the nutritional intervention. All participants reported their dietary intake over a 72-h period to estimate their daily caloric and macronutrient intake before and after the 12-week intervention. These dietary records, covering two weekdays and one weekend day, were analyzed using professional software (Dietsource®, Nestlé, Geneva, Switzerland). Physical activity was self-reported by the participants, who were instructed to engage in aerobic exercises—such as running, walking, and cycling—at least three times per week for 30 min each session.

2.2.1. Anthropometric parameters and clinical parameters

Data collection at the beginning of the study and after 12 weeks followed standardized procedures. Waist circumference was measured using a flexible tape measure (Omrom, LA, CA, USA) placed between the top of the iliac crest and the bottom rib. Height was measured in centimetres using a standard height scale (Omrom, LA, CA, USA). Body weight was recorded with subjects minimally clothed and barefoot, using digital scales (Omrom, LA, CA, USA). Two separate measurements were taken, and the average was used as the final value. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Total fat mass was measured using Bioelectrical Impedance Analysis with a precision of 5 g (EFG BIA 101 Anniversary, Akern, Italy), employing an alternating current of 0.8 mA at 50 kHz generated by a calibrated signal generator (EFG, Akern, Florence, Italy).[11]

Blood pressure readings were taken with a sphygmomanometer (Omrom, LA, CA, USA) after the subjects had been seated for 15 min. Three readings were taken for each patient, and the average of these readings was used.

Metabolic syndrome (MS) was defined according to the Adult Treatment Panel III (ATPIII) criteria.[12] Patients were diagnosed with MS if they met at least three of the following criteria: elevated fasting glucose or diabetes treatment, elevated triglycerides (>150 mg/dl) or dyslipidemia treatment, low HDL cholesterol (<40 mg/dl for males or < 50 mg/dl for females), elevated systolic or diastolic blood pressure (>130/80 mmHg or antihypertensive treatment), and increased waist circumference (>94 cm for males or > 80 cm for females).

2.3. Biochemical and genetic parameters

Biochemical measurements, including glucose, insulin, total cholesterol, HDL-cholesterol, triglycerides, and C-reactive protein (CRP) levels, were conducted using the COBAS INTEGRA 400 analyzer (Roche Diagnostic, Basel, Switzerland). LDL cholesterol was calculated using the Friedewald formula (LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5).[13] Insulin resistance was assessed using the homeostasis model assessment for insulin resistance (HOMA-IR) with the formula (glucose x insulin/22.5).[14]

Genomic DNA was extracted from oral mucosa cells using a

commercial kit (Applied Biosystems, Foster City, CA, USA). Genotyping of rs490683 was performed using the TaqMan® OpenArray™ Genotyping platform (Thermofisher, Pittsburgh, PA, USA). DNA samples were loaded using the AccuFill system, and DNA amplification was carried out on the QuantStudio 12 K Flex Real-Time qPCR instrument (Thermofisher, Pittsburgh, PA, USA). A final volume of 25 μl, containing 3.0 μl TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, CA, USA) and 3.0 μl of human DNA sample, was used and amplified following the manufacturer's instructions. During the polymerase chain reaction, DNA was denatured at 90 °C for 3 min, followed by 45 cycles at 95 °C for 15 s, annealing at 65 °C for 45 s, and an extension step at 60 °C for 5 min with hot start Taq DNA polymerase. Genotype calling and sample clustering for Open Array assays were performed using TaqMan Genotyper (LifeTechnologies, Carlsbad, CA, USA).

2.4. Statistical analysis

We examined the differences (after 12 weeks of the pMR diet) in adiposity parameters and biochemical variables among *GHSR* rs490683 genotypes. The dominant model (CC vs. CG + GG) was tested. Genotype distribution was assessed for deviation from Hardy-Weinberg equilibrium using a Chi-square test. The sample size was calculated to detect differences >5 kg with 90 % power and 5 % significance ($n = 90$). Results were reported as mean ± standard deviation. Within-group biochemical parameters at baseline and after 12 weeks of pMR were analyzed using paired *t*-tests. Between-group comparisons were made using independent t-tests. The Mann-Whitney *U* test was applied for non-parametric variables. Categorical variables were evaluated with the Chi-square test, with Yates's correction as necessary. We calculated odds ratios (OR) and 95 % confidence intervals (CI) to estimate the association of the rs490683 SNP with criteria for metabolic syndrome (MS). A p-value <0.05 was considered significant. Statistical analyses were performed using SPSS version 23.0 (Chicago, IL, USA).

