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Association of oxytocin levels and oxytocin receptor gene polymorphism (rs2254298) with cardiovascular risk factors in Brazilian elderly from Primary Health Care

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ABSTRACT

Background: Morbidity and mortality from cardiovascular disease is a typical phenomenon in the elderly, and are related to unfavorable genetic, hormonal and environmental (lifestyle) interactions. In this context, oxytocin (OT) seems plays a key role in the development of CVD by performing important actions in metabolism energy and hemodynamic variables.

Objective: To verify if there is an association between (OT) levels and the oxytocin receptor gene (OXTR) polymorphism (rs2254298) with cardiovascular risk factors (CRF) in the elderly.

Methods: This was a cross-sectional study in community-dwelling elderly attending primary health care. The genotyping was done using the polymerase chain reaction technique. The CRF factors investigated included hypertension, diabetes mellitus, dyslipidemia, sedentary lifestyle, and obesity. Levels of triglycerides (TGC) postprandial and glucose were measured in capillary blood. OT and cortisol levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The sample comprised 177 elderly individuals. OT levels showed a significant negative correlation with postprandial triglycerides ($p = 0.030$) and BMI ($p = 0.019$). OT levels were also associated with leanness ($p = 0.005$). On Poisson regression analysis, OT remained a predictor for leanness ($p = 0.010$). No significant associations were observed between the OXTR polymorphism and CRF.

Conclusion: The results suggest that Postprandial TGC levels are increased, while OT levels are decreased, and this hormone was significantly elevated in lean elderly. Future studies are needed to confirm these findings, and the role of OT in metabolic parameters.

1. Introduction

The demographic aspects of the Brazilian population's aging rate have been a recent source of concern, due to the increase in the prevalence of non-communicable chronic diseases (DCNT), mainly cardiovascular and metabolic diseases, which negatively impact the quality of life and the daily routine of these individuals (Mazzoccante, Moraes, & Campbell, 2012).

The mortality rate associated to cardiovascular diseases is the result of a series of interrelated risk factors, such as genetics, hormonal and behavioral (Mansur & Favarato, 2012). Within this context, oxytocin, a neuropeptide known as the “love hormone,” has been largely studied due to its relevance to many physiological processes in the body, which include food control and modulation of carbohydrate and lipid metabolism (Blevins et al., 2014; Cai & Purkayastha, 2013).

Oxytocin plays an important role in hemodynamic mechanisms, such as the regulation of blood pressure and the release of atrial natriuretic peptide (Japundžić-Žigon, 2013; Wsol, Kasarello, Kuch, Gala, & Cudnoch-Jedrzejewska, 2016). Furthermore, studies with rodents have shown that oxytocin can reverse insulin resistance and glucose intolerance due to the presence of oxytocin receptors in the islets of Langerhans (Zhang et al., 2013; Klement et al., 2017).

The main effects of oxytocin are mediated by its receptor, which is comprised by nine amino acids coupled to the G protein. In 1994, the

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oxytocin receptor gene (*OXTR*) was mapped by Inoue et al. (1994). The OXTR is located in the short arm of human chromosome 3p25 and it consists of three exons and four introns. The single nucleotide polymorphism on *OXTR* rs2254298 is characterized by the replacement of guanine (G) for adenine (A), which is located in the third intron (Inoue et al., 1994; Mizumoto et al., 1997).

Caucasians usually carry two copies of the G allele, whereas the A allele is quite common in Asian individuals. OXTR polymorphism (rs2254298) has been associated to the onset of anxiety disorders and depression (Brune, 2012; Chelala, Khan, & Lemoine, 2008; Gimpl & Fahrenholz, 2001). Moreover, the variation of this polymorphism has also been associated to overeating and endophenotypic traits, such as the preference for sweet and greasy foods. Davis, Patte, Zai, and Kennedy (2017) suggested an association between OXTR and eating disorders, as they that the A-allele carriers had a greater preoccupation with food consumption and weight. OXTR may be related to appetite and food ingestion that, if correlated with the relatively low serum oxytocin levels, may contribute to the onset of obesity (Davis et al., 2017).

Nevertheless, the influence of the *OXTR* polymorphism on others cardiovascular risk factors (CVD), such as hypertension, diabetes mellitus (DM), and dyslipidemia, has remained unclear in the literature, as well as the relationship of serum oxytocin with these diseases, especially in the elderly.

