Original article

Role of G308 promoter variant of tumor necrosis factor alpha gene on weight loss and metabolic parameters after a high monounsaturated versus a high polyunsaturated fat hypocaloric diets

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ABSTRACT

Background and objective: The aim of our study was to investigate the influence of G308 promoter variant of the tumor necrosis factor (TNF) alpha gene on metabolic changes and weight loss secondary to a high monounsaturated fat vs a high polyunsaturated fat hypocaloric diet in obese subjects.

Patients and method: A sample of 261 obese subjects were enrolled in a consecutive prospective way, from May 2011 to July 2012 in a tertiary hospital. In the basal visit, patients were randomly allocated during 3 months to Diet M (high monounsaturated fat hypocaloric diet) and Diet P (high polyunsaturated fat hypocaloric diet).

Results: One hundred and ninety seven patients (73.2%) had the genotype G-308G and 64 (26.8%) patients had the genotype G-308A. There were no significant differences between the effects (on weight, body mass index (BMI), waist circumference, fat mass) in either genotype group with both diets. With the diet type P and in genotype G-308G, glucose levels (−6.7(22.1) mg/dl vs −3.7(2.2) mg/dl; p = 0.02), HOMA-R (−0.6(2.1) units vs −0.26(3.1) units; p = 0.01), insulin levels (−1.7(6.6) UI/L vs −0.6(7.1) UI/L; p = 0.009), total cholesterol levels (−15.3(31.1) mg/dl vs −8.4(22.1) mg/dl; p = 0.01), LDL cholesterol levels (−10.7(28.1) mg/dl vs −3.8(21.1) mg/dl; p = 0.008) and triglycerides (−12.1(52.1) mg/dl vs −6.6(43.1) mg/dl; p = 0.02) decreased.

Conclusion: Carriers of the G-308G promoter variant of TNF alpha gene have a better metabolic response than A-308 obese with a high polyunsaturated fat hypocaloric diet.

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Papel de la variante del promotor G308 del factor de necrosis tumoral α sobre el peso y parámetros metabólicos tras una dieta rica en grasas monoinsaturadas frente a una dieta rica en grasas poliinsaturadas

RESUMEN

Fundamento y objetivo: El objetivo de este estudio es investigar la influencia de la variante G-308 del promotor del gen TNF-α sobre los cambios metabólicos y pérdida de peso secundaria a una dieta hipocalórica rica en grasas monoinsaturadas frente a una dieta rica en grasas poliinsaturadas.

Pacientes y método: Una muestra de 261 obesos fue reclutada de una manera prospectiva consecutiva, desde mayo de 2011 a julio de 2012 en un hospital terciario. En la visita basal fueron aleatorizados a recibir las siguientes dietas durante al menos 3 meses: dieta M (rica en grasa monoin saturada) y dieta P (rica en grasa poliinsaturada).

Resultados: Ciento noventa y siete (73.2%) obesos presentaron el genotipo G-308G, y 64 (26.8%), el genotipo G-308A. No hubo diferencias significativas en la mejoría de peso, IMC, circunferencia de la cintura y masa grasa con ambas dietas y en ambos genotipos. Tras la dieta P y con el genotipo G-308G, los niveles de glucosa (−6.7 [22.1] vs. −3.7 [2.2] mg/dl; p = 0.02), HOMA-R (−0.6 [2.1] vs. −0.26 [3.1] unidades; p = 0.01), insulina (−1.7 [6.6] vs. −0.6 [7.1] UI/L; p = 0.009), colesterol total (−15.3 [31.1] vs.
Introduction

Obesity and overweight are major public health problems that are estimated to affect a huge percentage of the population and have been linked as risk factors for many common diseases. Hypocaloric diets are known to be an effective treatment for overweight and obese subjects. The individual responses to lifestyle modification vary and it is partially genetically determined. Mutation analysis has identified a G→A transition in the promoter region of TNF-alpha gene at position –308. This polymorphic variant has been shown to affect the promoter region of the TNF-alpha gene leading to a higher rate of transcription compared to the wild allele. Considering obesity, insulin resistance and increased production of leptin. However, other studies have reported negative results, with no correlation between TNF alpha mutation and insulin resistance. An accumulating body of evidence shows that modest weight loss through dietary changes is an effective means for managing obesity. As far as we know, only two previous studies have described the effect of different hypocaloric diets on weight loss and metabolic parameters by analyzing G-308A promoter variant of TNF alpha gene in obese subjects. De Luis et al. have shown that, with a hypocaloric diet, carriers of the G308C genotype had larger improvements in serum glucose, HOMA-R and leptin levels. Also, in other study, the A allele genotype was associated with a lack of improvement on metabolic parameters after two different hypocaloric diets (low fat vs low carbohydrate). It is possible that the distribution of macronutrients and type of dietary fat, considering previous studies, may influence secondary metabolic responses to weight loss as a function of this polymorphism. Therefore, we designed this study evaluating two isocaloric diets with a different distribution of dietary fats.