3. Results

The average age of the entire cohort was 55.2 ± 8.7 years, and the mean BMI was 39.9 ± 1.6 kg/m^2, with a gender distribution of 29.2 % males and 70.8 % females. All participants completed the 12-week follow-up period without any dropouts (Fig. 1). The gender distribution was comparable between the two genotype groups (CC vs. CG + GG), with males accounting for 27.1 % in the CC group and 32.0 % in the CG + GG group, and females accounting for 72.9 % in the CC group and 68.0 % in the CG + GG group.

Age was similarly distributed between the genotype groups (CC: 55.4 ± 5.1 years vs. CG + GG: 55.0 ± 5.1 years; not significant) and between genders (males: 55.5 ± 6.9 years vs. females: 54.9 ± 4.2 years; not significant). The genotype distribution comprised 70 patients (72.9 %) with the CC genotype, 19 patients (19.8 %) with the CG genotype, and 7 patients (7.3 %) with the GG genotype. A dominant model analysis was performed (CC vs. CG + GG). This variant of the *GHSR* gene was in Hardy-Weinberg equilibrium ($p = 0.59$).

Within the cohort of 70 individuals with the CC genotype, the baseline nutritional intake was assessed using a 3-day written food

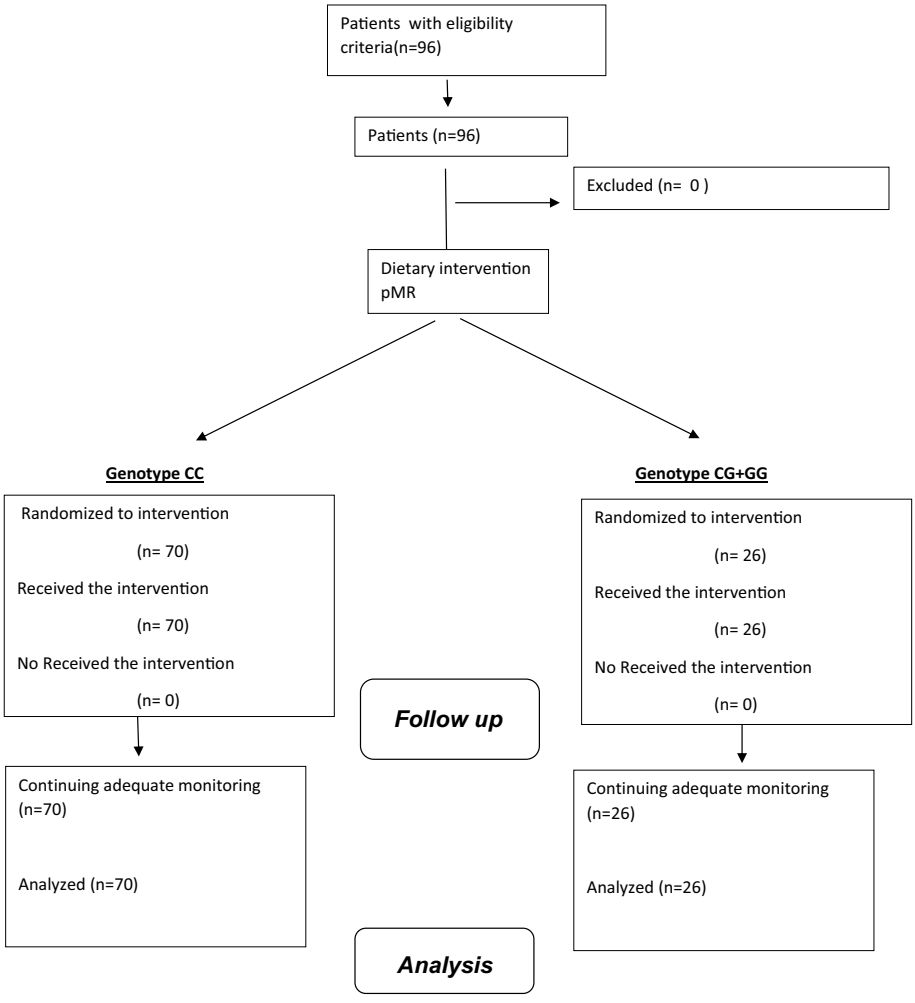

Fig. 1. Flow chart of patients.