This single-nucleotide polymorphism rs2254298 was chosen because there are many studies associating it with depression and rare with cardiovascular risk in the elderly. In addition, our results are part of a larger project investigating this polymorphism with affective and neuropsychiatric disorders.

Therefore, the hypothesis to be answered in this paper suggests that depressed individuals exposed to cardiovascular risk factors may carry the same predisposition or genetic trait, which would be associated to a social or food behaviour. Taking into consideration the ideas above, the objectives of this study were to investigate if there is an association between serum oxytocin concentration and OXTR polymorphism rs2254298 with cardiovascular and metabolic risk factors in a population of elderly individuals treated in Primary Health Care centres in Brazil.

2. Material and methods

2.1. Subjects and study design

This was a non-probabilistic, cross-sectional study which included 177 elderly individuals attending primary health care units in the eastern and north-eastern regions of Porto Alegre, a Brazilian municipality and the capital city of the southernmost state of Brazil - Rio Grande do Sul. The study was part of a Special Visiting Project (PVE), a collaborative network study among the Psychiatry Service of the University of Lausanne-Switzerland, the service of the Institute of Gerontology and Geriatrics (IGG) of PUCRS (Porto Alegre-Brazil), and the Department of Health of Porto Alegre (RS-Brazil).

The participants of this research were selected depending on their availability to attend a medical appointment scheduled by the nurse, doctor or Community Health Agent of the Primary Care Centre to which the elderly belong. These consultations occurred at the Psychiatry and Geriatrics outpatient clinic of the São Lucas Hospital of PUCRS. Individuals younger than 60 years old, with advanced cognitive decline or schizophrenia and undergoing cancer treatment were not included in the study.

Despite being a convenience sample, the sample size was calculated using the ANOVA test, considering the three possible groups of genotypes and a margin of error of 0.05 (α) and 0.8 (β). G * Power 3.1.7 software was used to calculate the sample size.

2.2. Measures

2.2.1. Postprandial glucose

It was measured by inserting a drop of capillary blood into a disposable biosensing tape containing glucose dehydrogenase, the measures were made on a glucometer ® (Accutrend plus GCT ®, Roche Diagnostics, Mannheim, Germany). The method used was Glucose Hexokinase, where glucose is phosphorylated by adenosine triphosphate (ATP) in the presence of hexokinase. The glucose-6-phosphate that is formed is oxidized in the presence of glucose-6-phosphate dehydrogenase. After this enzymatic action, an electrochemical reaction occurs directly proportional to the concentration of glucose test was released 2–4 h after a meal (Accutrend Plus, 2015; Brazilian Society of Diabetes, 2016).

2.2.2. Postprandial triglycerides (TGC)

Serum triglycerides concentration increases gradually after a meal, reaching a peak of 4 h after food consumption, and then it slowly decreases for 8 h after food intake (Masuda et al., 2009). If we assume that the period that an individual fasts within 24 h is a short one, the non-resistant lipid profile may be more useful than fasting when we measure lipids for the stratification of cardiovascular risk (Torres do Rego et al., 2013). Within this period, the oxytocin acts on the metabolism of fatty acids. The test consisted in collecting one drop of blood (30 μL), by the means of a capillary puncture in the digital pulp, and the blood was then placed on specific tapes which were inserted in a portable device ® (Accutrend plus GCT ®, Roche Diagnostics, Mannheim, Germany). Capillary TGC concentrations are 0.2–0.3 mmol/L higher than TGC concentrations in venous plasma (Accutrend Plus, 2015).

Capillary TGC concentrations are measured by a method which is based on the three-phase enzymatic reaction of Fossati with a Trinder endpoint. TGCs are converted to glycerol and free fatty acids by lipase, thereafter the glycerol is converted from glycerol-3-phosphate oxidase into hydrogen peroxide, and a coloured complex is produced through hydrogen peroxide, 4-aminophenazone, and 4-chlorophenocol. The sample is then submitted to a reflection-photometric method. The reflectance apparatus measures the reflected light of a surface, and the concentration is given by the intensity of the colour formed on the test strip after the chemical reaction. The measurement range is from 20 to 600 mg/dL and the reaction time for reading the result is of 12 s. Environmental factors, such as heat or cold (temperature equal to or above 300 °C, or equal to and below 50 °C, should be controlled, as they may interfere with the result of the test reading (Accutrend Plus, 2015).

