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Genetic variation in the promoter region of the TNF rs1800629 gene is not associated with adiposity index, but AA genotype is more likely to have low cellular membrane integrity

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ABSTRACT

Background: The complex pathophysiology of obesity includes low-grade chronic inflammation of white adipose tissue, an increase in plasma levels of pro-inflammatory cytokines as well as genetic factors, which have all emerged as important risk factors.
Objective: Evaluate the association between the TNFα-308G/A variant and sociodemographic, clinical and behavioral variables in an urban population using overall and central obesity as the measured outcome.
Methods: A cross-sectional population-based study was conducted. Anthropometric measures and genotyping were assessed in 808 individuals. The associations were tested using the hierarchical theoretical model and multiple logistic regressions.
Results: Thirty-one percent of subjects were overweight while 19.4% were obese. No association was observed between genotype frequencies of TNFα-308G/A and either obesity or other variables of adiposity, including waist circumference, waist to height ratio, conicity index, and fat mass. However, age, gender, physical inactivity, and other comorbidities were associated with measures of excess adiposity in the hierarchical model. The phase angle of AA genotype was lower than GA and GG genotypes ($p < 0.05$).
Conclusion: The rs1800629 SNP of the TNFA gene was not associated with the adiposity indicators evaluated in this based population study. However, AA genotype is more likely to have low cellular membrane integrity.

1. Introduction

Obesity is characterized by excessive accumulation of body fat and is influenced by genetic and environmental components (Kopelman, 2000). It has been suggested that the obesity and severe obesity rates will increase to 42%and 11.1% worldwide by 2030 (Finkelstein, 2014). Several studies have reported that central adiposity is associated with increased risk for coronary artery disease (Ladabaum et al., 2014; Sivasankaran et al., 2011). Body mass index (BMI) is an anthropometric indicator most commonly used to evaluate the level of obesity; however, this parameter has significant limitations (Flegal et al., 2009).

It has been hypothesized that an excessive accumulation of visceral adipose tissue may be partially associated with increased coronary risk as a result of inflammation (Panagiotakos et al., 2005). Although the pathophysiological mechanisms of obesity are complex (Hotamisligil, 2006), previous evidence supports the link between adiposity and

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excessive inflammation (Feltenberger et al., 2013; Santos et al., 2012). It is well known that adipose tissue is a major source of several endogenously produced inflammatory cytokines (Hotamisligil, 2006). Bioelectrical impedance analysis (BIA) is commonly used the method to evaluate body composition (Johnson Stoklossa et al., 2016). Advantages of BIA are related to portability, cost and relatively easy to use requiring minimal training. BIA utilizes electrical current to measure differences in resistance and reactance in tissues based upon water and electrolyte content (Johnson Stoklossa et al., 2016).

Importantly, the gene encoding the inflammatory cytokine tumor necrosis factor-α (TNFα) (Pennica et al., 1984) is highly expressed in obese individuals but may not be a useful marker of visceral fat deposition (Hotamisligil, 2006; Pennica et al., 1984; Angeli et al., 2011). TNFα, an inflammatory cytokine secreted by adipose tissue, has been linked to obesity as well as other related diseases. A genetic variant located in the promoter region of the TNFα gene (-308) has been shown to increase the rate of transcription of TNFα (rs1800629). The presence of the A allele at this position has been associated with increased levels of TNFα, as compared to the G allele (Wilson et al., 1997).

Conflicting data have been reported regarding the association between obesity and the TNFα-308G/A variant (Bouhaha et al., 2010; Vikram et al., 2011). As such, it is important to conduct a population-based study to analyze any potential association between these two parameters. The aim of this study was to evaluate the association between the TNFα-308G/A variant and sociodemographic, clinical as well as behavioral variables in an urban population with overall and central obesity.

2. Materials and methods

2.1. Design and study population and ethic aspects

A cross-sectional, population-based study was conducted in 2012 and 2013 in adults (age ≥ 18 years) living in the urban area of Montes Claros, Minas Gerais, Brazil (IBGE, 2010). The study was approved by the Institutional Review Board CAAE: 11, 858, 412.5.0000.5149 by National Health Council Resolution 196/96. All study participants were informed of their rights and the objectives of the research, following which they were asked to sign a consent form.

