Clinical Nutrition xxx (2016) 1–5

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Effect of two polymorphisms of the resistin gene (rs10401670 and rs1862513) on resistin levels and biochemical parameters in morbidly obese patients 1 year after a biliopancreatic diversion surgery

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ARTICLE INFO

Article history:
Received 11 December 2015
Accepted 2 April 2016

Keywords:
Biliopancreatic diversion
Resistin gene
SNPs
Morbid obesity
Surgery

SUMMARY

Background & aims: Two single nucleotide polymorphisms (SNPs) of the resistin gene *RETN* have been described: rs10401670 and rs1862513. The objective of this study was to investigate the effect of these SNPs on changes in serum resistin levels, biochemical parameters and weight after biliopancreatic diversion surgery in morbidly obese patients without diabetes mellitus.

Methods: A sample of 155 patients with morbid obesity without diabetes mellitus was enrolled. Anthropometric and biochemical evaluations were realized at the basal visit and at 12 months. The percentage of subjects with hypertension and hyperlipidemia was also reported.

Results: Initial percentage excess weight loss, body mass index, weight, waist circumference, fat mass, blood pressure, low-density lipoprotein cholesterol, total cholesterol, triglycerides levels, insulin and the homeostasis model assessment for insulin sensitivity (HOMA-IR) improve after 12 months. No differences in these improvements were detected between the two genotypes (wild vs mutant group) in each SNP analysis. Resistin levels only changed after surgery in wild genotypes of both SNPs (rs1862513 and rs10401670). The improvement in insulin levels was lower in the mutant group of rs1862513 (-3.4 ± 0.4 UI/dl vs -2.3 ± 0.2 UI/dl; $P < 0.05$) and rs1040167 (-3.3 ± 0.2 UI/dl vs -1.9 ± 0.3 UI/dl; $P < 0.05$). The decrease of HOMA-IR was lower in mutant group of rs1862513 (-1.4 ± 0.1 units vs -0.9 ± 0.3 units; $P < 0.05$) and rs10401670 (-1.2 ± 0.2 units vs -0.9 ± 0.3 units; $P < 0.05$).

Conclusions: The main result of this study was that the mutant genotype of two SNPs of the *RETN* gene (rs1862513 and rs10401670) was associated with a lack of change in resistin secondary to biliopancreatic diversion. The improvement in insulin levels and HOMA-IR was also lower in these patients.

1. Introduction

Adipose tissue is recognized as an endocrine organ. In the past few years, several proteins, called adipokines, produced by adipose tissue have been discovered [1]. Resistin is one of these adipokines; it was identified as a gene whose expression is induced by adipocyte differentiation and inhibited by peroxisome proliferators activated receptor ligands in 3T3-L1 cells [2]. In mice, resistin is produced by fat tissue, and resistin levels are increased in obesity. The over-expression of the resistin gene in the liver increases insulin resistance, whereas its disruption reduces blood glucose [3]. In humans, data on the role of resistin on insulin activity and adipose tissue remain controversial. Serum resistin levels are associated with obesity, visceral fat [4] and type 2 diabetes [5,6], while other studies failed to report such correlations [7] or a relationship with metabolic syndrome [8]. The gene encoding resistin (*RETN*) is located on chromosome 19p13. Genetic variants of *RETN* have been examined by many authors, and genetic factors are related with 70% of the variation in circulating resistin levels [9]. However, associations between *RETN* and parameters related with adiposity have shown very inconsistent results [10,11]. Single nucleotide polymorphisms (SNPs) in *RETN* also have been associated with indexes of insulin resistance in some reports [11,12]. Two of these SNPs are rs10401670 and rs1862513 [10–12]. The effect of these

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http://dx.doi.org/10.1016/j.clnu.2016.04.005

Please cite this article in press as: Antonio de Luis D, et al., Effect of two polymorphisms of the resistin gene (rs10401670 and rs1862513) on resistin levels and biochemical parameters in morbidly obese patients 1 year after a biliopancreatic diversion surgery, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.04.005

Table 1
Preoperative characteristics of the patients.

Morbidly obese	118
Super-obese	37
Gender (women/men)	27/12
Age (years)	45.3 ± 12.2
BMI (kg/m^2)	47.5 ± 7.1

Morbidly obese, body mass index (BMI) > 40 and <50; super-obese, BMI > 50.

SNPs on weight loss or metabolic changes after weight loss remained unchanged.

In contrast, the use of bariatric procedures for the treatment of morbid obesity has increased in the last decades [13]. Long-term outcomes after bariatric surgery have also shown resolution of comorbidities associated with obesity: recovery of diabetes mellitus, dyslipemia, hypertension and sleep apnea occur after bariatric surgery [14]. Biliopancreatic diversion (BPD) [15] is a mixed operation that has shown good results regarding weight loss. Conversely, some studies addressing the changes of resistin concentrations in morbidly obese patients after bariatric surgery have yielded conflicting results [16–19]. This topic area has unclear results and the effect of SNPS of *RETN* has not been evaluated.