2.3. Cardiovascular risk factors

Cardiovascular Risk Factors were identified from the drugs prescribed to the patients, who were instructed to bring all the current medical prescriptions on the day of the consultation. Patients were then diagnosed as having hypertension, diabetes, and dyslipidemia if they made use of medications for these diseases according to recommendations of the IV Brazilian Guidelines on Hypertension (Brazilian Society of Cardiology, 2014); VI Guidelines of the Brazilian Diabetes Society, 2016 and V Brazilian Guidelines on Dyslipidemias and Prevention of Atherosclerosis (Brazilian Society of Cardiology, 2013).

Blood pressure levels were measured using a calibrated aneroid sphygmomanometer and stethoscope. The patients were instructed to seat and keep their legs uncrossed, according to the VI Brazilian Hypertension Guidelines (Brazilian Society of Cardiology, 2014).

Obesity was classified through the Body Mass Index (BMI) and abdominal circumference. A trained nutritionist obtained the participants' anthropometric data. Weight was measured on a four-sensor bioelectrical impedance scale (Omron®) with the participants fasting for 3–4 h, in a standing position and barefoot, this procedure was contraindicated in patients with pacemakers or other implanted medical devices. BMI was measured, by bioelectrical impedance using Lipschitz (1994). Cut-

off values, for leanness and overweight – < 22 kg/m^2 and > 27 kg/m^2, respectively- (Lipschitz, 1994). The abdominal circumference, was measured, with an inelastic tape, at the midpoint between the lower costal border and the iliac crest with the individual in a standing position (since this is the most representative anthropometric measure of intra-abdominal fat) using cut-off values of ≥102 cm for men and ≥88 cm for women (Brazilian Society of Cardiology, 2005).

The participants were questioned, about their smoking status, and those who reported smoking were classified as smokers at the time of the study, while those who had never smoked or smoked less than 100 cigarettes during their lifetime were classified as non-smokers. Thus, it is important to emphasize that the smokers were not excluded from the study.

Regarding the presence of a sedentary lifestyle, a questionnaire was developed by the study authors to investigate habits related to the practice, frequency, and types of physical exercise performed.

2.4. Analysis of the oxytocin receptor gene (OXTR) polymorphism (rs2254298)

Genotyping of the OXTR gene polymorphism was performed using polymerase chain reaction - restriction fragment length polymorphism (PCR- RFLP) method (Grodzicker, William, & Sambrook, 1974), the following primers were used: 5′-TGA AAG CAG AGG TTG TGT GGA CAG G-3′ (forward) and 5′ AAC GCC CAC CCC AGT TTC TTC-3 (reverse). The reaction was carried out in a final volume of 50 µL which contained buffer (5 mM), $MgCl_2$ (1.5 mM), dNTPs (2.0 mM), DSMO (2.0 Mm), 2.0 mM of each primer, Taq polymerase (0.3 U/µL), water (32.2 Mm), and g DNA (3.0 µg/mL). The PCR reaction had an initial melting temperature of 95 °C (5 min) followed by 36 cycles of melting (95 °C; 1 min), annealing (61 °C; 1 min), and extension (72 °C; 2 min). An extension period of 7 min at 72E C followed the final cycle. Finally, genotyping was performed by PCR-RFLP. The amplified product of 100pb was digested with 1.0 µL of the restriction enzyme BsrI® (New England Biolabs, Inc., Beverly, MA, USA), 2.0 Mm Milique water, 20.0 Mm PCR product, and 2.0 Mm of enzyme buffer, being the final volume 25 Mm. The digested fragments were visualized by electrophoresis on 4% agarose gel stained with ethidium bromide at 100 V for 60 min. The A allele yields 164bp, 136bp and 8bp. The G allele yields 164bp, 101bp, 34, and 8bp. The Hardy-Weinberg equation was applied to verify the balance of genotypic frequencies.

