

The N363S polymorphism in the glucocorticoid receptor gene: effects on visceral fat assessed by abdominal computed tomography

Polimorfismo N363S do gene do receptor de glucocorticoide: efeito sobre a adiposidade visceral medida pela tomografia computadorizada

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ABSTRACT

Objective: To verify whether N363S polymorphism of the glucocorticoid receptor-gene can be associated to visceral fat by CT scan in obese individuals, and the impact of this variant on metabolic profile. **Methods:** The N363S variant was screened in 295 Brazilians, 195 were obese and 100 presented normal weight. Based on genotype, obese N363S SNP carriers were paired with obese wild-type subjects. This group was submitted to a CT scan and metabolic profile assessment. **Results:** Ten subjects were found to be heterozygous for the variant (A/G genotype frequency 3.4%), 8 (4.1%) obese and 2 (2.0%) non-obese. No differences were reported for visceral adiposity area (145.8 ± 49.9 vs. 147.7 ± 48.8 cm²; $p = 0.92$) based on CT scan results but N363S SNP carriers showed a proneness to unfavorable metabolic changes. **Conclusion:** The N363S polymorphism prevalence is low in the Brazilian population, although its presence may contribute to the worsening of individuals' metabolic profiles. *Arq Bras Endocrinol Metab.* 2009;53(2):288-292.

Keywords

Glucocorticoid; polymorphism; obesity; metabolic syndrome

RESUMO

Objetivo: Verificar se a presença do polimorfismo N363S do gene do receptor de glucocorticoide estaria associada, em indivíduos obesos, à presença de adiposidade visceral pela tomografia computadorizada, e sobre o impacto desta variante genética no perfil metabólico. **Métodos:** A variante N363S do receptor do glicocorticoide foi verificada em um grupo de 295 indivíduos brasileiros, sendo 295 obesos e 100 com peso normal. Com base na genotipagem, os indivíduos obesos carreadores do polimorfismo N363S foram pareados com obesos normais. O grupo com polimorfismo foi submetido a exames de tomografia computadorizada abdominal e laboratoriais para a caracterização de seu perfil metabólico. **Resultados:** Dez indivíduos eram heterozigotos para a variante AG (3,4%), sendo oito obesos (4,1%) e dois não-obesos (2%). Não foram encontradas diferenças na quantidade de adiposidade visceral ($145,8 \pm 49,9$ versus $147,7 \pm 48,8$ cm²; $p = 0,92$) baseados no TC de abdômen. No entanto, os indivíduos carreadores do N363S SNP (*single nucleotide polymorphism*) apresentaram tendência a perfil metabólico desfavorável. **Conclusão:** O polimorfismo N363S do gene do receptor de glucocorticoide teve prevalência baixa na população estudada. A sua presença pode contribuir para a deterioração do perfil metabólico desses indivíduos. *Arq Bras Endocrinol Metab.* 2009;53(2):288-292.

Descritores

Glicocorticoide; polimorfismo; obesidade; síndrome metabólica

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INTRODUCTION

Some obesity phenotypes – mainly those with abdominal adiposity – report clear characteristics of hypercortisolism despite keeping serum cortisol levels within normal ranges (1,2). Most glucocorticoid action is carried out through cortisol binding with an intracellular protein – the glucocorticoid receptor (GR) (3). The GR is part of the Type 1 subclass of nuclear receptors (4). The gene that codifies the GR (*NR3C1*) is located on the long arm of chromosome 5, in the 5q31-q32 region. GR expression level and receptor hormone binding affinity are the main determinants of tissue sensitivity (3). Recent data have suggested that GR phosphorylation may determine specificity to target receptors, interaction with co-factors, cellular localization, and receptor stability (5). In 1997, Koper and cols. (6) described a GR polymorphism located at nucleotide position 1220 (AAT to AGT), resulting in a change of asparagine into serine at codon 363 (N363S SNP) in the GR transactivation domain. The presence of a new serine site in the transcription-activating amino-terminal region could lead to phosphorylation increase, as well as to higher receptor activity. Recently, examination of this polymorphism by microarray analysis showed that there are significant differences between wild-type GR and the N363S SNP in their ability to regulate gene expression selectively (7). Several of these genes may define the link between the N363S SNP and the human disease. In a Dutch study, a group of 216 elderly individuals were examined for the N363S polymorphism gene, and 13 heterozygotes (6% of the group) were identified. Interestingly, these carriers exhibited an increased sensitivity to exogenously administered glucocorticoids as well as an increased insulin response and increased body mass index (BMI) (8). Other studies also associated the presence of the N363S variant with body weight increase by analyzing BMI in a population of Australians of British origin (9), obese individuals in Italy (10), and diabetics in France (11). The same association could not be found in Danish individuals (12), or Swedish males (13).

