Association between the -420 C> G polymorphism (rs1862513) in the human resistin gene and obesity in a sample of the Brazilian population

Associação entre o polimorfismo -420 C>G (rs1862513) no gene da resistina humana e obesidade em uma amostra da população brasileira

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Adaline Franco Rodrigues
Master's degree in Health Applied Sciences
Laboratory of Genetics and Molecular Biology, Academic Unit of Health Sciences, Graduate Program in Health Applied Sciences, Federal University of Jataí – UFJ, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, Goiás
E-mail: adalinefranco@gmail.com

Mohara Bruna Franco
Graduate in Biomedicine
Laboratory of Genetics and Molecular Biology, Academic Unit of Health Sciences, Department of Biomedicine , Federal University of Jataí – UFJ, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, Goiás
E-mail: moharabfc@gmail.com

Kyrla Cartynalle das Dores Silva Guimarães
Nursing student
Laboratory of Genetics and Molecular Biology, Academic Unit of Health Sciences, Department of Nursing , Federal University of Jataí – UFJ, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, Goiás
E-mail: kynally83@hotmail.com

Vitor Gabriel Felipe
Biomedicine student
Laboratory of Genetics and Molecular Biology, Academic Unit of Health Sciences, Department of Biomedicine, Federal University of Jataí – UFJ, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, Goiás
E-mail: vitor.gabriel.felipe@gmail.com

Wagner Gouvêa dos Santos
Ph.D in Biochemistry and Immunology
Laboratory of Genetics and Molecular Biology, Academic Unit of Health Sciences, Department of Biomedicine, Federal University of Jataí – UFJ, Câmpus Jatobá, Cidade Universitária, BR 364, km 195, nº 3800, CEP 75801-615, Jataí, Goiás
E-mail: wagner_santos@ufg.br

ABSTRACT

Purpose: Obesity became a serious public health problem in the last decades and is associated with several comorbidities such as type 2 diabetes, cardiovascular diseases, and some types of cancer. Adipose tissue cells produce various proinflammatory cytokines including resistin (RETN). Several studies have shown that RETN polymorphisms may be potentially related to the predisposition to obesity, but the results are contradictory. Herein we aimed to investigate the frequency of the RETN -420 C>G (rs1862513) polymorphism and its association with obesity in a sample of adults of both sexes residing in the city of Jataí - GO, Brazil.

Subjects and Methods: This cross-sectional study was conducted with 117 participants from which 72 were genotyped for RETN polymorphism. Anthropometric measurements such as body mass index (BMI) and waist circumference (WC) were obtained and used to classify individuals in eutrophic, overweight and obese. Systolic and diastolic blood pressure (SBP and DBP) were measured and lipidic and fasting blood glucose profiles were determined. Polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: There was no statistical difference between the RETN -420 C >G polymorphism and the variables BMI and lipidic profile. However, there was a significant difference in the frequency of C/C genotype in the group with WC values associated with high risk of cardiovascular disease compared to the lower risk group both in the codominant model (C/C; C/G, G/G) and in the dominant genetic model (C/C, C/G + G/G).

Conclusion: RETN -420 C >G polymorphism is not associated with obesity in the sample of Brazilian population analyzed in this study, however the RETN C/C genotype may be associated with cardiovascular risk and deserves further investigation.

Keywords: PCR-RFLP, RETN, Waist Circumference, Metabolic Syndrome, Body Mass Index.

RESUMO

Objetivo: A obesidade tornou-se um grave problema de saúde pública nas últimas décadas e está associada a várias comorbidades, como diabetes tipo 2, doenças cardiovasculares e alguns tipos de câncer. As células adiposas do tecido produzem várias citocinas pró-inflamatórias, incluindo a resistência (RETN). Vários estudos mostraram que os polimorfismos RETN podem estar potencialmente relacionados à predisposição à obesidade, mas os resultados são contraditórios. Neste estudo, objetivamos investigar a freqüência do polimorfismo RETN -420 C>G (rs1862513) e sua associação com a obesidade em uma amostra de adultos de ambos os sexos residentes na cidade de Jataí - GO, Brasil.

Temas e Métodos: Este estudo transversal foi realizado com 117 participantes dos quais 72 foram genotipados para o polimorfismo RETN. Medidas antropométricas como índice de massa corporal (IMC) e circunferência da cintura (CC) foram obtidas e utilizadas para classificar indivíduos em eutróficos, sobrepeso e obesos. A pressão arterial sistólica e diastólica (SBP e DBP) foram medidas e foram determinados os perfis lipídicos e de glicemia em jejum. Os polimorfismos foram determinados pelo polimorfismo de reação em cadeia da polimerase - comprimento do fragmento de polimorfismo (PCR-RFLP).

