Med Clin (Barc). 2013;**141(5)**:189–193

MEDICINA CLINICA

www.elsevier.es/medicinaclinica

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

Daniel A. de Luis *, Rocío Aller, Olatz Izaola, Manuel Gonzalez Sagrado, Rosa Conde

Institute of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Río Hortega, University of Valladolid, Valladolid, Spain

ARTICLE INFO

Article history:
Received 21 September 2012
Accepted 20 December 2012
Available online 17 April 2013

Keywords:
G308 TNF-alpha polymorphism
Hypocaloric diet
Obesity

ABSTRACT

Background and objective: The aim of our study was to investigate the influence of G-308 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.

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

Palabras clave:
Polimorfismo G-308 del factor de necrosis tumoral α
Dieta hipocalórica
Obesidad

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 monoinsaturada) 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.

* Corresponding author.
E-mail address: dadluis@yahoo.es (D.A. de Luis).

0025-7753/$ – see front matter
http://dx.doi.org/10.1016/j.medcli.2012.12.021

−8,4 [22,1] mg/dl; p = 0,01), colesterol LDL (−10,7 [28,1] vs. −3,8 [21,1] mg/dl; p = 0,008) y triglicéridos (−12,1 [52,1] mg/dl vs. −6,6 [43,1] mg/dl; p = 0,02) disminuyeron.
Conclusión: Los portadores del genotipo G-308G presentan mayores beneficios metabólicos tras la pérdida de peso generada por la dieta rica en grasas poliinsaturadas.

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.[1] 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.[2] Several association studies have been conducted on the G-308 variant, with conflicting results. Fernandez Real et al.[3] have reported a significant association between the G-308A variant and increased BMI, insulin resistance and increased production of leptin. However, other studies have reported negative results, with no correlation between TNF alpha mutation and insulin resistance.[4-6]

An accumulating body of evidence shows that modest weight loss through dietary changes is an effective means for managing obesity.[8] As far as we know, only two previous studies[7,8] 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.[7] have shown that, with a hypocaloric diet, carriers of the G308G genotype had larger improvements in serum glucose, HOMA-R and leptin levels. Also, in other study,[8] 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 dietitian 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 dietician and analyzed with a computer-based data evaluation system. National composition food tables were used as reference.[9]

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®, LA, CA). 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 denaturized 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.