Hypercortisolism patients present visceral obesity as a marked feature, although BMI in the same individuals is not always higher. Higher sensitivity to glucocorticoids may also be a predominant influence on abdominal adiposity distribution, as demonstrated by Dobson and cols., 2001 (14), who showed the association between N363S polymorphism and waist-hip ratio in men. However, that has not been proven by other studies, which

also used only anthropometric measures for the assessment of body fat distribution (10,13). Nowadays, it has also been demonstrated that the best method for the assessment of visceral adiposity is abdominal computed tomography (CT) scan (15) – not used in any study with carriers of this kind of polymorphism. The purpose of this study was to verify whether N363S polymorphism could be associated to visceral adiposity when assessed by abdominal CT scan on phenotypes associated with a metabolic syndrome such as insulin resistance, systemic arterial hypertension, increased levels of plasminogen 1 activator inhibitor (PAI-1).

METHODS

Study Population

This study was approved by the Ethics Committee and all participants signed informed consent form. The assessment included 295 individuals, of whom 195 are obese, and 100 are of normal weight according to BMI values. In the obese individuals (n = 195) group, 168 were females (86.1%) and 27 were males (13.9%). All participants were healthy individuals, in the age range of 18-55 years, and BMI ≥ 30 kg/m² and < 40 kg/m², with central distribution of adiposity (abdominal circumference ≥ 80 cm and waist-hip ratio > 0.8 in women, and abdominal circumference ≥ 94 cm and waist-hip ratio > 0.95 in men). Individuals of Asian ancestry or those reporting serious systemic conditions or persons with a history of systemic use of glucocorticoid in the previous 5 years were excluded.

Genetic analysis

Genomic DNA was extracted from peripheral blood samples by standard procedures. The N363S SNP was examined by a restriction fragment length polymorphism (RFLP) technique following polymerase chain reaction (PCR) amplification with primers 5'-TGCCATTTCTGTTCATGGTG-3' (forward) and 5'-CTGAACTTCCCTGGTCTGAAC-3' (reverse). PCR conditions were initial denaturation at 94 °C for 4 min, and 35 cycles at 94 °C for denaturation, 58 °C for annealing and 72 °C for extension, each step lasting 1 min, with a final extension of 7 min at 72 °C. Amplification yielded a 210-bp fragment that contained two Tsp509I restriction sites (117+74+19 bp) for the A-allele (N363) and only one (117+93 bp) for the G-allele (363S). Following enzymatic digestion, PCR products were resolved on 3% agarose gel electrophoresis and visualized by ethidium bromide staining.

Study protocol

All individuals were submitted to genetic analysis for the identification of N363S SNP. Based on genotype identification, N363S SNP obese carriers were carefully paired with non-carriers, at a 1:5 ratio following gender, race, age group, body weight, height and BMI, and were submitted to regional body fat measurements with CT scans, in addition to analysis of metabolic parameters such as glycemia, insulin, HOMA-IR, total cholesterol and fractions, triglycerides, APOB, blood pressure and PAI-1.

In order to determine intra-abdominal and abdominal subcutaneous adiposity, a CT scan was carried out following the technique standardized by Sjöström and Kvist in 1988 (16). Plasma glucose level was determined by Roche Cobas Integra Model, through colorimetric enzyme assay, using hexokinase. Serum insulin concentration was determined by radio immunoassay by Linco Research, Inc. (USA) HI-14K. Analysis of insulin resistance was carried out through the homeostasis model assessment (HOMA) using the following formula: insulin resistance (IR) = insulin ($\mu\text{U}/\text{mL}$) x glucose (mmol/L)/22.5 (17). Total cholesterol (TC), HDL-C and plasma triglyceride (TG) levels were determined by COBAS MIRA automated system (F. Hoffmann-La Roche, Basel, Switzerland), by Roche's commercial enzyme kits (Mannheim, Germany). PAI-1 plasma concentration was determined by IMUNOBIND® Plasma PAI-1 ELISA using the CPT 85400 kit by American Diagnostica Inc. (USA).