Resultados: Não houve diferença estatística entre o polimorfismo RETN -420 C >G e as variáveis IMC e perfil lipídico. Entretanto, houve uma diferença significativa na freqüência do genótipo C/C no grupo com valores de CC associados a alto risco de doença cardiovascular em comparação com o grupo de menor risco tanto no modelo codominante (C/C; C/G, G/G) quanto no modelo genético dominante (C/C, C/G + G/G).

Conclusão: O polimorfismo RETN -420 C >G não está associado à obesidade na amostra da população brasileira analisada neste estudo, porém o genótipo RETN C/C pode estar associado ao risco cardiovascular e merece mais investigação.

Palavras-chave: PCR-RFLP, RETN, Circunferência da Cintura, Síndrome Metabólica, Índice de Massa Corporal.

1 INTRODUCTION

According to the World Health Organization more than 1.9 billion adults worldwide (39%), 18 years and older, are overweight and over 650 million (13%) are obese.[1] In the last decades, obesity has become a serious public health problem mainly because it increases the risk of chronic diseases such as cardiovascular disease, hypertension, type 2 diabetes, and several cancers.[2,3] Additionally, it has been reported that the economic burden in healthcare expenditures is greater in obese individuals than in their normal weight peers.[4,5]

The pathophysiology of obesity is complex, and many factors are involved including environmental, sociocultural, physiological, behavioral, genetic and epigenetic.[6,7] Excessive fat accumulation occurs, most of the time due to a chronic dysfunction of the body's energy balance led by a constant imbalance between food consumption and energy expenditure.[8,9] However, Data from genome-wide association studies suggest a genetic predisposition, with the identification of more than 140 chromosomal regions related to obesity.[10] Furthermore, gene expression related to body mass index (BMI) and general adiposity has been shown to be highly enriched in the central nervous system.[11] However, only a few genes with large effect size has been identified such as genes related to leptin and melanocortin signaling. Thus, it is believed that in contrast to monogenetic diseases, common obesity seems to be associated with many genes with small effect sizes. On the other hand, it has been largely accepted that obesity results from an interaction between environment/lifestyle and genetic susceptibility.[12,13]

It is now well recognized that adipose tissue, besides serving as depot of energy is also an endocrine tissue able to produce several biological mediators that regulates blood pressure, appetite, glucose homeostasis and immune function.[14] Also, adipose tissue produces both pro- and anti-inflammatory cytokines that promotes local and systemic inflammation, as well as adipokines that functions as hormones.[15]

Resistin, also known as FIZZ3 (Found in Inflammatory Zone 3) is a pro-inflammatory adipokine, characterized as a cysteine-rich polypeptide hormone with 108

amino acids and a molecular weight of 12.5 kD.[16,17] It belongs to the family of resistin-like molecules (RELM) and is secreted by monocytes and adipocytes[16]. Human resistin gene (RETN) is located on chromosome 19p3.2 and the encoded protein may form oligomers that can circulate in the human serum in several different low molecular weight and high molecular weight isoforms.[18,19] Resistin protein levels have shown to be elevated in obese mouse and decreased by insulin sensitizing thiazolidinediones.[16] It was also shown that in obese mouse model, although mRNA resistin expression is suppressed, the circulating resistin level is significantly increased and positively correlated with insulin, lipids and glucose.[20] In addition, several studies have shown a positive correlation between obesity, insulin resistance and elevated serum resistance in humans.[21-23] In contrast, there are reports showing no correlation between resistin status and obesity demonstrating the controversy on the real relationship of this gene and obesity.[24-26] Several genetic polymorphisms have been identified in the resistin gene both in promoter region as well as in introns and 3'UTR (untranslated region). Considering that the link between resistin polymorphisms, obesity and metabolic syndrome is not clear, herein we investigated if the identified single nucleotide polymorphism (SNP) -420C>G (rs1862513) in the promoter region of the resistin gene is associated to obesity in a sample of Brazilian population.

2 MATERIAL AND METHODOS

2.1 STUDY DESIGN AND SUBJECTS

This is a cross-sectional study conducted with 117 participants over 18 years old from both sexes that were recruited in the Regional Blood Center, Jataí city, Goiás, Brazil in the first semester of 2017. Of these 72 participants were investigated for the presence of the RETN polymorphism. The research protocol was approved by the Ethical Research Committee of the Federal University of Goiás, UFG-Brazil (protocol: 1.500.297/2016) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject after explanation of the nature of the study.