record (Table 2). This evaluation revealed an average daily calorie intake of 1698.1 ± 413.6 kcal, with carbohydrate consumption averaging 167.6 ± 45.1 g/day, which constituted 44.6 % of the total caloric intake. The fat intake was 58.9 ± 10.3 g/day, accounting for 37.3 % of calories, while protein intake averaged 79.9 ± 10.4 g/day, representing 18.1 % of calories. During the dietary intervention, these patients reached the goals (Table 2). In the group of 26 subjects carrying the G allele (CG + GG genotypes), the baseline nutritional intake was similarly assessed using a 3-day written food record (Table 2). This evaluation showed an average daily caloric intake of 1605.8 ± 321.1 kcal. Carbohydrate intake was 168.8 ± 33.3 g/day, constituting 45.3 % of the total caloric intake, while fat intake was 61.2 ± 13.2 g/day, making up 36.4 % of calories. Protein intake averaged 77.8 ± 9.3 g/day, accounting for 19.3 % of the caloric intake. During the dietary intervention, these patients did not meet the recommended dietary restriction (Table 2).

The anthropometric measurements of the participants at baseline and after 12 weeks of intervention are detailed in Table 3. Both genotype groups exhibited significant improvements in BMI, body weight, fat mass, and waist circumference at the 12-week mark. However, the reductions were more pronounced in patients with obesity without the G allele. Specifically, the BMI reduction was (−3.5 ± 0.4 kg/m^2 versus −1.0 ± 0.2 kg/m^2 ($p = 0.01$)), body weight (−8.5 ± 1.0 kg versus −2.6 ± 1.1 kg (p = 0.01)), fat mass reduction was (−7.7 ± 0.3 kg versus −2.6 ± 0.2 kg (p = 0.01)), and waist circumference decreased (−7.2 ± 0.3 cm versus −2.9 ± 0.1 cm (p = 0.01)) in C allele carriers versus G allele carriers, respectively. Additionally, the reduction in systolic blood pressure was −8.3 ± 2.1 mmHg compared to −4.1 ± 1.0 mmHg (p = 0.01), and diastolic blood pressure decreased by −5.8 ± 2.0 mmHg versus −2.1 ± 1.1 mmHg (p = 0.01), with statistical significance observed only in C allele carriers. The baseline and post-intervention values were comparable across both genotype groups.

Table 4 presents the modifications in biochemical parameters. Among patients lacking the G allele, there was a notable improvement in glucose, insulin levels, HOMA-IR, CRP, and lipid profile after 12 weeks. Conversely, these changes were not observed in patients possessing the G allele. In those without the G allele, the changes reached statistical significance, including reductions in glucose levels (−12.1 ± 1.4 mg/dl vs −3.1 ± 1.8 mg/dl, $p = 0.01$), insulin (−10.8 ± 1.2 UI/L vs −3.9 ± 1.1 UI/L, p = 0.01), HOMA-IR (−2.1 ± 1.0 units vs −0.58 ± 0.2 units, p = 0.01), CRP (−1.2 ± 0.1 mg/dl vs −0.7 ± 0.2 mg/dl, p = 0.01), triglycerides (−22.1 ± 4.1 mg/dl vs −5.1 ± 3.2 mg/dl, p = 0.01), total cholesterol (−22.2 ± 1.3 mg/dl vs −8.8 ± 1.9 mg/dl, p = 0.01), LDL-cholesterol (−15.2 ± 1.1 mg/dl vs −4.7 ± 1.2 mg/dl, p = 0.01), and HDL-cholesterol (6.2 ± 0.4 mg/dl vs −2.9 ± 1.2 mg/dl, p = 0.01) when compared to those with the G allele.