2.2. Sampling plan and data collection

This study used population data from a previously published study that analyzed leptin receptor polymorphism (Gln223Arg) and its association with obesity and cardiovascular disease (Pena et al., 2014). Correction for the design effect was performed by adopting a deff equal to 2.0. The prevalence of obesity used was 30% (Matsushige et al., 2012) as well as the prevalence of the TNFα-308G/A polymorphism (10%) (Patente et al., 2015), the adequate sample size was determined to be at least 625 adults (Matsushige et al., 2012; Pena et al., 2014; Reis et al., 2015). For more details, please see the Pena et al. (2014) and Reis et al. (2015)

Data were collected by trained interviewers and supervised throughout the collection process. An interview involved recording the responses to an in-person survey questionnaire covering various demographic characteristics (i.e. gender, age, skin color, marital status, and level of education) and lifestyle characteristics (i.e. physical activity, smoking habits, and alcohol consumption), as described below.

2.3. Interviews and measures.

The level of physical activity was assessed by applying the International Physical Activity Questionnaire (IPAQ-8, long version), which has previously been validated in Brazil (Matsudo et al., 2002). The individuals were considered active if the level of physical activity was > 150 min per week. Current or previous smoking habits were also noted. Individuals that smoked at least 100 cigarettes over a lifetime were considered smokers. Participants were categorized as current smokers, ex-smokers, and non-smokers. An assessment of alcohol use was based on whether or not the individual consumed alcohol as described before (Matsushige et al., 2012).

Anthropometric evaluation was performed according to the recommendations of the World Health Organization (WHO); (WHO, 1995). All anthropometric measurements were performed in triplicate, and the mean of these results was calculated. Weight was measured using a portable scale (Model PL 150, GTech®, São Paulo, Brazil) with an accuracy of 0.1 kg when participants were wearing light clothing and no shoes. Height was measured with a portable stadiometer (AlturaExata®, São Paulo, Brazil) with an accuracy of 1 mm. Individuals were stratified based on normal or high levels of adiposity, as determined using anthropometric indices. The cutoff points for body mass index (BMI) were as follows: < 24.9 kg/m^2 (normal weight), > 25 kg/m^2 (overweight) and ≥ 30 kg/m^2 (obese). Waist circumference (WC) was measured using a non-extendable tape and measurements were recorded halfway between the lowest rib and the iliac crest. The cutoffs for high WC (central obesity) were WC ≥ 102 cm for men and ≥ 88 cm for women. Conicity index (CI) was determined using the weight, height, and WC. A CI of > 1.25 for men and > 1.18 for women was considered high (Valdez, 1991). Finally, the waist-to-height ratio was calculated by simple division and considered high when the ratio was > 0.5 (Pitanga and Lessa, 2005).

Blood pressure was measured using a calibrated digital sphygmomanometer (HEM-7200, ONROM®, São Paulo, Brazil). After an initial rest period of 5 min, three measurements were performed, with a two-minute interval between measurements using the participant's right arm, by the seventh report of the Joint National Committee Position Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al., 2003). Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg (Chobanian et al., 2003). Also, participants were considered hypertensive if they reported the regular use of medication to manage hypertension. A capillary sample was obtained from all subjects in a non-fasting state. Glucose levels were analyzed using the Accucheck Roche® blood glucose monitor (Mannheim, Germany). A blood glucose level of ≥ 140 mg/dL was considered high (WHO, 2006a). Finally, the prevalence of dyslipidemia among the study participants was determined based on their response to whether they had high cholesterol levels.

2.3.1. Bioelectrical Impedance Analysis (BIA)

BIA is a simple, accurate and noninvasive method to evaluate body composition. It estimates the body water volume accordingly body resistance against the low amplitude electrical current (500-800 mA) and high frequency (50 kHz). This analysis was performed using a bioimpedance analyzer with a quadruple frequency (BIA 310, Biodynamics®, Shoreline, USA). Data were estimated as follow: weight, fat mass percentage, impedance, resistance, and reactance. Additionally, phase angle (PA) was calculated to each genotype. Fat mass percentage (FM) of > 20% for men and > 30% for women was considered high (Lohman et al., 1988) (Lohman et al., 1988).(Barbosa-Silva et al., 2017).