2.2 ANTHROPOMETRIC PARAMETERS

Weight and height were measured for each patient included in the study. The Body Mass index (BMI) was calculated by applying the formula: BMI = weight(kg)/height(m2). Waist circumference (WC) was measured in the horizontal plane midway between the lowest ribs and the iliac crest. Cardiovascular disease risk based on

WC measures was classified according to Lean et al, (1995)27 as level 1 (Moderate risk): WC ≥ 94 cm and ≥ 80 cm for men and women respectively; level 2 (Higher risk): WC ≥ 102 cm and ≥ 88 cm for men and women respectively. Systolic and diastolic blood pressure (SBP and DBP) were obtained considering as normal SBP of 90 to 140 mmHg and DBP of 60 to 90 mmHg. A standard questionnaire was applied to evaluate the clinical parameters and medical history. Individuals were classified as obese, overweight, or eutrophic according to the International Diabetes Federation (BMI ≥30 kg/m2, 25 ≤ BMI< 30 and 18,5≤ BMI <25 respectively).

2.3 BIOCHEMICAL ANALYSIS

Serum samples were obtained from participants and biochemical assays were performed by using enzymatic colorimetric assay and commercial kits at the clinical laboratory at the Regional Blood Center of Jataí city after physician prescription. The following components were measured in the serum samples: glucose (GLU), total cholesterol (CT), triglycerides (TG), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The LDL was calculated using the Friedewald equation: LDL (mg/dl) = CT - HDL - TG/5 as described by Fukuyama et al, (2008)28. Reference values established for glucose and lipids were considered according to the American Heart Association: GLU (70-99 mg/dl), CT (< 200 mg/dl), TG (< 150 mg/dl), LDL (< 100 mg/dl) and HDL (40–60 mg/dl).

2.4 DNA EXTRACTION

Blood samples were drawn from the antecubital vein following a 10 -12 h overnight fast and collected into a Vacutainer® Plus plastic sterile tube containing EDTA K3 anticoagulant. Total genomic DNA was extracted from white blood cells according to the protocol described by Bartlett and White (2003)29.

2.5 GENOTYPING PCR-RFLP

RETN genotyping was determined for 72 participants of the study using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The following set of primers were used: Forward- 5´-TGTCATTCTCACCCAGAGACA-3; and reverse 5´-TGGGCTCAGCTAACCAAATC-3´ as described by Kunnari et al, (2005) and Takhshid et al, (2015). 30,31 The amplification was performed in a volume of 25 □l reaction containing 100 ng DNA, 1

mM MgCl2, 0.2 mM dNTPs, 0.5 □M primers and 2U Taq polymerase in 75 mM Tris-HCl, 50 mM KCl, 20 mM de (NH4)2SO4, pH 9.0) buffer. The PCR conditions consisted of initial denaturation at 95 oC for 5 min followed by 35 cycles of denaturation at 95 oC for 1min, annealing at 58 oC for 1 min, extension at 72 oC for 1 min and a final extension at 72 oC for 5 min. The 533 bp PCR products were digested with the restriction endonuclease BbsI for 16 hours at 37 oC in 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl2, 100 □g/ml BSA, pH 7.9 buffer. Then, the digested PCR products were analyzed on a 2% agarose gel stained with ethidium bromide. The G allele was visualized as 533bp band whereas the C allele yield two fragments (329 bp and 204 bp).

2.6 STATISTICAL ANALYSIS

Statistical analysis was performed by using the Action 3.0 software statistical package. The normality of the data was assessed by the Kolmogorov-Smirnov test. The Chi-squared test was used to compare categorical variables according to the frequencies of the determined genotypes and to expectations of the Hardy Weinberg equilibrium (HWE). Continuous variables were compared using the t-test or Mann-Whitney. To analyze the probability of independence or association of the presence of SNP -420 C> G and the risk of developing obesity, as well as for correlations between body mass index, waist circumference, lipid profile and the SNP it was used the multivariate analysis of variance (MANOVA) considering the general linear model. Results were expressed as mean ± standard deviation, unless noted otherwise. P-values < 0.05 (two-tailed) were considered statistically significant.

3 RESULTS

3.1 SUBJECTS CHARACTERISTICS

The total number of participants in the research comprised a sample size of 117 individuals, consisting of 75 women (64.10%) and 42 men (35.9%). The mean age, body mass index, waist circumference as well as systolic and diastolic blood pressure were similar for men and women in the sample analyzed with no statistically significant differences except for waist circumference . The mean age of both groups was approximately 30 years, with a minimum age of 18 years and a maximum age of 61 years. The mean body mass index (BMI) was close to 26, with minimum value of 15.3 and maximum 39. Of all anthropometric variables analyzed only the WC values were significantly higher for men compared to women (p=0.0047) **Table 1**. The participants in

the study were divided into groups classified as malnourished, eutrophic, overweight or obese according to the calculated BMI Most of the individuals showed BMI greater than 25 Kg/m^2, which includes the overweight and obese individuals (56.4%). Obesity (BMI≥ 30 Kg/m^2) was more frequent in women (24%) compared to men (16.7%) although these differences between men and women were not significant statistically (p=0.397) as shown in **Table 2**. The BMI values obtained for the different groups after stratification by genre did not show statistically significant differences using the Mann-Whitney test considering a significance level of 5%. The mean value for the BMI group ≤ 25 was approximately 21 Kg/m^2 for both sexes with a minimum value of 15.3 in the women group and 18.37 in the men group. The mean value for the group 25 < BMI ≤ 30 was 26.97 Kg/m^2 considering the whole population and similar value was obtained when both sexes were analyzed separated. Also, no differences were observed between men and women presenting BMI > 30 Kg/m^2 where the mean value was approximately 33 Kg/m^2 (**Table 3**).

