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Original article

The rs10401670 variant in resistin gene improved insulin resistance response and metabolic parameters secondaries to weight loss after a hypocaloric diet

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SUMMARY

Background: The SNP 3′UTR C/T (*rs10401670*), it is a polymorphism that has been associated with diabetes mellitus and it has been scarcely studied before. As far as we know, no studies on interaction among diet intervention, *rs10401670* variant of *RETN* and metabolic response has been realized.
Objective: Our aim was to analyze the effects of the rs10401670 *RETN* gene polymorphism on insulin resistance response and metabolic changes secondary to weight loss after 3 months of a hypocaloric diet in adults obese patients without diabetes mellitus.
Design: A Caucasian population of 135 obese patients without diabetes mellitus was analyzed. Before and after 3 months on a low fat hypocaloric diet, an anthropometric evaluation, an assessment of nutritional intake and a biochemical analysis were performed. The statistical analysis was performed for the combined *CT* and *TT* as a group (minor allele group) and wild type CC as second group (major allele group) (dominant model).
Results: Forty nine patients (36.3%) had the genotype *CC* (major allele group) and 86 (63.7%) patients had the next genotypes; *CT* (67 patients, 49.6%) or *TT* (19 patients, 14.1%) (minor allele group). After dietary treatment and in major allele group, weight, BMI, fat mass, systolic blood pressure and waist circumference decreases were similar than minor allele group. In T allele carriers, fasting plasma glucose, insulin, HOMA-IR, total cholesterol and LDL cholesterol levels decreased significantly. In non T allele carriers and after dietary treatment, only LDL cholesterol and total cholesterol decreased. In non T Allele carriers, the decrease in total cholesterol was −15.1 ± 18.3 mg/dl (decrease in T Allele carriers −18.3 ± 15.7 mg/dl: $p > 0.05$), LDL-cholesterol was −14.3 ± 18.5 mg/dl (decrease in T Allele carriers −17.3 ± 10.1 mg/dl:$p > 0.05$), fasting glucose plasma −2.2 ± 1.5 mg/dL (decrease in T Allele carriers −4.8 ± 1.2 mg/dL: $p = 0.02$), insulin −1.1 ± 2.0 mUI/L (decrease in T Allele carriers −6.3 ± 1.9 mUI/L: $p = 0.001$) and HOMA-IR −0.2 ± 1.0 (decrease in T Allele carriers −1.8 ± 1.4: $p = 0.005$). Leptin levels decrease in both genotypes after dietary treatment (−21.1 ± 8.5 ng/dL in nonT Allele carriers vs −16.2 ± 10.2 ng/dL in T Allele carriers:$p > 0.05$). Resistin remained unchanged in both groups.
Conclusion: In our study in non-diabetic obese subjects, we describe an association of rs10401670T allele with a better metabolic response (glucose, insulin and HOMA-IR) secondary to weight loss with a hypocaloric diet.

1. Introduction

The actual view of adipose tissue is that of an active secretor organ, sending out and responding to signals that modulate inflammation, immunity, appetite, insulin sensitivity, and energy expenditure. These signals are the so call adipokines, one of them are resistin. Resistin was identified as a gene whose expression is

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List of abbreviations

(BMI) body mass index
(*RETN* GENE) Resistin gene
HOMA-R Homeostasis model assessment
CRP C reactive protein
MCEMP1 (Mast cell-expressed membrane protein 1)

induced by adipocyte differentiation and inhibited by peroxisome proliferators activated receptor ligands in 3T3-L1 cells [1]. Resistin is an adipokine secreted by adipocytes and macrophages in adipose tissue and liver. The over-expression of the resistin gene in the liver increases insulin resistance, whereas its disruption reduces blood glucose [2]. In humans, data on the role of this adipokine in insulin sensitivity and obesity are controversial. Serum resistin levels are associated with increased obesity, visceral fat [3] and type 2 diabetes mellitus [4], while other groups failed to observe such correlations [5].