2.4. DNA extraction and genetic analysis

Genomic DNA was obtained using a buccal swab and extracted using the previously described silica method (Guimaraes et al., 2006). TNFα-308 (G > A; rs1800629) was assessed by RFLP-PCR, as previously described (Guimaraes et al., 2007), using the following TNFA primers: 5′-ATCTGGAGGAAGCGGTAGTG-3′ and 5′-AGAAGACCCCCC-TCGGAACC-3′. Digestion was performed using the NcoI restriction enzyme (37 °C/12 h, Promega®, Madison, USA). The G allele was

cleaved by the enzyme into two fragments, one of 87 bp and another of 20 bp (GG). The haplotype GA allele was composed of three fragments: 107, 87, and 20 bp. The A allele was not cleaved by the enzyme, and only one fragment of 107 bp was observed (Bouhaha et al., 2010).

2.5. Statistical analysis.

The questionnaires were analyzed immediately after collection to ensure there was no missing or inadequate information. Once complete, the data were input by two independent researchers. Consistency was maintained using EpiInfo© version 3.5.4 (available at www.cdc.gov/epiinfo/) and any inconsistencies were corrected. Because the data showed low response rate in both individuals and households, post-stratification weights were constructed using the rake method (Kalton, 1983). Several dependent variables (adiposity indices) were tested, including BMI, conicity index, waist to height ratio, body fat percentage and waist circumference. The following parameters were selected as independent variables: genotype (mainly exposure), skin color, gender, age, marital status, income, education, hypertension, diabetes and dyslipidemia, the level of physical activity, smoking and alcohol consumption. In the hierarchical model proposed, the distal level included both block demographic characteristics and the genetic variant, the intermediate level was comprised of socioeconomic variables, and the proximal level included comorbidities and health-related behavior (Fig. 1). The independent variables were grouped into blocks according to a hierarchical theoretical model that guided the composition of the variable blocks and the order of entry of the same models (Richiardi et al., 2013; Victora et al., 1997).

Initially, descriptive analysis of all variables was performed. Proportions for categorical variables and the respective 95% confidence intervals for numerical variables as well as the mean and standard errors were estimated. Bivariate analysis using the odds ratio was performed to assess the association between outcomes and each independent variable. To estimate the odds ratio and confidence interval, a binary logistic regression model was used. Variables with a p-value < 0.25 were selected for multivariate analysis. The block of genetic and demographic variants was included in the model first and used as the adjustment factor for both intermediate and proximal determinants. Only the intermediate variables (e.g. socioeconomic status), which displayed a descriptive level of $p < 0.05$, after adjusting for variables from the distal level, were included in the model.

Finally, only the blocks composed of comorbidities and health-related behaviors that presented a descriptive level of $p < 0.05$, after adjusting for the variables of the distal and intermediate levels, were included in the model. The adjusted odds ratio for each variable was estimated when the variable was first included in the model. At all stages of modeling, the significance of the estimated coefficients was verified using the Wald test. To evaluate the fit of the binary logistic models, we used the pseudo-R-squared measure. Analyses were carried out using both survey commands and Predictive Analytics Software (PASW) 17.0 to account for strata and weights in the complex composition of the sample survey data, following adjustments to account for any effect of the experimental design.

Additionally, we used an analysis of variance and post hoc of Bonferroni to investigate differences in PA between the three genotypes (AA, GA, and GG). Then, we performed a cross tabulation to calculate the percentage of individuals classified as normal weight or above. All of these analyses were performed the data of BIA (resistance and reactance) and BMI accordingly. A p-value of ≤ 0.05 was considered, and SPSS 22.0 was used.

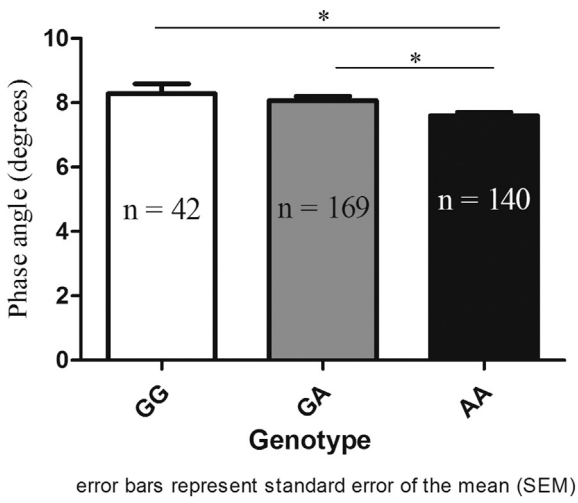

Fig. 1. Phase angle comparison between genotypes (AA, GA and GG). Divided bars indicate the percentage of individuals with normal weight or above. Dashed line shows a cut-off point of cellular membrane integrity (Kumar et al., 2012). *$P < 0.05$.