2.4.1. Oxytocin and cortisol

The measurements were performed by immunoenzymatic enzyme-linked immunosorbent assay (ELISA) using commercial kits (IBL International-TECAN, Hamburg, Germany), and followed the manufacturer's protocol. The measurements were performed in a private laboratory in Porto Alegre (RS-Brazil). Reference values ranged between 1.5 and 250 ng/mL for oxytocin and between 7 and 23 ng/mL for cortisol when collected between 7 a.m. and 9 a.m. The blood samples were collected at 8 a.m. It is important to highlight that the participants were instructed to disregard undergoing a 12-h fast. In order to avoid disrupting the logistics of the study, as it was part of a larger project. Other tests were being performed on the same day, there was no physical space to offer snacks after blood collection and, taking advantage of the fact that the elderly would not be fasting, we expected to observe the relationship between oxytocin and postprandial metabolism.

2.5. Ethical aspects

The present study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) and CONEP (National Council of Ethics in Research; Opinion No. 948,938).

2.6. Statistical analysis

The analysis of the allele and genotypic frequencies were performed using the Hardy-Weinberg formula (genotype frequency = p2 + 2pq + q2 and allelic frequency; p + q = 1), the expected genotype frequencies were calculated and compared using the nonparametric chi-square test. Before statistical tests, the normality of the variables was verified through the Kolmogorov-Smirnov test. For the variables that presented within normality, Student's *t*-test was used for continuous, independent and univariable variables, and ANOVA analysis of variance to compare more than two categorical and continuous variables. For the calculation of non-parametric variables, the tests of kruskall Waliis and Mann Whitney were used.

The chi-square test measured categorical variables, and Pearson's correlation compared two numerical variables. Since oxytocin emerged as an asymmetric variable with a large standard deviation (SD), it was transformed into a logarithm before analysis with the parametric tests Student's *t* and ANOVA.

Variables exhibited p = or below 0.20 in the bivariate analysis were carried forward to the adjusted analysis. As it was a cross-sectional study, the measure of effect used was prevalence ratio (PR) obtained by means of Poisson Regression with robust variance adjustment. *P* values ≤ 0.05 were considered significant.

3. Results

The sample consisted of individuals with an average age of 72.6 ± 6.9 years, being most of them females (n = 129; 72.9%), Caucasian (n = 121; 74.2%), African Brazilian (n = 30; 18.45%), brown (11; 6.7%), and one (0.7%) was Asian Brazilian.

Table 1 lists the general characteristics of the studied population, being the distribution of the genotype frequencies of this population the following: GG = 62 (44.3%), AG = 58 (41.4%) and AA = 20 (14.3%).These frequencies were in Hardy-Weinberg equilibrium ($\chi 2 = 1.12$, p = 0.29). Among the cardiovascular risk factors, the most prevalent ones were sedentary lifestyle (84.8%) and hypertension (69.1%). Regarding the anthropometric measurements, the overall

Table 1
Cardiovascular risk factors, metabolic parameters and genotypic frequencies of the elderly in southern Brazil.

Genotypes OXTR	N (%)
GG	62 (44.3)
AG/AA	78 (55.7)
Cardiovascular risk factors	
Hypertension	114 (69.1)
Diabetes Mellitus	54 (32.7)
Dyslipidemia	91 (55.5)
Sedentary	140 (84.8)
Obesity	64 (36.2)
Central Obesity	74 (55.6)
Smoking	11 (6.7)
Hormones ng/mL	**Mean ± Standard deviation**
Cortisol	15.0 ± 5.2
Oxytocin	51.1 ± 81.7
Log*Oxytocin	3.0 ± 1.4
Cardiovascular and Metabolic Parameters	
SBP (mmHg)	127 ± 20.0
DBP (mmHg)	75.4 ± 11.4
Postprandial TGC (md/L)	297.8 ± 173.4
Postprandial glucose (md/L)	118,1 ± 50,7
Anthropometric measurements	
WC (cm)	94.6 ± 14.0
Man	97.5 ± 13.0
Woman	93.7 ± 14.2
BMI (kg/m^2)	28.2 ± 6.0
Man	26.9 ± 5.4
Woman	28.5 ± 6.1

Table 2
Lack of association of the polymorphisms oxytocin receptor gene (rs 2254298) with cardiovascular and metabolic risk factors in elderly individuals in southern Brazil.