Statistical analysis

Results were expressed as mean \pm standard deviation (SD) for quantitative variables. Absolute and relative frequencies were calculated for qualitative variables. Student's *t*-test was used for equality hypothesis between averages in both groups. Fisher's exact test was used for group homogeneity testing compared to ratios. The significance level used for testing was 5%.

RESULTS

A group of 295 individuals was examined for the N363S polymorphism, and 10 heterozygotes were identified (A/G genotype frequency was 3.4%; G-allele frequency was 1.7%). The obese individuals presented a double frequency of N363S SNP as compared to normal body weight individuals (Table 1). Waist, waist-hip, abdominal visceral adiposity (VA) and abdominal subcutaneous adiposity (SA), as well as the ratio VA/SA were compared be-

tween N363S SNP carriers and their paired controls, and no differences were found between the groups (Table 2).

Metabolic parameters between N363S SNP carriers and paired non-carriers can be found in Table 3. In patients with N363S SNP a tendency to higher insulin resistance by HOMA-IR assessment, higher levels of systolic blood pressure (SBP) and lower HDL-cholesterol levels was noted.

Table 1. Genotype frequencies according to BMI levels

BMI	N363/N363 (genotype AA)	N363/363S (genotype AG)
< 25 kg/m ²	98 (98.0%)	2 (2.0%)
≥ 30 kg/m ²	187 (95.9%)	8 (4.1%)
Total population	285 (96.6%)	10 (3.4%)

Table 2. Demographic and anthropometric characteristics according to genotype

	N363/363S (genotype AG)	N363/N363 (genotype AA)	P
Subjects	8	40	
Females	100%	100%	
Age (years)	35.0 \pm 7.9	33.8 \pm 6.3	0.64
Body weight (kg)	88.6 \pm 7.0	88.9 \pm 7.2	0.93
Height (m)	1.59 \pm 0.04	1.58 \pm 0.04	0.96
BMI (kg/m ²)	35.3 \pm 2.4	35.4 \pm 2.6	0.88
Fat mass (kg)	38.2 \pm 3.7	36.9 \pm 5.2	0.51
Fat mass (%)	43.1 \pm 2.9	41.4 \pm 3.8	0.25
Abdominal subcutaneous area (cm ²)	479.3 \pm 78.8	470.7 \pm 101.2	0.82
Abdominal visceral area (cm ²)	145.8 \pm 49.9	147.7 \pm 48.8	0.92
VA/SA Ratio	0.32 \pm 0.14	0.33 \pm 0.13	0.82

(mean \pm SD)

Table 3. Metabolic profile according to genotype

	N363/363S (genotype AG)	N363/N363 (genotype AA)	P
Subjects	8	48	
Glucose (mg/dL)	88.0 \pm 10.4	87.0 \pm 8.3	0.52
HOMA-IR score	8.0 \pm 3.2	6.6 \pm 2.1	0.07
Total cholesterol (mg/dL)	174.8 \pm 35.2	171.9 \pm 23.0	0.82
HDL-cholesterol (mg/dL)	47.2 \pm 13.6	42.0 \pm 5.3	0.08
LDL-cholesterol (mg/dL)	104.5 \pm 32.7	103.2 \pm 21.8	0.92
Triglycerides (mg/dL)	115.5 \pm 48.5	133.8 \pm 65.7	0.36
APO B (mg/dL)	98.3 \pm 20.2	90.4 \pm 22.5	0.36
PAI-1 (mg/mL)	51.5 \pm 6.7	51.7 \pm 7.4	0.94
SBP (mmHg)	125.0 \pm 10.7	117.0 \pm 11.8	0.08
DBP (mmHg)	84.4 \pm 11.2	79.5 \pm 7.8	0.14

SI conversion factors: to convert serum glucose to mmol/L, multiply by 0.0555; total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