3. Results

The final sample consisted of 808 participants who were selected from 46 census tracts; however, there was a loss of 15% at the time of DNA extraction. Of the participants, 47.3% were males, and 52.7% were females. 54.6% were married, and the most prevalent group was aged 18 to 29 years (33.4%). 60.6% of participants had an education level ≥ 8 years. The prevalence of obesity in the studied population was 19.4%. With regards to the clinical variables, 34.9% had high blood pressure, and the capillary blood glucose test revealed increased glycemia in 6.3% of the subjects. 9.5% reported smoking and 34.2% frequently consumed alcohol.

The genotype distribution of the three SNPs was as follows: GG (58.6%), GA (30.2%), and AA (11.3%) (Table 1). The genotype frequencies were not consistent with Hardy-Weinberg equilibrium ($p < 0.001$). it is important to highlight that Hardy-Weinberg equilibrium could not be applied in population with natural selection is acting on the locus in question. Moreover, the Hardy-Weinberg equilibrium theorem should be applied to infinite size population. Distribution of anthropometric indicators related to excess weight, comorbidities, and lifestyle was stratified according to the genotype (Table 2). No association was observed between the genetic variants and obesity ($p = 0.621$), high blood glucose ($p = 0.430$) or hypertension ($p = 0.931$).

Table 3 shows that, after multiple logistic regression analysis, no association was observed between the genetic variant and obesity, WC, waist to height ratio, CI or FM. However, age, gender, physical inactivity, and other comorbidities were associated with measures of excess adiposity in the hierarchical model.

PA was analyzed of 673 individuals who had enough data to calculate it (resistance and reactance). This analysis showed a statistically significant difference between the genotypes ($p = 0.005$). Differences were found between AA ($n = 259$) vs. GA ($n = 327$) ($p = 0.011$) and AA vs. GG ($n = 87$) ($p = 0.043$), but no difference was seen between GA vs. GG ($p = 1.00$). Interestingly AA genotype showed lower PA and majority of individuals with BMI above of normal weight (7.61° and 54.1%, respectively) than GA (8.48° and 51.7%, respectively) and GG (8.14° and 48.3%, respectively). Data are shown in Fig. 1.

4. Discussion

Obesity has nearly doubled since the 1980s. Currently, there are approximately 500 million obese adults worldwide. It has previously been reported that 65% of the world's population lives in countries where the majority of the population is overweight and obese (WHO, 2006b). The high percentage of overweight individuals has also been observed in the Brazilian population (Matsushige et al., 2012). The current study corroborates with previous study (Carmienke et al., 2013)

Table 1
Distribution of adults according to genotypes, anthropometric indices, demographic, socioeconomic characteristics, comorbidities, and health-related behavior.