	Genotypes N (%)		
	GG	AG/AA	P*
Risk Cardiovascular			
Hypertension			0.569
Yes	41(46.1)	48 (53.9)	
No	16 (40.0)	24 (60.0)	
Diabetes Mellitus			0.849
Yes	19 (46.3)	22 (53.7)	
No	38 (43.2)	50 (56.8)	
Dyslipidemia			0.289
Yes	33 (49.3)	34 (50.7)	
No	24 (39.3)	37 (60.7)	
Sedentary			0.639
Yes	11 (50.0)	11 (50.0)	
No	46 (43.0)	61 (57.0)	
Central Obesity			0.840
Yes	26 (43.4)	34 (56.7)	
No	17 (39.5)	26 (60.5)	
Nutritional Status			0.727
Leanness/Eutrophy	23 (41,8)	32 (58,2)	
Overweight	27 (51.0)	26 (49.0)	
Postprandial Glucose md/L			0,932
Equal or below 160	40 (44.0)	51 (56.0)	
Above 160	04 (50.0)	04 (50.0)	
Postprandial TGC md/L			0.859
Equal to or below 150	09 (40.9)	13 (59.1)	
Between 150 and 300	18 (45.0)	22 (55.0)	
Above 300	15 (46.8)	17 (53.2)	
Cardiometabolic parameters	**Mean ± Standard deviation**		**P****
SBP(mmHg)	126.7 ± 21,1	127.2 ± 21.6	0.769
DBP (mmHg)	75.0 ± 11.4	75.2 ± 11.3	0.939
Postprandial TGC (mg / dL)	304.4 ± 165.4	288,3 ± 173.5	0.642
WC (cm)	96.4 ± 15,1	94.1 ± 13.7	0.415
BMI (kg/m ^{2})	29.4 ± 6.8	27.8 ± 5.6	0.186
Postprandial glucose md/L	110,6 ± 34,6	112.9 ± 34.9	0.751
Hormones			
Cortisol (μg/mL)	14.5 ± 4.0	14.8 ± 5.7	0.737
Oxytocin (μg/mL)	3.0 ± 1.3	2.9 ± 1.4	0.766

SBP = systolic blood pressure; DBP = dyastolic blood pressure; TGC = postprandial capillary triglycerides WC = waist circumference; BMI = body mass index.

waist circumference was of 94.6 ± 14.0 cm, and regarding the nutritional status, the average BMI of the participants was in the overweight category (28.3 ± 17 kg/m^2) according to the Lipschitz criteria (Lipschitz, 1994). The average concentration of the hormones evaluated in the sample were 15.0 ± 5.2 μg/dL for cortisol (which is within the borderline value) and 51.1 ± 81.7 ng/mL (median 19.1 ng/mL, range −5.9 ng/mL to 396 ng/mL) to serum oxytocin.

Table 2 shows that OXTR genotypes showed no association with cardiovascular risk factors and metabolic parameters. Pearson's correlation resulted in a negative correlation between serum oxytocin and postprandial TGC concentrations (r = −0,196, p = 0.035) and BMI (r = −0,213, p = 0.019) as shown in Figs. 1 and 2. However, we did not observe any correlations between serum oxytocin and systolic blood pressure (r = −0.106 p = 0.240), diastolic blood pressure (r = −0.081, p = 0.366), waist circumference (r = −0.167 p = 0.065), postprandial glucose (r = 0.134 p = 0.137) and cortisol (r = 0.009 p = 0.921).

Table 3 shows that when obesity was classified according to the Lipschitz classification, the association remained significant among lean participants in relation to eutrophic and overweight ones (p = 0.005), being the findings confirmed by the Tukey's test. When the stratification of the triglycerides was done (ANOVA test), the individuals who presented values of triglycerides of 150 mg/dL or below also presented the highest oxytocin values (p = 0.027). Such association was

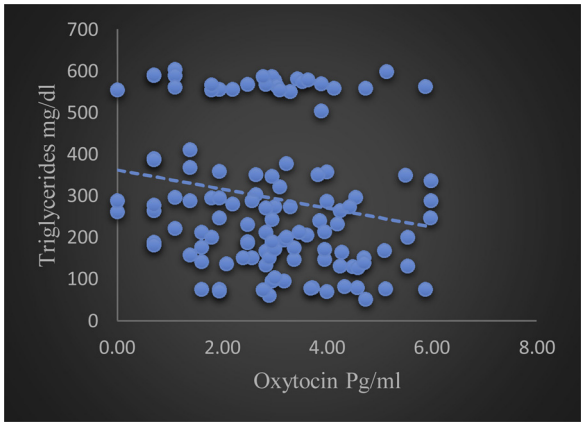

Fig. 1. Correlation between oxytocin level and triglycerides in elederly attendend by Primary Health Care (r = −0,196 e *P* = 0,035.