The gene encoding resistin (*RETN*) is located on chromosome 19p13 and genetic variants in *RETN* have been examined by many groups. A high heritability of resistin levels has been suggested [6], and it is estimated that up to 70% of the variation in circulating resistin levels can be explained by genetic factors. Several polymorphisms of a single nucleotide (SNPs) in *RETN* have been associated with indexes of insulin resistance in some reports [7,8]. The SNP 3′UTR C/T (*rs10401670*), it is a polymorphism that has been associated with diabetes mellitus in Framingham Offspring Study [9]. This SNP has been related with lipoprotein metabolism. Ortega et al. [10] have been described an association between the rs10401670 and LD-cholesterol levels in 12–16 years old boys, and between the polymorphism and HDL-C levels in girls. As far as we know, no studies on interaction among diet intervention, *rs10401670* variant of *RETN* and metabolic response (insulin resistance and lipid profile) has been realized.

Our aim was to analyze the effects of the *rs10401670 RETN* gene polymorphism on insulin resistance response and metabolic changes secondary to weight loss after 3 months of a hypocaloric diet in adult's obese patients without diabetes mellitus.

2. Subjects and methods

2.1. Subjects

The study population (Caucasian) included 135 adults obese non-diabetic outpatients, they were enrolled in a prospective way. These patients were recruited in a Department of Endocrinology and Nutrition and all participants provided informed consent to a protocol approved by the local ethical review boards. This study was approved by the HURH ethics committee. Inclusion criteria were age >18 years, body mass index >30 and absence of a diet during the 6 months previously to the study. Exclusion criteria included history of cardiovascular disease or stroke during the previous 24 months, total cholesterol >200 mg/dl, triglycerides >250 mg/dl, blood pressure >140/90 mmHg, fasting plasma glucose >126 mg/dl, as well as the use of as well as the use of metformin, sulphonilurea, dipeptidyl type IV inhibitors drugs, thiazolidinediones, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other lipid drugs.

2.2. Procedure

Biochemical parameters: Fasting (12 h) venous blood samples were obtained by venipuncture and collected in Vacutainer tubes. Basal fasting glucose, C reactive protein (CRP), insulin, insulin resistance (HOMA-IR), total cholesterol, LDL cholesterol, HDL-cholesterol, plasma triglycerides concentration and adipokines levels (leptin, adiponectin, resistin) were measured within the start of the trial and repeated after 3 months of dietary intervention. Genotype of *RETN* gene was studied rs1862513.

Anthropometric parameters: A tetrapolar bioimpedance was realized to in order to measure fat mass. Weight, height, and blood pressure measures were measured within the start of the trail and repeated 3 months of intervention. These measures were realized at same time of the day (morning).

2.3. Genotyping of rs10401670 RETN gene polymorphism

- Genomic DNA was prepared from leukocytes. Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International ®, LA, CA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5′-ACGTTGGATGGCTGTTGACGTGCTAATGAG-3'and reverse 5′-ACGTTGGATGAGCCACCCTCAGCGATCTAA-3′). DNA was denaturated at 95 °C for 3 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 59.3 °C for 45 s). The PCR were run in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase. If in a patient's sample grow both strands, this patient was classified as heterozygous. If in a patient's sample only grows one strand, this patient was classified as homozygous. Thermocycler software classifies each patient as wild homozygous (CC), heterozygous (CT) and mutant homozygous (TT).

2.4. Dietary intervention

The lifestyle modification program was a hypocaloric diet (1520 calories per day) during three months, the distribution of macronutrient was; 52% of carbohydrates, 25% of lipids and 23% of proteins. Distribution of fats was: 50.7% of monounsaturated fats, 38.5% of saturated fats and 11.8% of polyunsaturated fats. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day Records were reviewed by a dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference [11]. The adherence of this diet was assessed each 14 days with a phone call by a dietitian in order to improve compliment of the calorie restriction and macronutrient distribution. The exercise activity allowed was aerobic exercise for at least 3 times per week (60 min each) and it was recorded by the patient.

2.5. Assays

Lipid metabolism: Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL-cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Glucose metabolism: Fasting plasma glucose levels were determined by using an automated glucose oxidase method