The percentage of patients not carrying the G allele with MS was 51.4 % and in patients carrying the G allele, 50 %, with no significant differences between both groups at basal time. After 12 weeks of treatment, the percentage of MS in patients who were C allele carriers was 28.7 % and in patients who were G allele carriers was 42.3 % ($p = 0.02$). After dietary intervention, the odds ratio (OR) of presenting MS in the group of patients not carrying the G allele improved OR 0.48 (95% CI: 0.31–0.73; p = 0.02), however this change was not significant in patients with the G allele improved OR 0.85 (95%CI: 0.47–1.53; $p = 0.62$).

4. Discussion

Our study shows how the G allele of rs490683 worsens the response in the reduction of body weight and the associated change in the lipid profile and parameters related to carbohydrate metabolism in Caucasian patients with obesity after a pMR hypocaloric diet. Patients who do not carry this risk allele achieve lower caloric restriction values with a pMR diet and present a significant decrease in the presence of metabolic syndrome (MS).

The *GHSR* gene, situated on chromosome 3q26.31, encodes a protein classified within the G protein-coupled receptor family.[15] There are two known transcripts of the *GHSR* gene: GHSR type 1a, which functions as the ghrelin receptor, and GHSR type 1b, a truncated and pharmacologically inactive variant of GHSR 1a. GHSR 1a is predominantly expressed in the hypothalamus and pituitary gland, while GHSR type 1b mRNA is also present in various peripheral tissues, including immune cells. This suggests that ghrelin might play diverse roles in these tissues, though their significance remains unclear and models with impaired ghrelin signalling can exhibit complex phenotypes related to energy homeostasis.[16] In humans, the *GHSR* gene is located within a quantitative trait locus that is highly associated with various phenotypes related to obesity and metabolic syndrome.[17]

Mager et al.[18] reported that the rs490683-CC genotype is a "beneficial" genotype after dietary intervention. This investigation showed in a sub-analysis of the Finnish Diabetes Prevention Study (DPS), a highest body weight loss and glucose levels improvements in obesity subjects with this genotype. To evaluate the possible functional significance of SNPs in the 5′-region of the *GHSR* gene, these authors conducted an in-silico promoter analysis to identify regions where this SNP might be disrupting potential transcription factor binding sites.[18] These investigators observed that nuclear proteins were binding to the sequence containing the rs490683-G allele with much higher affinity than to that of rs490683-C allele. At this position a putative nuclear factor-1 transcription factor (NF-1) binding site exists and is disrupted by the SNP rs490683. NF-1 is known to activate transcription, and it is hypothesized that in individuals with the rs490683-GG genotype, where the NF-1 half-site remains intact, GHSR expression may be upregulated, potentially resulting in heightened receptor signalling and increased appetite.[19,20] Indeed, our study demonstrates that individuals with the rs490683-G allele experienced less body weight loss and metabolic improvements during a pMR hypocaloric diet than those with the rs490683-CC genotype. And in addition, the data from our study show how patients carrying the G allele were unable to achieve the caloric restrictions proposed in the pMR hypocaloric diet, and subsequently this lower loss of body weight could be explained by having a lower caloric restriction during the 12 weeks of intervention, always taking into account the limitations of collecting intake as self-reported by the patient.

In line with the previously mentioned hypotheses, recent findings indicate that GHSR exhibits approximately 50 % signalling activity even without an agonist.[21] This suggests that the expression level of the

Table 2
Daily consumption and exercise levels at baseline and post-dietary intervention, along with changes in GHSR rs490683 after 12 weeks of intervention (mean ± SD).