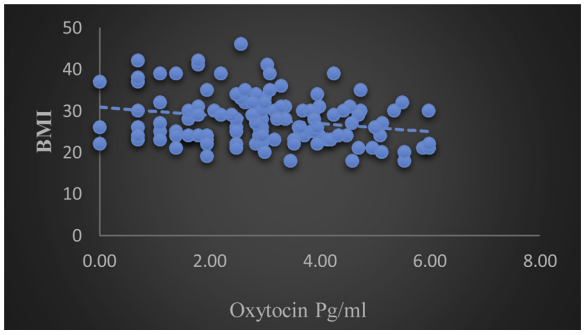

Fig. 2. Correlation between oxytocin level and body mass index BMI in elederly attendend by Primary Health Care (r = −0,019 e *P* = 0,019).

significant in Tukey's test in the group of individuals whose trigyceride concentration was the same or below 150 mg/dL, and above 300 mg/dL.

The assessment of prevalence ratio with the introduction of potentially confusing variables observed a statistically significant association in the elderly classified as lean with higher levels of oxytocin (PR = 1.866, IC95%1.125–3.095) and lower levels of postprandial triglycerides (PR = 0.997,IC95%:0.993–1.000) (Table 4).

Regarding postprandial lipemia, binary tests were performed to adjust the confounding values. The Poisson regression model showed that male individuals had 80.5% of (PR = 0.805, IC95%:0.695–0.933) and the elderly with higher mean values of oxytocin had 96.6% of PR (PR = 0.966, IC95%:0.937–0.996). Explaining that these factors are associated with values of postprandial TGC equal to or less than 150 mg/dL (Table 5).

4. Discussion

The purpose of this study was to verify if there was an association between serum oxytocin levels and *OXTR* gene polymorphism with cardiovascular and metabolic risk factors in elderly people. The first hypothesis was confirmed, demonstrating a correlation between oxytocin, triglycerides and the nutritional status of the investigated individuals. Indeed it is a pioneering study in aging and cardiogeriatrics areas, and it may be used as a guide for further scientific advances about the relationship of the oxytocin with metabolic and cardiovascular mechanisms in elderly people. However, we found no significant associations between *OXTR* gene polymorphism with cardiovascular and metabolic risk factors in our sample, and it is important to highlight that studies using this approach are still rare in the literature.

One of the first studies in this area analysed the socioeconomic

Table 3
Association between serum OT concentration and cardiovascular and metabolic risk factors in elderly individuals in southern Brazil.

Cardiovascular risk factor	Oxytocin Mean ± Standard deviation	P**
Hypertension		0.955
Yes	3.0 ± 1.4	
No	3.0 ± 1.4	
Diabetes Mellitus		0.617
Yes	3.2 ± 1.3	
No	3.0 ± 1.4	
Dyslipidemia		0.209
Yes	2.9 ± 1.4	
No	3.2 ± 1.4	
Sedentary		
Yes	3.0 ± 1.3	
No	3.0 ± 1.4	
Central Obesity		0.168
Yes	2.8 ± 1.3	
No	3,1 ± 1.4	
Smoking		0.148
Yes	3.8 ± 1.6	
No	3.0 ± 1.4	
Postprandial Glucose md/L		0,113
Equal or below 160	3,0 ± 1,4	
Above 160	3,7 ± 1,3	
Nutritional Status		**0.005*****
Skinny [ab]	4.1 ± 1.5	
Eutrophic [b]	2.8 ± 1.4	
Overweight [b]	2.8 ± 1.2	
Postprandial Triglycerides md/L		**0.027*****
Equal or below 150[ab]	3.5 ± 1.2	
Between 150 e 300[b]	2.9 ± 1.4	
Above de 300[ab]	2.6 ± 1.4	

** *t*-test *** variance analysis ANOVA mean values with different letters are significantly different (Tukey test).

Table 4
Poisson's Log Regression Model of associated factors (prevalence ratio) to leanness in elderly individuals in southern Brazil.