The aim of our study was to investigate the influence of G-308A promoter variant of the TNF alpha gene on metabolic changes and weight loss secondary to a high monounsaturated fat vs a high polyunsaturated fat hypocaloric diets in obese subjects.

Subjects and methods

Subjects

A sample of 261 obese subjects were enrolled in a consecutive prospective fashion, from May 2011 to July 2012 in a tertiary Hospital. These patients were studied in a Nutrition Clinic Unit and signed an informed consent (Ethical Committee of our Hospital approved this protocol). Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol > 300 mg/dl, triglycerides > 400 mg/dl, blood pressure > 140/90 mmHg, fasting plasma glucose > 110 mg/dl, as well as the use of sulphonylurea, thiazolidinedione, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and psychoactive medications.

Procedure

Patients were randomly allocated to one of the two diets for a period of 3 months. Diet M (high monounsaturated fat hypocaloric diet, enriched with foods including 30–40 ml per day of extra virgin olive oil and 40–50 g per day of walnuts or almonds) consisted of a diet of 1342 kcal with the following distribution of percentage of macronutrients: 46.6% of carbohydrates, 34.1% of lipids and 19.2% of proteins. The distribution of fats was: 21.7% of saturated fats, 67.5% of monounsaturated fats and 10.8% of polyunsaturated fats. Diet P (high polyunsaturated (PUFAs) fat hypocaloric diet enriched with foods including 30–40 ml per day of sunflower oil and 3 servings of oily fish a week) consisted of a diet of 1459 kcal, 45.7% of carbohydrates, 34.4% of lipids and 19.9% of proteins. The distribution of fats 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 exercise program consisted of an aerobic exercise at least 3 times per week (60 min each). A diettian assessed the adherence of these diets each 7 days with a phone call in order to improve compliment of the calorie restriction and macronutrient distribution. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference.

Weight, blood pressure, basal glucose, C-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and adipokines (leptin, adiponectin, TNF alpha, and interleukin 6) levels were measured at basal time and at 3 months, after both hypocaloric diets. Genotype of G308A promoter variant of the tumor necrosis factor-alpha gene was studied.

Genotyping of G308A promoter variant of the TNF alpha gene: Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International, CA, LA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA from peripheral blood, 0.5 μl of each oligonucleotide primer (primer forward: 5′-CTG TCT GGA AGT TAG AAG GAA AC-3′; primer reverse: 5′-TGT GTG TAG GAC CCT GGA G-3′), and 0.25 μl of each probes (wild probe: 5′-Fam-AAC CCC GTC CTC ATG CCC-Tamra-3′ and (mutant probe: 5′-Hex-ACC CCG TCT TCA TGC CCC-Tamra-3′) in a 25 μl final volume (Termociclador iCycler IQ (Bio-Rad®, Hercules, CA)). 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 °C for 45 s. The PCR were run in a 25 μl final volume containing 12.5 μl of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase. One probe was labeled at the 5-end with a LightCycler–Red fluorophore (Fam) and the other probe is labeled at the 3-end with Hex. Only after hybridization to the template DNA do the two probes come in close proximity, resulting in fluorescence resonance energy transfer between the two fluorophores. The emitted fluorescence of the LightCycler fluorophore was measured (Real Time polymerase chain reaction). Hardy Weimberger equilibrium was assessed.

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula.
Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2; Beckman Instruments, Fullerton, CA). Insulin was measured by enzymatic colorimetry (Insulin; WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin resistance (HOMA-R) was calculated using these values. CRP was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg/dL) and analytical sensitivity 0.5 mg/dL.

Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/mL and a normal range of 10–100 ng/mL. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, USA) with a sensitivity of 0.246 ng/mL and a normal range of 8.65–21.43 ng/mL. Interleukin 6 and TNF alpha were measured by ELISA (R&D Systems, Inc., Minneapolis, USA) with a sensitivity of 0.7 pg/mL and 0.5 pg/mL, respectively. Normal values of IL-6 was (1.12–12.5 pg/mL) and TNF alpha (0.5–15.6 pg/mL).

**Anthropometric measurements**

Body weight was measured to an accuracy of 0.5 kg and body mass index computed as body weight/(height²)(kg/m²). Waist circumference was measured in the midway between the lowest rib and the iliac crest using an anthropometric tape. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g. 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. Precautions taken to insure valid BIA measurements were: no alcohol within 24 h of taking the test, no exercise or food for 4 h before taking the test. Blood pressure was measured twice after a 10 min rest with a random zero mercury sphygmomanometer, and averaged.

**Statistical analysis**

Sample size was calculated to detect differences over 3 kg in body weight with 90% power and 5% significance (n = 125, in each diet group). The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables were analyzed with a 2-way ANOVA model. 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. Non-parametric variables were analyzed with the Wilcoxon test. The statistical analysis was designed for the combined G-308A and A-308A as a mutant type group and wild type group G-308C as second group. Dominant model was used to analyze the results. A p-value under 0.05 was considered statistically significant.

**Results**

Two hundred and sixty one patients gave informed consent and were enrolled in the study. The mean age was 45.8 ± 16.4 years and the mean BMI 36.1 ± 5.6, with 65 males (24.9%) and 196 females (75.1%).

All patients completed the 3-month follow-up period without dropouts in both branches (diet M vs diet P). One hundred and ninety seven patients (73.2%) had the genotype G-308C and 64 (26.8%) patients had the genotype G-308A. Age was similar in both groups (G-308C genotype: 46.9(11.4) years vs G-308A genotype: 44.9(12.1) years; p = 0.12). Sex distribution was similar in both groups, males (27.4% vs 20.8%) and females (72.6% vs 79.2%).

The 128 subjects (91 G-308G genotype and 37 G-308A genotype) treated with diet M showed in the basal assessment of nutritional intake with a 3 days written food record a calorie intake of 1992.1(811.6) kcal/day, a carbohydrate intake of 219.3(78.9) g/day (44.3% of calories), a fat intake of 80.2(39.3) g/day (36.2% of calories) and a protein intake of 90.5(36.8) g/day (19.5% of calories). During the intervention, these subjects reached the recommendations of diet: 1418.3 calories (44.9% of carbohydrates, 34.0% of lipids and 21.1% of proteins). The distribution of dietary fats was: 20.5% of saturated fats, 68.0% of monounsaturated fats and 11.5% of polyunsaturated fats.

The 133 subjects (106 G-308G genotype and 27 G-308A genotype) treated with diet P showed in the basal assessment of nutritional intake with a 3 days written food record a calorie intake of 1882.9(553.1) kcal/day, a carbohydrate intake of 189.9(62.3) g/day (40.3% of calories), a fat intake of 74.1(28.2) g/day (35.4% of calories) and a protein intake of 85.1(21.6) g/day (24.3% of calories). During the intervention, these patients reached the recommendations of diet: 1439.8 calories (45.1% of carbohydrates, 34.0% of lipids and 20.9% of proteins). The distribution of dietary fats was: 20.8% of saturated fats, 53.7% of monounsaturated fats and 23.5% of polyunsaturated fats (6.9 g per day of w-6 fatty acids, 2.3 g per day of w-3 fatty acids and a ratio w6/w3 of 3.0).

Anthropometric characteristics of participants at baseline and at month 3 of intervention are shown in **Table 1**. With the diet type M (high monounsaturated fat hypocaloric diet) and in both genotype groups (G-308G vs G-308A), body mass index (BMI) (−1.6(1.5) kg/m² vs −1.8(1.5) kg/m²; p = 0.09), weight (−4.2(3.7) kg vs −3.9(3.1) kg; p < 0.05), fat mass (−3.4(3.6) kg vs −3.0(2.6) kg; p > 0.05) and waist circumference (−4.3(4.4) cm vs −4.4(5.1) cm; p > 0.05) decreased. With the diet type P (high polyunsaturated fat hypocaloric diet) and in both genotypes, BMI (−2.0(1.6) kg/m² vs −1.9(1.8) kg/m²; p > 0.05), weight (−4.0(3.7) kg vs −4.2(3.6) kg; p < 0.05), fat mass (−3.6(3.7) kg vs −4.2(3.7) kg; p > 0.05) and waist circumference (−3.5(3.6) cm vs −3.8(3.2) cm; p > 0.05) decreased. There were no significant differences between the effects (on weight, BMI, waist circumference, fat mass) in either genotype group with both diets. No