Parameters	CC ($n = 70$) CG + GG ($n = 26$)					
	Basal	12 weeks	p	basal	12 weeks	p
Total calorie intake (kcal/day)	1698.1 ± 413.6	1032.1 ± 32.1*	0.01	1605.8 ± 321.2	1222.9 ± 61.1*,#	0.02
Carbohydrate intake (g/day) (PTC%)	167.62 ± 451.2 (44.6 %)	129.1 ± 13.4 *(36.9 %)	0.03	168.8 ± 33.3 (45.3 %)	147.1 ± 20.3 *,# (38.0 %)	0.03
Fat intake (g/day) (PTC%)	58.9 ± 10.3 (37.3 %)	29.2 ± 2.3 *(33.0 %)	0.02	61.2 ± 13.2 (36.4 %)	48.9 ± 2.5 *,# (33.9 %)	0.03
Protein intake (g/day) (PTC%)	79.9 ± 10.4 (18.1 %)	59.2 ± 7.6 * (30.1 %)	0.02	77.8 ± 9.3 (19.3 %)	64.1 ± 8.1 (28.1 %)*,#	0.03
Fiber intake (g/day)	16.5 ± 4.1	15.3 ± 4.1	0.39	16.8 ± 4.2	15.4 ± 3.0	0.44
Physical activity (minutes/week)	124.2 ± 8.3	128.4 ± 6.1	0.51	125.2 ± 8.1	130.1 ± 7.1	0.51

PTC = Percentage of total calorie; * $P < 0.05$, in each genotype group; # $p < 0.05$ in different genotype after dietary treatment.

Table 3
Changes in GHSR rs490683 polymorphism, adiposity parameters, and arterial pressure after 12 weeks of intervention (mean ± SD).

Parameters	CC (n = 70)			CG + GG (n = 26)		
	Basal	12 weeks	p	Basal	12 weeks	p
BMI (kg/m2)	39.9 ± 2.1	36.4 ± 3.1*	0.01	39.8 ± 1.9	38.8 ± 1.1*	0.03
Weight (kg)	100.3 ± 5.2	91.8 ± 2.1$	0.01	100.5 ± 4.0	97.9 ± 3.0$	0.03
Fat mass (kg)	45.2 ± 3.0	37.5 ± 3.1#	0.01	45.9 ± 2.1	42.3 ± 1.3#	0.03
WC (cm)	117.3 ± 3.1	110.1 ± 2.0&	0.001	115.9 ± 5.0	113.2 ± 4.9&	0.02
SBP (mmHg)	135.9 ± 6.2	126.1 ± 5.3* *	0.01	135.9 ± 3.1	131.1 ± 2.9	0.11
DBP (mmHg)	82.9 ± 2.1	77.1 ± 2.2+	0.01	81.3 ± 2.4	79.7 ± 3.1	0.32

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference. Statistical differences $P < 0.05$, in each genotype group (* BMI, $ weight, # fat mass, & WC, **SBP, +DBP).

Table 4
"Changes in GHSR rs490683 polymorphism and biochemical parameters after 12 weeks of intervention (mean ± SD)."

Parameters	CC (n = 70)			CG + GG (n = 26)		
	Basal	12 weeks	p	Basal	12 weeks	p
Glucose (mg/dl)	108.8 ± 1.0	94.7 ± 1.9+	0.01	106.9 ± 3.0	103.1 ± 4.3	0.23
Total Cholesterol (mg/dl)	201.8 ± 5.0	179.5 ± 3.9$	0.02	200.9 ± 4.8	192.1 ± 4.2$	0.43
LDL-cholesterol (mg/dl)	145.3 ± 4.1	120.2 ± 3.2 #	0.01	143.6 ± 5.1	138.0 ± 4.2#	0.38
HDL-cholesterol (mg/dl)	46.1 ± 2.2	52.9 ± 1.3	0.02	46.3 ± 2.9	49.5 ± 2.8	0.23
Triglycerides (mg/dl)	139.7 ± 3.0	117.1 ± 7.2*	0.01	142.1 ± 9.1	137.7 ± 9.1	0.32
Insulin (mUI/l)	27.8 ± 2.1	17.0 ± 1.9&	0.01	25.8 ± 2.9	21.8 ± 4.9&	0.34
HOMA-IR	6.2 ± 0.4	4.1 ± 07**	0.01	5.9 ± 0.8	5.1 ± 1.5	0.41
CRP (mg/dl)	5.4 ± 0.4	4.2 ± 0.3++	0.01	5.0 ± 1.0	4.8 ± 1.8	0.49