The purpose of the present study was to investigate the effect of two polymorphisms of the *RETN* gene (rs10401670 and rs1862513) on changes in serum resistin levels, biochemical parameters and weight after BPD in morbidly obese patients.

2. Materials and methods

2.1. Subjects and surgical procedures

A prospective, non-randomized, observational study of morbid patients undergoing open BPD by the Scopinaro technique was realized. Written informed consent was obtained from all subjects. This study was performed according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by our Local Ethical Committee (HCUVA Committee).

One hundred and fifty-five morbidly obese patients without diabetes mellitus (body mass index (BMI) > 40) were operated on from December 2006 to December 2013 (Table 1). Consecutive consenting patients were enrolled and all patients consented. The BPD consisted of an average of 75-cm common limb and 175-cm alimentary limb. Gastric volume was measured with sterile water after stapling. Intestinal limbs were measured during surgery with a sterile tape measure.

Follow-up visits were carried out at basal time and after 12 months. The following variables were reported: weight, height, BMI, waist circumference, fat mass, initial percentage excess weight loss (IEWL%), blood pressure, lipid profile, insulin, the homeostasis model assessment for insulin sensitivity (HOMA-IR), resistin and comorbidities. The genotype of the RETN gene (rs10401670 and rs1862513) was studied.

2.2. Anthropometric and biochemical parameters after surgery

Weight, BMI, IEWL%, fat mass, blood pressure, basal glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, insulin, and HOMA-IR were measured at the basal visit and at 12 months after BPD. The percentages of patients' hypertension and hyperlipidemia were recorded at this time.

Hyperlipemia and hypertension were diagnosed in patients taking hypolipemic and hypotensive drugs, respectively. Hypertension or hyperlipidemia was also diagnosed according to National Cholesterol Education Program standards [20]. Diabetes mellitus and fasting hyperglycemia was excluded in patients taking hypoglycemic drugs or insulin or according to the American Diabetes Association standard of diagnosis [21].

2.3. Genotyping of RETN gene polymorphisms

Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc., San Diego, California CA). The polymerase chain reaction (PCR) was carried out with 20–25 ng of genomic DNA, 0.1–0.15 μl each of oligonucleotide primer for rs1862513 (primer forward: 5′-ACGTTGGATGGAGCCTTCCCACTTCCAACA-3′ and reverse 5′-ACGTTGGATGCACAGCCCCTGGCATTATC-3′ in a 2-μl final volume (Termociclador Lifetecnologies, LA, CA) and under the same conditions with these primers for rs10401670 (primer forward: 5′-ACGTTGGATGGCTGTTGACGTGCTAATGAG-3′ and reverse 5′-ACGTTGGATGAGCCACCCTCAGCGATCTAA-3′). DNA was denatured at 85 °C for 5 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 58.1 °C for 45 s). The PCRs were run in a 2-μl final volume containing 0.1 μl of iPLEx Termination mix (Bio-Rad®, San Diego, CA) with hot start Taq DNA polymerase. Hardy Weinberg equilibrium was calculated with a statistical test (Chi-square). The two variants were in Hardy Weinberg equilibrium in both genotypes.

2.4. Biochemical assays

Resistin was measured by enzyme-linked immunosorbent assay (ELISA; Biovendor Laboratory, Inc., Brno, Czech Republic). Assay sensitivity was 0.2 ng/ml and intra-assay and inter-assay coefficients of variation were less than 5% and less than 10%, respectively.

Fasting glucose levels were measured by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was determined by enzymatic colorimetric (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) [22] and the HOMA-IR was calculated using these parameters [23]. C-reactive protein (CRP) was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of 0–7 mg/dl and analytical sensitivity of 0.5 mg/dl.

Serum HDL-cholesterol, total-cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany).

2.5. Body composition measurements and blood pressure

Body weight was executed to an accuracy of 0.1 kg and BMI calculated as body weight/(height [2]). Waist circumference was determined in the narrowest diameter between xiphoid process and iliac crest. Percentage of initial excess weight percent loss (IEWL%) was calculated (%IEWL = (preoperative weight − current weight) divided by (preoperative weight − ideal weight) × 100). Tetrapolar body electrical bioimpedance was used to calculate body composition with an accuracy of 50 g. An electric current of 0.8 mA and 50 kHz was produced by a signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Reactance and resistance were used to calculate total body water and fat mass. Blood pressure was determined twice after a 10-min rest with a sphygmomanometer OMRON Mx3 (Omron Matsusaka Co. Ltd, Tokyo Japan), and averaged.

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