<table>
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<th>Characteristics</th>
<th>DIET M (n = 128)</th>
<th>DIET P (n = 133)</th>
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<tr>
<td></td>
<td>G-308G (n = 91)</td>
<td>G-308A (n = 37)</td>
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<td>0 time</td>
<td>36.8(5.7)</td>
<td>35.2(5.5)</td>
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<tr>
<td>3 months</td>
<td>36.8(6.8)</td>
<td>35.2(5.5)</td>
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<tr>
<td>6 months</td>
<td>36.8(6.8)</td>
<td>35.2(5.5)</td>
</tr>
<tr>
<td>9 months</td>
<td>36.8(6.8)</td>
<td>35.2(5.5)</td>
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BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; WHR: waist to hip ratio; WC: waist circumference.

*p < 0.05, in the same genotype group with basal values.*
differences were detected before and after weight loss of anthropometric variables between both genotypes.

Table 2 shows the biochemical parameters. With the diet type M and in both genotype groups, no significant changes were detected before weight loss. With the diet type P and in genotype G-308G, glucose levels (−6.7(22.1) mg/dl vs. −3.7(2.2) mg/dl; p = 0.02), HOMA-R (−0.6(2.1) units vs. −0.26(3.1) units; p = 0.01), insulin levels (−1.7(6.6) U/l vs. −0.6(7.1) U/l; p = 0.009), total cholesterol levels (−15.3(31.1) mg/dl vs. −8.4(22.1) mg/dl; p = 0.01), LDL cholesterol levels (−10.7(28.1) mg/dl vs. −3.8 ± 21.1 mg/dl; p = 0.008) and triglycerides (−12.1(52.1) mg/dl vs. −6.4(31.1) mg/dl; p = 0.02) decreased. There was no effect on these parameters in A allele carriers. No differences were detected among basal and post-treatment values of biochemical variables between both genotypes.

Table 3 shows levels of adipokines. With the diet type M and in both genotype groups, leptin levels (−7.7(13.1) ng/ml vs. −10.8(19.7) ng/ml; p > 0.05) decreased. With the diet P and in both genotype groups, leptin levels (−7.1(12.9) ng/ml vs. −9.1(18.9) ng/ml; p > 0.05) also decreased. The decrease of leptin levels was similar in both genetic types and with both diets. No changes were observed in the other adipokine levels.

Discussion

In subjects with G-308G genotype of G-308A promoter variant of the TNF alpha gene treated with two different hypocaloric diets during 3 months (high monounsaturated fat “M” and high polyunsaturated fat “P” hypocaloric diets), we observed a significant decrease of weight, BMI, fat mass, waist circumference and leptin levels. Our study shows that A allele carriers had a different metabolic response with P diet, with a lack of effect on total cholesterol, LDL cholesterol, glucose, insulin, HOMA-R and triglyceride levels.

We have detected an improvement in insulin concentrations after weight loss secondary to a high polyunsaturated fat hypocaloric diet, only in subjects with G-308G genotype. Some previous studies have proposed that the effects of the G-308A promoter variant of the TNF-alpha gene on insulin action may depend on body weight.12 The G-308A substitution was associated with high RQ (resting quotient), indicating high rates of glucose oxidation and lipid synthesis, depending of BMI.13 However, our study showed a significant loss of weight and fat mass with both diets and in both genotypes but only non-A allele carriers showed this improvement in insulin levels, HOMA-R and lipid profile. A hypothesis to explain this interaction gene-diet is that the G-308A promoter variant could play a direct role in this different response. The promoter variant at position −308(TNF-308 G→A) leads to a higher rate of TNF alpha gene transcription, followed by raised TNF alpha concentrations and decreased insulin resistance.13 The mechanism of insulin resistance involves down-regulation of PPARgamma and C/EBPs (C fibreonectin binding protein α), which have been shown to be the propagators of adipocyte differentiation.14 Other mechanism is the down-regulation of GLUT-4 (glucose transporter type 4) or other cellular components that mediate the metabolic effects of insulin such as cytokines.15 Nevertheless, basal levels of adipokines, IL-6 and TNF-alpha in our obese participants have not been influenced by genotype. In this topic area, data in the literature are contradictory,16,17 with some studies not demonstrating a major role of the −308 substitutions of the TNF alpha gene in the pathogenesis of high levels of TNF alpha or insulin resistance.