Variable	PR adjusted	CI 95% adjusted	*P**
Age	1.049	0.899-1.058	0.283
Gender (Men)	0.274	0.961-1.145	0.190
Postprandial Glucose (mg/dL)	0.985	0.968-1.003	0.096
Postprandial TGC (mg/dL)	0.997	0.993-1.000	**0,031**
Oxytocin (µg/mL)	1.866	1.125-3.095	**0,016**

PR = Prevalence Ratio, CI = confindence interval, *P* = Wald Test.

Table 5
Poisson's Log Regression Model of associated factors (prevalence ratio) to postprandial triglycerides in elderly individuals in southern Brazil.

Variable	PR adjusted	CI 95% adjusted	*P*
DBP	1.000	0.995–1.005	0.918
BMI	0.993	0.977–1.008	0.354
SBP	1.002	0.937–0.996	0.288
WC	1.005	0.999–1.012	0.126
Age	0.995	0.987–1.002	0.165
Oxytocin	0.966	0.937–0.996	**0.028**
Gender (Men)	0.805	0.695–0.933	**0.004**

PR = prevalence ration, CI = confiance Interval, *P* = Wald Test, DBP = Diastolic blood pressure, SBP = Sistolyc blood presure, BDI = body mass index, WC = Waist circumference.

influence of the OXTR gene polymorphism on obesity in a cohort of children and found that carriers of the A allele (AG/AA) from families with higher financial and social status presented lower BMI and that this relationship was not verified in GG individuals (Bush et al., 2017). Another study conducted in a group of undergraduate students homozygous for the G allele revealed alterations in blood pressure and serum cortisol after a simulation of ostracism, effects that were not evident among carriers of the A allele (McQuaid, McInnis, Matheson, & Anisman, 2015).

It is important to highlight the close relationship between these genotypes and ethnicity, according to the data for the polymorphism OXTR gene. (Brune, 2012). Nations such as Japan, China and Korea presented higher frequency of A allele in comparison with the population of the United States, United Kingdom, Australia, Canada, Netherlands, Italy, Sweden, Germany and Finland which had a higher frequency of G allele (Luo & Han, 2014). Elderly people with a heterogeneous ethnic and cultural profile composed our sample (Pena et al., 2011).

Which is a striking feature of the population living in the south of Brazil, with an extensive genomic mix and a strong imprint of the wave of massive immigration that occurred in the centuries XIX and XX, resulting in a high genetic variability ancestral (Gottlieb, Schwanke, Gomes, & Cruz, 2011). It suggests that Brazilians have a unique proportion of Amerindians, Europeans and Africans in their genomic mosaic, which may have implications in the epidemiology and diagnosis of diseases that have an etiology in genetic polymorphisms (Pena et al., 2011).

Therefore, elements such as culture and environment have often caused confusion in the attempts to replicate the earliest discoveries about the OXTR gene polymorphism, making the genotype alone unable to predict certain phenotypes. Accordingly, the OXTR is regulated by a combination of hormones, inflammatory cytokines and epigenetics mechanisms (Gregory et al., 2009).

Regarding the correlation between the serum oxytocin levels and the cardiometabolic variables, we found that the higher the hormone's serum concentration, the lower the postprandial levels of triglycerides in the elderly. Experiments with central and peripheral oxytocin infusion in animal models have showed an increased expression of the messenger RNA of some genes related to lipolysis and beta-oxidation of fatty acids (Deblon, Veyrat-Durebex, & Bourgoin, 2011). A more recent study suggested that the oxytocin has a role in the endogenous synthesis and extracellular uptake of fatty acids (Ding, Leow, & Magkos, 2019).

In a clinical trial conducted by Lawson et al. (2015), intranasal oxytocin was administered to a group of healthy men and, at the end of the experiment a slight reduction in serum TGC levels was observed. It is important to emphasize that in our study the measures of lipid marker in capillary blood were conducted 2–4 h after a meal. The choice of using a postprandial method took into consideration the fact that the oxytocin acts on the regulation of the energetic balance, on the regulation of dietary intake (due to its anorectic mechanisms), and on lipolysis, thermogenesis and insulin sensitivity (Leslie, Leppanen, Paloyelis, & Treasure, 2018; Maejima, Yokota, Nishimori, & Shimomura, 2018).