Variables	n	% ($CI_{95\%}$)[a]	Deff
Genotypes -308 TNFa G/A			
GG	401	58.6 (54.6–62.4)	1.08
AG	210	30.2 (26.7–33.9)	1.06
AA	74	11.3 (8.6–14.6)	1.47
Anthropometric indices			
BMI			
Non-obese	634	80.6 (77.0–83.8)	1.49
Obese	170	19.4 (16.2–23.0)	1.49
BMI			
Non-overweight	370	49.6 (45.6–53.6)	1.29
Overweight	434	50.4 (46.4–54.4)	1.29
Conicity index			
Normal	331	49.1 (45.2–53.1)	1.22
High	468	50.9 (46.9–54.8)	1.22
WHeR			
Normal	364	51.2 (47.1–55.3)	1.34
High	438	48.8 (44.7–52.9)	1.34
Body fat			
Normal	320	50.9 (47.5–54.3)	0.83
High	398	49.1 (45.7–52.5)	0.83
WC[b]			
Normal	362	52.8 (49.1–56.5)	1.08
High	443	47.2 (43.5–50.9)	1.08
Demographic characteristics	n	%	
Skin color			
White	154	17.7	1.53
Non-white	652	82.3	1.53
Gender			
Female	526	52.7	0.90
Male	282	47.3	0.90
Age (years)			
18–29	209	33.4	1.47
30–39	150	22.3	2.02
40–49	138	16.6	1.34
50–59	140	14.6	1.12
≥ 60	171	13.1	1.13
Socioeconomic characteristics	n	%	
Marital status			
With spouse	447	54.6	1.51
Without spouse	361	45.4	1.51
Income (monthly minimum salary)[c]			
< 2	370	46.1	4.31
≥ 2 and < 4	348	43.2	3.75
≥ 4	89	10.7	4.40
Education (years)			
Illiterate	62	5.5	1.33
≥ 1 and ≤ 4	200	20.6	1.31
≥ 5 and ≤ 8	108	13.3	1.41
> 8	435	60.6	1.45
Comorbidities			
Hypertension			
No	476	65.1	0.86
Yes	332	34.9	0.86
Glucose level			
Normal	735	93.7	1.26
High	70	6.3	1.26
Dyslipidemia			
No	508	70.2	1.59
Yes	299	29.8	1.59
Health-related behavior			
Physical activity			
Active	638	80.5	2.46
Sedentary	170	19.5	2.46
Smoking			
Non-smoking	604	75.3	1.57
Former Ex-smoker	135	15.1	1.02
Smoker	69	9.5	2.61
Alcohol consumption			
No	561	65.8	2.71
Yes	246	34.2	2.71

[a] Values corrected for the effect of drawing; Deff: design effect;
[b] 3 missing values.
[c] In Brazil, income is often reported as multiples of the monthly minimum salary, which is currently approximately USD 350.00.

Table 2
Distribution of anthropometric parameters, comorbidities, and lifestyle, stratified by genotype.

Variables	Genotypes			
	AA n (%)	GA	GG	*P*-value*
Obesity				0.621
No	58 (11.3)	161 (29.2)	314 (59.5)	
Yes	16 (11.2)	48 (33.7)	86 (55.1)	
Abdominal obesity				0.950
Normal	32 (11.1)	98 (30.9)	180 (58)	
High	42 (11.5)	112 (29.6)	218 (58.8)	
Body fat (%)				0.969
Normal	32 (11.8)	85 (30.8)	160 (57.4)	
High	36 (11.4)	105 (30.1)	195 (58.5)	
Conicity index				0.792
Normal	29 (10.4)	84 (30.0)	165 (59.5)	
High	45 (12.2)	123(30.2)	232 (57.6)	
Waist/height				0.859
Normal	36 (11.8)	94 (29.5)	184 (58.7)	
High	38 (10.8)	115 (31.1)	213 (58.1)	
Glycemia (mg/dL)				0.430
Normal	(10.8)	(29.1)	(60.2)	
High	(13.7)	(32.6)	(53.7)	
Hypertension				0.931
Normal	44 (11.5)	130 (30.5)	233 (58.0)	
High	30 (10.9)	80 (29.6)	168 (59.6)	
Physical activity				0.379
Sedentary	13 (9.0)	52 (35.1)	87 (55.9)	
Active	61 (11.9)	158 (28.9)	314 (59.3)	
Smoking				0.764
Smoke	07 (11.7)	15 (26.2)	40 (66.2)	
Former smoker	09 (8.2)	39 (34.2)	66 (57.5)	
No smoke	58 (11.8)	156 (29.9)	295 (58.3)	
Alcohol consumption				0.085
Yes	16 (8.5)	73 (35.6)	120 (55.9)	
No	58 (12.8)	137 (27.4)	280 (59.8)	

* Chi-square test.

which found that 50.4% of overweight and 50.9% high frequency of central obesity indicators. Evidences demonstrated that is not necessary, and possibly even harmful, to test the Hardy-Weinberg equilibrium assumption before testing for association between alleles and disease Zou, 2006.