The waist circumference measurements data showed that 51.3 % of the analyzed population were at risk for cardiovascular disease (WC ≥ 80 cm for women and ≥ 94 cm for men) including 58.7% of the women and 38.1% of men. Considering these combined frequencies for moderate and high risk of cardiovascular disease, women were significantly at higher risk than men (**Table 4**).

To evaluate if there are differences in the group of individuals with BMI < 25 Kg/m^2 and BMI ≥25 Kg/m^2 regarding the WC, SBP and DBP we compared all these parameters values in both groups (**Table 5**). There were statistically significant differences between the two groups for all the three variables at the level of significance of 5%. However, when we separate the population by sex only the WC values were significant between the two groups, what was expected since male WC values are physiologically higher than women, mainly if we consider the different established values for cardiac risk between genders (WC ≥ 80 for women and ≥ 94 for men) (**Table 6**).

3.2 RETN POLYMORPHISM FREQUENCY AND ASSOCIATION WITH OBESITY MARKERS

From the initial 117 subjects participating in the study, only 72 accepted to have molecular analysis performed with their blood sample provided, therefore this was also the sample used for genotyping of the RETN gene. The most frequent genotype was the heterozygous C/G (51,4%), followed by the homozygous containing the SNP G/G (29.2 %) and the homozygous wild type C/C (19.4%) (**Table 7**). RETN -420C>G

polymorphism was analyzed evaluating three genetic models: dominant (C/C and C/G + G/G); codominant (C/C, C/G and G/G) and recessive (C/C + C/G, G/G) in relation to anthropometric and biochemical values. The analysis of genotypic frequencies in the population sample when it was divided into two groups based on the values of BMI (BMI ≤ 25 Kg/m^2 and BMI >25 Kg/m^2) is shown in the **Table 8**. There were not statistically significant differences in the allelic frequencies in the two groups nor in the genotype frequencies even considering different genetic models. However, the odds ratio for the allele C was 1.33 considering a confidence interval of 95%. In the dominant genetic model, the odds ratio was 2.87 for the genotype C/C associating a BMI >25 Kg/m^2 with the genotype C/C. Moreover, considering the allelic and genotype frequencies in the two groups separated by genre there were not statistically significant differences between the frequencies of the genotypes in men and women (**Table 9**).

The analysis of allelic and genotypic RETN – 420 C>G polymorphism frequencies related to the values of WC converted into risk of cardiovascular disease are shown in **Table 10**. Moderate risk is associated with WC ≥ 80 cm for women and ≥ 94 cm for men, whereas high risk is associated with WC ≥ 88cm for women and ≥ 102 cm for men. The C/C genotype was more frequent in the group with risk for cardiovascular disease in both codominant and dominant genetic model (p=0.02 and p=0.005 respectively). However, when we combined the genotypes frequencies of moderate and high risk for cardiovascular disease and compared with the frequencies of lower risk, no statistical differences were observed (p=0.292, c^2=2.46).

The association analysis between RETN – 420 C>G polymorphism and the variables blood pressure and fasting glucose levels are shown in **Tables 11** and **12**. The participants were divided into two groups for each variable: reference blood pressure (BP < 140x90 mmHg) and high blood pressure (BP ≥ 140x90) or reference fast glucose levels (<100mg/dl) and hyperglycemic (≥ 100mg/dl). There were not statistically significant differences in none of the variables analyzed in relation to the allelic or genotypic frequencies using different genetic models. Also, there was not association between the polymorphism -420 C > G and the lipidic profile among participants with BMI ≤ 25 Kg/m^2 and BMI ≥25 Kg/m^2 using different genetic models, however as expected there was differences between lipidic profile and BMI where the participants with BMI ≥25 Kg/m^2 showed unfavored values towards CT, LDL, HDL and TG (**Table 13**).