The results of different interventional studies with this polymorphism gene have shown unclear data. In other study,7 no carriers of the A allele had a greater reduction in glucose levels, insulin levels, leptin levels and weight than A allele carriers in response to a 3-month hypocaloric diet. Other interventional study8 during 3 months with a low fat hypocaloric diet showed a better metabolic improvement secondary to weight loss in G-308G genotype. In this previous study,8 the improvement of glucose, insulin levels, HOMA-R, LDL cholesterol, total cholesterol and triglyceride levels was statistically significant in G-308G subjects.

Table 3
Changes in circulating adipokinetones levels after two hypocaloric diets.

<table>
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<tr>
<th>Characteristics</th>
<th>DIET M (n = 128)</th>
<th>DIET P (n = 133)</th>
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<tbody>
<tr>
<td></td>
<td>G-308G (n = 91)</td>
<td>G-308A (n = 37)</td>
</tr>
<tr>
<td></td>
<td>0 time</td>
<td>At 3 months</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.52 ± 0.2</td>
<td>1.44 ± 0.3</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.40 ± 0.6</td>
<td>1.81 ± 1.6</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>10.5 ± 6.3</td>
<td>9.4 ± 5.1</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>33.1 ± 24.1</td>
<td>30.2 ± 20.6</td>
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IL-6: interleukin 6; TNF: tumor necrosis factor.

* p < 0.05, in the same genotype group with basal values.
with a low fat hypocaloric diet and only insulin levels improved in the same genotype with low carbohydrate diet. And A allele carriers did not show metabolic improvements after weight loss. On the other hand, leptin levels decreased more in G-308G genotype subjects than in A allele carriers with both diets.8

Finally, a surgical study with bariatric procedure18 has shown that this polymorphism is not related to biochemical outcomes after a massive weight loss with a biliopancreatic diversion procedure.

These different results in metabolic changes secondary to weight loss in the literature could be due to the duration and style of dietary intervention in the protocols or differences on background characteristics in the study populations (basal weight, sex distributions, average age and so on). Some specific reasons could explain these contradictory results. Firstly, the distribution of macronutrients in the prescribed diets and the type of dietary fat may influence on secondary metabolic responses and weight loss. For example, in one study1 the distribution of macronutrient was: 52% carbohydrates, 25% fats and 23% proteins with a total of 1520 kcal per day yet type of dietary fat was not reported. In other study,9 low fat diet had the following distribution of macronutrients: 53% carbohydrates, 27% fats and 20% proteins and the percentage of polyunsaturated fatty acids was around 18%, a percentage close to that used in our study, which could explain the similarity in metabolic results. Finally, the duration of dietary intervention may influence secondary metabolic responses to weight loss as a function of this polymorphism. The duration of interventions has been around 8 weeks,7 12 weeks,8 and until 3 years.18 Perhaps the interaction G-308A polymorphism and weight loss secondary to diet is modulated during the time.

Our data show that the amount and type of dietary PUFA may have an important effect based on presenting polymorphism and we recommended to include in hypocaloric diets foods such as 30–40 ml per day of sunflower oil and 3 servings of oily fish a week. Previously, Fotaine-Bisson et al.19 have reported that G-308A genotype modifies the relation between dietary PUFA intake and HDL cholesterol concentrations. Moreover, the consumption of 6 g/d of fish oil during 12 weeks showed an interaction between this polymorphism and change in triglyceride concentrations.20

In conclusion, weight loss is associated with different changes depending on TNF-alpha genotype. Carriers of GG genotype have a better metabolic response than GA genotype obese subjects with a high polyunsaturated fat hypocaloric diet. The first genotype has a significant decrease in glucose, HOMA-R, total cholesterol, LDL cholesterol, triglyceride and insulin concentrations. The weight loss secondary to a high monounsaturated fat hypocaloric diet did not show an interaction between this polymorphism and metabolic changes. Additional studies will be needed to clarify the contribution of lifestyle modification in the weight loss response of obese patients and the effects of personalized diets.21

Conflict of interest

The authors declare no conflict of interest.

References