In the present study, no association was observed between the genetic variant of TNFα and other characteristics of adiposity, including BMI, WC, WHeR, CI and FM. Interestingly, in this study, neither the GA nor AA genotypes were associated with overall or central obesity overall. The findings reported in this study corroborate other studies (Santos et al., 2006). Also, no association was found between the A allele and obesity, worth mentioning this study differs in its design because it is a case-control. A previous study has found no association between the A allele and fat deposition in women (Hoffstedt et al., 2000). In an Asian Indian population from Northern India, measures of body composition, abdominal fat distribution and TNF-α levels were not influenced by the presence of the -308G/A polymorphism (Vikram et al., 2011). Conversely, some studies have suggested that the -308G/A TNF-α polymorphism is associated with obesity in several European populations (Bendesky and Bargmann, 2011; De Luis et al., 2011; Herrmann et al., 1998). These studies discuss the potential effect of modifiable variables, including population stratification and health

Table 3
Results of multiple logistic regression analysis: association between dependent and independent variables.

Variables	Obesity		(C-index)		(WHtR)		(FM)		(WC)	
	OR_a ($CI_{95\%}$)	*P*-value	OR_a ($CI_{95\%}$)	*P*-value	OR_a ($CI_{95\%}$)	*P*-value	OR_a ($CI_{95\%}$)	*P*-value	OR_a ($CI_{95\%}$)	*P*-value
Block 1: genetic and demographic characteristics*										
TNFa										
AA	0.96 (0.49–1.88)	0.912	1.19 (0.56–2.54)	0.647	0.81 (0.45–1.48)	0.484	0.88 (0.45–1.71)	0.701	0.93 (0.48–1.83)	0.838
GA	1.28 (0.79–1.58)		1.04 (0.65–1.68)		1.07 (0.73–1.58)		0.93 (0.56–1.53)		0.95 (0.59–1.51)	
GG	1.00		1.00		1.00		1.00		1.00	
Sex										
Female	1.79 (1.09–2.60)	**0.021**	1.49 (1.02–2.18)	**0.004**	n.s	n.s	n.s	n.s	3.62(2.21–5.94)	< **0.001**
Male	1.00		1.00						1.00	
Age (years)										
≥ 60	2.66 (1.19–5.94)	**0.018**	28.52 (15.03–54.09)	< **0.001**	15.62 (8.54–28.55)	< **0.001**	17.19 (7.90–37.0)	< **0.001**	11.58(6.05–22.17)	< **0.001**
50 and 59	3.41 (1.57–7.41)	**0.003**	20.44 (12.94–32.29)	< **0.001**	12.98 (7.33–22.98)	< **0.001**	9.02 (5.19–15.67)	< **0.001**	7.40 (3.91–14.00)	< **0.001**
40 and 49	2.42 (1.16–5.02)	**0.019**	6.90 (3.96–12.02)	< **0.001**	5.25 (3.17–8.69)	< **0.001**	6.29 (3.74–10.56)	< **0.001**	6.31 (3.45–11.53)	< **0.001**
30 and 39	3.76 (1.79–7.90)	**0.001**	3.79 (2.34–6.12)	< **0.001**	4.67 (2.75–7.89)	< **0.001**	3.72 (2.09–6.64)	< **0.001**	5.10 (3.05–8.51)	< **0.001**
18 and 29	1.00		1.00		1.00		1.00		1.00	
Block 2: socio-economic characteristics**										
Status marital										
With Spouse	n.s	n.s	n.s	n.s	1.45 (1.03–2.04)	**0.031**	1.83 (1.26–2.66)	**0.002**	1.61(1.08–2.14)	**0.020**
Without spouse					1.00		1.00		1.00	
Income (minimum monthly salary) &										
< 2	n.s	n.s	1.76 (1.10–2.84)	**0.021**	1.83 (1.04–3.20)	**0.036**	n.s	n.s	n.s	n.s
≥ 2 and ≤ 4	n.s	n.s	1.44 (0.85–2.46)	0.173	1.80 (1.11–2.90)	**0.018**	n.s	n.s	n.s	n.s
> 4			1.00		1.00		1.00	1.00	1.00	
Block 3: comorbidities***										
Hypertension										
Yes	1.81 (1.04–3.15)	**0.036**	2.51 (1.38–4.57)	< **0.001**	2.72 (1.74–4.26)	< **0.001**		**n.s**	2.46 (1.62–4.29)	< **0.001**
No	1.00		1.00		1.00					
Diabetes										
Yes	n.s		1.74 (1.03–2.95)	**0.040**	1.76 (1.18–2.63)	**0.007**	1.73 (1.03–2.90)	**0.039**	1.88 (1.21–2.93)	**0.006**
No			1.00		1.00		1.00		1.00	
Dyslipidemia										
Yes	1.78(1.05–3.04)	**0.033**	1.87 (1.09–3.22)	**0.024**	n.s	n.s	2.44 (1.45–4.11)	**0.001**	1.88 (1.08–3.28)	**0.027**
No	1.00		1.00				1.00			
Block 4: health-related behavior***										
Physical activity										
Sedentary	n.s	n.s	n.s	n.s	n.s	n.s	1.94 (1.14–3.30)	**0.016**	n.s	n.s
Active							1.00			
Smoking	n.s	n.s	n.s	n.s						
Non-smoking					0.47 (0.24–0.95)	**0.035**	0.38 (1.14–1.03)	**0.057**		
Former smoker					0.69 (0.35–1.37)	**0.028**	0.89 (0.48–1.66)	0.712	n.s	n.s
Smoker					1.00		1.00			
	Pseudo-R^2 = 0.124		Pseudo-R^2 = 0.409		Pseudo-R^2 = 0.346		Pseudo-R^2 = 0.335		Pseudo-R^2 = 0.364	