4 DISCUSSION

Obesity is currently considered one of the major epidemiologic challenges worldwide. Additionally, obesity is associated with the incidence of several diseases such as diabetes, hypertension, cardiovascular diseases, and cancer. Body mass index (BMI) has been used to predict adverse cardiovascular outcomes, but it has limitations when used as a single predictor.[32,33] However, BMI associated with waist circumference and other anthropometric measurements has shown to strengthen its predictive power.[34] We studied a population from the Brazilian city Jataí, located on the southwestern part of the State of Goiás in the Center-West Region of Brazil. We showed that 57% of the subjects are overweight or obese based on BMI value, which corroborate the global concerns related to obesity becoming an epidemic. More than 51% of the subjects showed waist circumference measurements associated with risk to develop cardiovascular disease, a very alarming indicator. Moreover, the assessed cardiovascular risk was higher for women compared to men. These data are similar to the ones reported in studies done with populations from different regions of Brazil like Bahia State and Pernambuco.[35,36] The significant increased systolic blood pressure in overweight and obese individuals demonstrate the evidence of association between waist circumference and BMI with increased blood pressure. This shows the importance of the distribution of body adiposity, where the waist adiposity seems to be an active element that influences insulin resistance and may result in metabolic syndrome and cardiovascular risk in obese individuals.

Inflammatory adipokines such as interleukin IL-6, tumor necrosis factor-α (TNF-α) and resistin are usually secreted by adipose tissue and therefore, obesity has been associated to chronic inflammation.[37,38] Also, it has been shown that resistin exerts several pleiotropic biological effects through endocrine, paracrine and autocrine pathways.[39-41] There are conflicting results in the literature regarding the relationship of RETN −420C>G polymorphism with obesity. It has been shown that this polymorphism is associated with levels of resistin mRNA in overweight individuals.[42] Some studies show a positive correlation between this polymorphism and insulin sensitivity, BMI and obesity [16,43]; while other studies show no association.[24,25] A meta-analysis study that included a Mexican population among 10 studies with populations from different countries showed increased risk of obesity among carriers of the G allele of the RETN −420C>G polymorphism considering the heterozygous (CG) and dominant (CG + GG) models. However, when the Mexican population was examined separated no association was found.[44] On the other hand, another meta-analysis done by Chinese researchers did

not found association between RETN-420 C>G polymorphism and obesity in none of the genetic models evaluated.[45] In fact, only when they omitted one of the studies used in the meta-analysis it was possible to observe a significant association within the allelic model and the dominant genetic model. The authors claimed that this particular omitted study [46], although described a gender-specific association of -420 C>G polymorphism with BMI and waist circumference, it was restricted to the premenopausal women, which was considered to have had influence in the results after sensitivity analysis. Our results demonstrated that in the Brazilian population sample composed of 72 individuals from both sexes the allelic frequencies were C = 45.1% e G = 54.9%. The genetic model heterozygous (C/G) was the most frequent (51.4%), followed by the homozygous (G/G), 29.2% and homozygous (C/C), 19.4%. These frequencies are different from those reported for populations from different countries. For instance, in the Mexican population described by Montiel-Tellez et al, 2016,[44] the frequencies for C/G, G/G and C/C were 16.4%, 3.4% and 80.2% respectively. On the other hand, in a Chinese population sample, the frequencies of C/G, G/G and C/C, reported by Fu et al, (2017) [47] were respectively 43.9%, 45.9% and 10.8%.

Our results showed no association between the -420 C>G polymorphism and obesity (BMI ≥25 Kg/m^2) in none of the genetic models analyzed indicating that this polymorphism independently does not influence the body mass index. This finding agrees with other data published in the literature for different populations.[25,44,47] However, when we analyzed the association of the polymorphism with the waist circumference measures, we found that the C/C genotype was significantly more frequent in the group with elevated risk for cardiovascular risk (WC ≥ 88 cm for women and ≥ 102 cm for men). This was significant for both codominant (p=0.002) and dominant genetic model (p=0.005) suggesting that the C allele may be associated with increase accumulation of fat in the abdominal region and consequently increase in the waist circumference. This is an important finding if we consider the correlation between waist circumference and the risk for cardiovascular disease. In this case our results suggest that the wild type RETN genotype (CC) would have a negative impact on the risk of cardiovascular disease and the polymorphism -420 C>G would be protective. Interestingly, Bouchard et al, (2004)[48] showed that in men, carriers of the G allele in homozygosis has less visceral fat than carriers of the C allele, what agrees in part with our results. Nevertheless, the mechanism by which the wild type (C) version of RETN -420C>G may lead to predisposition to cardiovascular disease needs to be better investigated. The polymorphism RETN -420