DISCUSSION

In this population the A/G genotype frequency was 3.4%: one of the lowest described for this type of polymorphism when compared to other distinct populations. The prevalence of this type of polymorphism has ranged between 3.9% and 9.3% for Caucasian populations of European origin (8-14), and has reached 19.8% for Australian individuals in Sidney (18). On the other hand, a recent description has reported extremely low frequency for this polymorphism in South Asian individuals living in England (19). Therefore, it seems there is some variation in polymorphism prevalence based on ethnic background. This study did not include Asian ancestry individuals, which could contribute to low polymorphism prevalence in our environment. However, high miscegenation in our country (Brazil) makes the analysis of ethnic influence on individuals' genotypes more difficult to be carried out.

GR's N363S SNP has been associated with obesity, but few studies have assessed the association with body adiposity distribution. In 2001, Dobson and cols. (14) reported increased waist-hip ratio between polymorphism carriers and non-carriers. A CT scan is the golden standard to determine visceral adiposity levels. This is the only study in literature to have assessed the association between N363S SNP and visceral adiposity through abdominal CT scan. Study results have shown no differences in abdominal visceral adiposity levels between polymorphism carriers as compared to paired non-carriers. The fact that no association was found may be an indication that the G-allele has little influence on visceral adiposity deposits in the Brazilian population at least at baseline. Perhaps some interventions – such as use of glucocorticoids at pharmacological dosing, or exposure to fat rich diets – may show differences in visceral adiposity levels in N363S SNP carriers.

Other studies have shown that excess of cortisol is associated with insulin resistance (20,21). There are different techniques to assess insulin sensitivity (22,23). This study assessed insulin resistance through HOMA-IR, which has reported positive correlation with hyperinsulinemic euglycemic clamp (24). The present study has shown a tendency of higher insulin resistance through HOMA in N363S SNP carriers.

Hypercortisolism patients presenting different changes in the metabolism of lipoproteins, including increased plasma triglyceride levels, reduced HDL-cholesterol levels, and increased APO B levels (25) have been

reported. This study has detected a tendency towards lower HDL-cholesterol levels in N363S SNP carriers. In 1999, Fraser and cols. (26) investigated the association between cortisol and cardiovascular risk factors in a Scottish population and found a significant inverse relationship between cortisol and HDL-cholesterol. The author suggested cortisol may affect peripheral cholesterol metabolism due to changes in HDL-cholesterol formation. The 363S allele may lead to lower HDL-cholesterol levels through that mechanism.

It has been described that adipocytes secrete PAI-1 (27). The present study has found that obese patients presented quite high PAI-1 levels, but no differences were found when comparing polymorphism carriers and non-carriers. PAI-1 secretion is known to be higher at omental adiposity deposits – even after a dexamethasone stimulus (28). No difference in visceral adiposity levels between N363S SNP carriers and non-carriers was detected by the present study. Therefore, this may have contributed to the fact that no differences in PAI-1 serum levels were found between the groups.

Glucocorticoid in excess may lead to systemic hypertension and cardiovascular adverse effects (29). This study has shown a tendency for SBP higher levels in N363S SNP carriers. The mechanisms through which glucocorticoids cause hypertension are not yet fully understood. Cortisol may directly increase vascular resistance and intravascular volume, thus increasing blood pressure (30). However, such salt and water retention mechanism as a result of glucocorticoid action seems to be induced by cortisol binding to type 1 mineralocorticoid renal receptors. The other glucocorticoid-induced blood pressure increase mechanism is indirect, resulting from insulin resistance. It is known that insulin resistance sponsors hypertension, especially as a result of sympathetic nervous activity, thus causing higher vascular resistance and higher water retention, thus contributing to blood pressure increase (31). Study data – showing a tendency to higher blood pressure levels in addition to higher insulin resistance in N363S SNP carriers – suggest both phenomena may be associated in the study population.

Therefore, N363S SNP carriers in this population showed proneness to unfavorable metabolic changes, such as increased insulin resistance, increased blood pressure levels, and decreased HDL-cholesterol levels, irrespective of visceral adiposity increase.

To conclude, the impact of this polymorphism is small in the study population as prevalence is only 3.4%, although its presence may contribute to the worsening of an individual's metabolic profile.

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