HOMA-IR (homeostasis model assessment). CRP (C reactive protein). Statistical differences $P < 0.05$, in each genotype group (+ glucose, total cholesterol $, LDL cholesterol #, triglycerides*,insulin &, HOMA IR **, CRP++).

receptor is directly linked to its signalling activity.[22] It has also been observed that during prolonged fasting, GHSR expression in the hypothalamus increases, potentially enhancing the action of ghrelin and leading to a ghrelin-independent rise in receptor signalling, which could subsequently elevate appetite.[20] In our study population, the *GHSR* polymorphism rs490683 was associated with body weight change, and the rs490683-G allele, where the NF-1 site remains intact, may result in increased ghrelin receptor expression and may have an effect on appetite regulation.

Studies in the literature that evaluate the effect of this genetic variant are scarce. Another work is that of Matzko et al.,[10] these authors identified significant correlations between the ghrelin receptor SNP rs490683 and the pattern of weight loss over the first 30 months following RYGB surgery in a cohort of 657 patients with obesity and BMI over 40 kg/m^2. The previous above-mentioned cohort of DPS[18] involving 507 Finnish individuals with impaired glucose tolerance reported an association between the C/C genotype of rs490683 and weight loss during a 3-year dietary intervention. This bariatric intervention study[10] demonstrated that RYGB patients with the C/C genotype at this locus experienced approximately 5 % greater excess body weight loss compared to those without this genotype, whereas in the Finnish lifestyle study,[10] individuals with the C/C genotype lost 1–3 % more body weight. In our present work with a low-calorie pMR diet, patients with the CC genotype lost 5 % more weight than weight than patients with the risk G allele, being consistent with the literature, but at the same time being the first study that demonstrates this effect with a hypocaloric diet pMR. These results could be explained taking to account the functional analysis of SNP rs490683 in an adult mouse hypothalamic cell line demonstrated that the homozygous C/C genotype reduced promoter activity by approximately 20 %. This reduction may lead to decreased expression levels of GHSR1, potentially diminishing ghrelin binding signalling, thereby reducing appetite and contributing to weight loss,[20] as a potential theory to explain our present findings.

Despite the interest of our findings as they are the first in the literature to show a clear effect on dietary restriction and body weight change, our study has several limitations. First, the inclusion of adult patients with obesity and low cardiovascular risk restricts the generalizability of the findings to other populations, such as younger individuals or those with previous cardiovascular events. Second, we examined only one SNP of the *GHSR* gene, leaving the possibility that other variants could be associated with our observations, for example rs490683 and rs509035 are in strong linkage disequilibrium with each other and this may further explain the similar results concerning glucose metabolism phenotypes.[15] Third, various uncontrolled factors, such as epigenetic influences and meal timing, could have impacted our results. Additionally, the lack of a control group introduces potential bias in the analysis. Fourth, our intervention is a diet with significant caloric restriction, which produces significant weight loss as well as the study mentioned with RYGB,[10] however other bariatric studies have not been able to demonstrate this association,[23] without having a clear explanation for these differences. Lastly, self-reported dietary intake may be subject to under-reporting of energy and macronutrient intake, and similarly, the accuracy of physical activity data collected from patients could be compromised.

In summary, G allele of rs490683 have a deleterious effect on dietary restrictions, body weight, lipid profile, insulin resistance and risk of metabolic Syndrome response after a pMR diet. Taking into account our results, genotyping this genetic variant in patients with obesity may become a mandatory strategy to predict the metabolic response to weight loss in dietary interventions.

CRediT authorship contribution statement

Daniel de Luis: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Olatz Izaola:** Resources, Methodology, Investigation. **David Primo:** Project administration, Methodology, Investigation. **Daniel Rico:** Validation, Software, Project administration, Methodology. **Juan Jose López:** Writing – original draft, Investigation, Funding acquisition, Data curation.

Statement of ethics

This study protocol was reviewed and approved by [HCVUA Commitee], approval number (HVUVA committee 2/2018) Written

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

Funding sources

The authors have no funding sources to declare.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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