C>G is located in the promoter region of the gene and may interfere with binding of transcription factors and ultimately transcription of the gene. High serum resistin has been shown to be a risk factor for cardiovascular disease and all-cause mortality in diabetic patients of European ancestry.[49] Therefore, although -420 C>G polymorphism independently may not be associated with obesity directly, it may be a contributing factor for the comorbidities associated with obesity. Of note, Suriyaprom et al, 2015,[50] found that resistin concentrations were significantly higher in metabolic syndrome diagnosed subjects than in healthy controls. However, among the metabolic syndrome diagnosed subjects, those with the G allele (CG/GG) presented higher resistin plasma concentrations than subjects with CC genotype in a population from Thailand. Despite this association, there was no difference in the frequencies of any genotypes from healthy controls when compared to the metabolic syndrome group. In contrast, in another study with Tunisian population there was no association between the -420 C>G polymorphism and the levels of circulating resistin, however when the data was adjusted for cofounding factors such as age, gender, smoking status, diabetes, dyslipidemia and cardiovascular disease, there was a significant association between the polymorphism and obesity.[51] In our study, there was no significant differences in the frequencies of RETN polymorphism neither in the allelic model nor in the genotype model when we compared obese and eutrophic group regarding the lipidic profile. Therefore, our results suggest that resistin polymorphism alone is not associated with obesity. The contradictory results found in the literature may be due to differences in ethnic origin of the population studied. Also, obesity is a multifactorial disease where genetic, epigenetic, metabolic, and environmental factors are involved. Nevertheless, it would be important to investigate what genes in combination with resistin could contribute significantly to the obesity and its known consequences. Moreover, it is an open question if different combinations of all known different polymorphisms in the resistin gene could be associated to predisposition of obesity. In this context, it would be important to investigate the frequencies and the effect of these combined polymorphisms in obese individuals. Thus, we will have a more conclusive information on the effective role of RETN gene in the pathophysiology of obesity. This type of analysis started to be performed and was reported for three polymorphisms (-167C>T, +157C>T, and -299G>A) identified in Japanese subjects with type 2 diabetes but did not included -420 C>G.[52] The role of resistin polymorphisms in the pathophysiology of obesity is still a subject of debate and may depend on ethnic

differences and other genetic and epigenetic factors. Therefore, this field of research warrants further investigation using large populations and multivariate analysis.

5 CONCLUSION

RETN -420 C>G polymorphism was not associated with obesity in a sample of Brazilian population; however, it may be a protective factor for cardiovascular disease.

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ANEXOS

Table 1. Anthropometric and physiologic parameters of the total population analyzed

Variable	Total (n=117)		women(n=75)		men (n=42)		*p*
	Mean ± sd)		Mean ± sd		mean ± sd		
Age (years)	30.23	± 11.17	30.68	± 11.41	29.43	± 10.85	0.5452
BMI (Kg/m^2)	25.92	± 4.97	26.04	± 5.26	25.77	± 4.44	0.9208
WC (cm)	86.73	± 13.13	84.49	± 13.61	90.74	± 11.2	0.0047*
SBP (mmHg)	117.39	± 11.27	116.57	± 11.24	118.86	± 11.32	0.2906
DBP (mmHg)	79.24	± 10.11	78.71	± 10.06	80.19	± 10.24	0.5587
Hypertension n (%)	13 (11.11)		8 (10.7)		5 (11.9)		-

p values were analyzed using Mann-Whitney test with a confidence interval of 95% and significance of $p < 0.05$. BMI=Body mass index, WC=waist circumference, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure.

Table 2. Classification and frequency of different groups in the population distributed according to BMI values

Classification*	Population	Women	Men	*p*
	n (%)	n (%)	n (%)	
Malnourished	3 (2.6)	3 (4.0)	0 (0)	
Eutrophic	48 (41.0)	30 (40.0)	18 (42.9)	
Overweight	41 (35.0)	24 (32.0)	17 (40.5)	
Obese	25 (21.4)	18 (24.0)	7 (16.7)	
Total	117 (100)	75 (100)	42 (100)	0.397

*Malnourished BMI ≤ 18 Kg/m^2; Eutrophic 18 Kg/m^2 < BMI <25 Kg/m^2; Overweight 25 Kg/m^2 ≤ BMI < 30 Kg/m^2; Obesity BMI ≥ 30 Kg/m^2. Data was analyzed using chi-square test or Mann-Whitney test. A value of $p < 0.05$ was considered statistically significant.

Table 3. Distribution of the population according to the body mass index values

Variable (kg/m^2)	**Total (n=117)**	**Women(n=75)**	**Men (n=42)**	*p*
	Mean ± sd	Mean ± sd	Mean ± sd	
BMI ≤25	21.57 ± 2.13	21.39 ± 2.16	21.85 ± 2.09	0.715
25 < BMI ≤30	26.92 ± 1.43	26.85 ± 1.51	27.01 ± 1.35	0.573
BMI >30	33.30 ± 2.74	33.47 ± 2.73	32.83 ± 2.94	0.534

p values were analyzed using Mann-Whitney test with a confidence interval of 95% and significance of $p < 0.05$.