OR_a: odds ratio adjusted-$IC_{95\%}$: range 95% confidence adjusted according to study design- n.s: not statistically significant.
In Bold significant results ($p < 0.05$).
* Block 1 variables adjusted for each other.
** Block 2 variables adjusted for the variables in Block 1.
*** Variables in Block 3 and 4 adjusted for variables in Blocks 1and 2.
& In Brazil, income is often reported as multiples of the monthly minimum salary, which is currently approximately USD 350.

status, which seem to influence the various associations that were observed in these populations (Bendesky and Bargmann, 2011). A possible role for the TNF-α locus in obesity is supported by a linkage study, which reported a relationship between this locus and several global measures of adiposity in Pima Indians and Caucasians (WHO, 2006b). The hierarchical approach allowed us to evaluate the importance of each block of variables on the anthropometric indices of obesity. The order in which the blocks were entered into the model was determined by known factors associated with obesity. In the present study, by epidemiological associations previously reported in literature, excess body fat was found to be related to several anthropometric parameters ($p < 0.001$). These parameters included both demographic and socio-economic characteristics as well as comorbidities and health-related behavior.

In the present study, the genotype frequency of the three variants GG, GA, and AA were similar to other studies (Correa et al., 2011; Romeo et al., 2001). However, Brazil has a heterogeneous population, and rs1800629 allele frequencies did not differ markedly in the population utilized in this study, an observation which contradicts previously reported data (Correa et al., 2011; Romeo et al., 2001; Guimaraes et al., 2007). It is possible that this conflicting observation is because previous studies did not use a population-based approach thus leading to differences in the observed frequency of the three alleles.

The differences of PA between genotypes are important to be observed in the present study. PA estimates cellular membrane integrity (permeability and hydration), and when it is below of eight degrees, it may be associated with poor cellular integrity (Llames et al., 2013; Kumar et al., 2012). The PA of AA genotype (7.61° ± 1.5) was lower than GA (8.48 ± 1.77º) and GG (8.14 ± 2.06°). The presence of A allele may be associated not only with obesity but other many diseases and needs to be more investigated. Surprisingly, our study has shown the majority of subjects with AA genotype (54.1%) above of normal weight compared to GA (51.7%) and GG (48.3%).

It is important to note that the validity and reliability of this study were ensured through careful planning, training, and management of examiners, as well as validation of census tracts and data collection. Furthermore, quality control was maintained with all instruments used for data collection and, during analyses, corrections were applied to account for design effect. The design effect (Deff) of dependent variables was lower than the 2.0 estimated in the sample plan, ensuring the power of inference for these variables. However, the present study did have some limitations. This is a cross-sectional study and, as such, we cannot assert a causal relationship between environmental variables. To confirm a cause and effect relationship, it may be necessary to perform longitudinal studies and intervention trials where serum levels of TNFα are concurrently measured.

5. Conclusions

In conclusion, in this study, no association was observed between genotype frequencies of rs1800629 SNP of the TNFA gene and either obesity or other variables of adiposity, including waist circumference, waist to height ratio, conicity index, and fat mass. However, AA genotype is more likely to have low cellular membrane integrity. Besides that age, gender, physical inactivity, and other comorbidities were associated with measures of excess adiposity in the hierarchical model.

Declaration of interest

There are no conflicts of interest.

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