Table 4. Classification and frequency of risk for cardiovascular disease based on waist circumference values in men and women

Classification	Population	Women	Men	*p*	*c2*	*p*
	n (%)	n (%)	n (%)			
Low risk	57 (48.7)	31 (41.3)	26 (61.9)			
Moderate risk	34 (29.1)	24 (32.0)	10 (23.8)			
High risk	26 (22.2)	20 (26.7)	6 (14.3)			
Total	117 (100)	75 (100)	42 (100)	0.089937	4.5	0.03272*

Cardiovascular risk: WC ≥ 80 cm for women and WC ≥ 94 cm for men according to Lean *et al*. (1995). Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant. * p-value combining moderate and high risk compared to low risk.

Table 5. Comparison of waist circumference, systolic and diastolic blood pressure in the population stratified into two groups according to body mass index

Variable	BMI < 25 Kg/m² Mean ± sd	BMI ≥ 25 Kg/m² Mean ± sd	*p*
WC (cm)	77.1 ± 8.1	94.2 ± 11.3	0.0003*
PAS (mmHg)	112.2 ± 9.3	121.4 ± 11.0	0.00025*
PAD (mmHg)	75.2 ± 8.7	82.4 ± 10.1	0.0001*

Data was analyzed using Mann-Whitney test. A value of $p < 0.05$ was considered statistically significant at a confidence interval of 95%.

Table 6. Comparison of waist circumference, systolic and diastolic blood pressure between two different groups stratified per sex according to body mass index.

Variable	Population (n = 117)			BMI < 25 Kg/m² (n = 51)		
	BMI ≥ 25 Kg/m² (n = 66)					
	Women (n = 75) Mean ± sd	Men (n= 42) Mean ± sd	*p*	Women (n = 33) Mean ± sd	Men (n =18) Mean ± sd	*p*
WC (cm)	84.5 ± 13.6	90.7 ± 11.3	0.0047*	73.9 ± 7,8	82.4 ± 7.2	0.001*
	89.2 ± 13.0	97.0 ± 9.67	0.010*			
PAS (mmHg)	116.6 ± 11.2	118.9 ± 11.3	0.2906	110.9 ± 9.3	114.4 ± 10.4	
	0.258	119.1 ± 11.2	122.2 ± 11.0	0.264		
PAD (mmHg)	78.7 ± 10.1	80.2 ± 10.2	0.5587	74.3 ± 8.0	76.7 ± 9.7	
	0.407	80.6 ± 10.3	82.8 ± 10.0	0.386		

*Statistically significant ($p < 0.05$)

Table 7. Genotypic and allelic frequencies of RETN -420 C>G polymorphism

Genotype/allele	Frequency n (%)
C/C	14 (19.4)
C/G	37 (51.4)
G/G	21 (29.2)
Total	72 (100)
C-allele	65 (45.1)
G-allele	79 (54.9)
Total	144 (100)

Table 8. Association between resistin polymorphism and body mass index

Genetic model	Parameter				
	BMI ≤25 kg/m² n (%)	BMI > 25 Kg/m² n (%)	X²	*p*	OR
Alleles					
C	29 (41.4)	36 (48.65)			
G	41 (58.6)	38 (51.35)			
Total	70 (100)	74 (100)	0.757	0.384	1.33
Codominant					
C/C	4 (11.4)	10 (27.03)			
C/G	21 (60.0	16 (43.24)			
G/G	10 (28.6)	11 (29.73)			
Total	35 (100)	37 (100)	1.270	0.260	-
Recessive					
C/C + C/G	25 (71.43)	26 (70.27)			

G/G	10 (28.57)	11 (29.73)			
Total	35 (100)	37 (100)	0.01	0.913	0.94
Dominant					
C/C	4 (11.43)	10 (27.03)			
C/G + G/G	31 (88.57)	27 (72.97)			
Total	35 (100)	37 (100)	2.794	0.094	2.87

Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant.

Table 9. Genotypic frequencies in men and women distributed into two groups according to body mass index

Genotype/Sex	**Parameter**				
	BMI ≤ 25 kg/m²	**BMI > 25 Kg/m²**	**X²**	***p***	
	n (%)	**n (%)**			
Women					
C/C	3 (13.04)	8 (29.63)			0.836813
C/G	13 (56.52)	14 (51.85)			0.082424
G/G	7 (30.43)	5 (18.56)			0.256299
Total	23(100)	27(100)	2.33	0.310	
Men					
C/C	1 (8.33)	2 (20)			0.836813
C/G	8 (66.67)	2 (20)			0.082424
G/G	3 (25)	6 (60)			0.256299
Total	12 (100)	10 (100)	4.79	0.09	

BMI = Body mass index. Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant.

Table 10. Association of genotypic and allelic frequencies with the risk of cardiovascular disease based on WC measures

Genetic model	**Cardiovascular risk according to WC**					
	No risk	**Moderate risk**	**High risk**	**X²**	***p***	**OR**
	n (%)	**n (%)**		**n (%)**		
Alleles						
C	32 (43.24)	15 (35.71)		18 (64.29)		
G	42 (56.76)	27 (64.29)		10 (35.71)		
Total	74 (100)	42 (100)	28 (100)	1.996	0.369	1.17
Codominant						
C/C	5 (13.51)	2 (9.52)	7 (50.00)			
C/G	22 (59.46)	11 (52.38)	4 (28.57)			
G/G	10 (27.03)	8 (38.10)	3 (21.43)			
Total	37 (100)	21 (100)	14 (100)	11.265	0.024*	-
Recessive						
C/C + C/G	27 (72.97)	13 (61.90)		11 (78.57)		
G/G	10 (27.03)	8 (38.10)		3 (21.43)		
Total	37 (100)	21 (100)	14 (100)	1.298	0.523	0.80
Dominant						
C/C	5 (13.51)	2 (9.52)	7 (50.00)			
C/G + G/G	32 (86.49)	19 (90.48)	7 (50.00)			
Total	37 (100)	21 (100)	14 (100)	10.495	0.005*	2.21

Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant. OR-Odds ratio.

Table 11. Association of genotypic and allelic frequencies with the risk of hypertension

Genetic model	Blood Pressure			
	Normal	Hypertension	X^2	*p*
	n (%)	n (%)		
Alleles				
C	56 (45.92)	9 (54.55)		
G	72 (54.08)	7 (45.45)		
Total	128 (100)	16 (100)	0.897	0.343
Codominant				
C/C	11 (17.19)	3 (37.50)		
C/G	34 (53.13)	3 (37.50)		
G/G	19 (29.69)	2 (25.00)		
Total	64 (100)	8 (100)	1.900	0.397
Recessive				
C/C + C/G	45 (70.31)	6 (75,00)		
G/G	19 (29.69)	2 (25.00)		
Total	64 (100)	8 (100)	1.178	1.000
Dominant				
C/C	11 (17.19)	3 (37.50)		
C/G + G/G	53 (82.81)	5 (62.50)		
Total	64 (100)	8 (100)	0.801	0.371

Hypertension=Systolic blood pressure > 140 mmHg, Diastolic Blood pressure > 90 mmHg. Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant.

Table 12. Association of genotypic and allelic frequencies with fasting glucose levels

Genetic model	Fasting glucose levels			
	Normal	hyperglycemic	X^2	*p*
	n (%)	n (%)		
Alleles				
C	56 (45.45)	9 (60.00)		
G	74 (54.55)	5 (40.00)		
Total	130 (100)	14 (100)	0.298	0.1297
Codominant				
C/C	11 (16.92)	3 (42.86)		
C/G	34 (52.31)	3 (42.86)		
G/G	20 (30.77)	1 (14.28)		
Total	65 (100)	7 (100)	2.884	0.236
Recessive				
C/C + C/G	45 (69.23)	6 (85.71)		
G/G	20 (30.77)	1 (14.29)		
Total	65 (100)	7 (100)	1.310	0.3619
Dominant				
C/C	11 (16.92)	3 (42.86)		
C/G + G/G	54 (83.08)	4 (57.14)		
Total	65 (100)	7 (100)	0.801	0.0995

Data was analyzed using chi-squared test. A value of $p < 0.05$ was considered statistically significant.

Table 13. Association between lipidic profile and specific genotype/allele carriers' individuals grouped according to body mass index.

Genetic Model	BMI < 25 Kg/m² (n = 35)					BMI ≥ 25 Kg/m² (n = 37)				
			p (Genotype)	*p* (BMI)						
	CT	LDL	HDL	TG		CT	LDL	HDL	TG	
	Mean (mg/dl)									
Alleles										
C	159.08	84.44	62.38	100.63	196.96	114.08	55.77	142.28		
G	164.07	84.36	65.92	96.68	200.01	114.46	60.54	132.35	0.728	8.0E-6*
Codominant										
C/C	155.17	89.02	52.53	86.22	191.43	113.19	49.26	167.89		
C/G	159.83	83.57	64.26	103.37	200.41	114.63	59.84	126.28		
G/G	172.99	86.00	69.39	82.64	199.43	114.21	61.55	141.16	0.391	-
Recessive										
C/C + C/G	159.08	84.44	62.38	100.63	196.96	114.08	55.77	142.28		
G/G	172.99	86.01	69.396	82.64	199.43	1142.1	61.55	141.16	0.613	0.001*
Dominant										
C/C	155.17	89.02	52.53	86.22	191.43	113.19	49.26	167.89		
C/G + G/G	164.07	84.36	65.92	96.68	200.01	114.46	60.54	132.35	0.121	0.001*

BMI- body mass index, CT-total cholesterol, LDL- low density lipoprotein, HDL- high density lipoprotein, TG- triglyceride. Data were analyzed by multivariate analysis (MANOVA). *Statistically significant ($p < 0.05$).