We have found no similar results in the literature, which may be explained by the fact that the measurement of postprandial serum lipid levels is still a novelty in clinical practice and research studies. However, this approach should be considered, as during most of the day an individual remains in a permanent postprandial state (Bansal, 2007), which exposes the body to the circulating lipids when the individual is awake, while fasting occurs only during sleep, which lasts, in adults, between 6–8 h (Nakamura, Miyoshi, Yunoki, & Ito, 2016).

Although triglyceride levels are typical, when obtained during fasting, the postprandial hypertriglyceridemia may play an important role in atherosclerosis, as it is related to the production of proinflammatory cytokines and to the production of oxidative stress, resulting in endothelial dysfunction (Pirillo, Norata, & Catapano, 2014).

The elderly classified as lean in our study presented a significantly higher serum oxytocin levels. Studies in animal models have suggested that this hormone exerts anorexic effects and regulates hunger (Sabatier, Leng, & Menzies, 2013; Takayanagi et al., 2008). Ott et al. (2013) observed that the administration of intranasal oxytocin to a

healthy cohort decreased the consumption of foods with sweet flavors and induced satiety. Another explanation for these results could be given by the gastric distension modulation along with the activation of GABAergic synapses, and secretion of Cholecystokinin (CCK) from the duodenum, an anorexic hormone (Wu, Doong, & Wang, 2008).

Lawson et al. (2015), conducted a clinical trial in which intranasal oxytocin was administered to a cohort of men aged 20–40 years and found that the intervention group presented elevation of serum CCK. It is also speculated, that the presence of oxytocin receptors in the ventral tegmental area of the brain may interfere with the signaling of dopamine in the nucleus accumbs, which contribute to the regulation of the intake of palatable foods such as carbohydrates and sweets (Lawson et al., 2015; Sabatier et al., 2013).

Studies that addressed the role of oxytocin in aging are scarce. However, one study, which measured oxytocin in a population of 540 volunteer men aged 50–85 years and correlated it with body mass index, found a reverse result to ours (Szulc et al., 2016). Such results support the hypothesis that acute gastric distension, which is the result of a large intake of food, may cause a negative feedback on the satiety centre. Such results had been previously found for rodents that after ingesting a large meal had their blood levels of oxytocin increased (Nelson, Alberts, Tian, & Verbalis, 1998). Qian et al. (2014) showed lower serum OT levels in T2DM patients which correlated with a variety of metabolic markers (BMI, waist-to-hip ratio), but the sample was formed mostly by women.

Differences in the sample size, methodological heterogeneity, and a limited number of publications in humans suggest that further studies are needed to determine the magnitude of the influence of this neuropeptide in obesity. The main points that should be further addressed are: evaluation of food intake in the last 24 h, as it influences the body metabolism; and gender-specific studies, evaluating the potential effects of estrogen and progesterone on oxytocin secretion and receptor distribution.

An experimental study with ovariectomized Wistar rats demonstrated that estrogen deprivation reduced oxytocin expression in OTergic neurons of the paraventricular nucleus of the hypothalamus (De Melo et al., 2016). In humans, the results of a cross-sectional study showed that oxytocin levels decrease in postmenopausal women compared to premenopausal women (Maestrini et al., 2018).

The present study had some limitations. First, the sample size was small. These patients were part of a larger longitudinal study and their consultation was scheduled well in advance and, sometimes, they had problems and could not attend the consultation on the schedule day. Secondly, we used some methods to measure triglycerides and fasting glucose that have not been well established within the scope of research studies. We chose to use the capillary methods to determine the lipid profile and fasting glucose to avoid disrupting the logistics of the other studies. However, the benefits offered by the capillary tests, such as dry chemistry, and the availability of the results within a few minutes allowed some health education activities with family members and the participants of the study.

According to Donato et al. (2015), outpatient and public health screening services based on lipid measurements would benefit from a method that demands shorter periods of fasting and offers an accurate evaluation of the patient's cardiovascular risk. Furthermore, some studies have showed that the results from the capillary tests for glucose and triglycerides (accutrend®) were not significantly different from the standard method. Studies that investigated the accuracy and precision of the Accutrend Plus system to determine blood glucose, total cholesterol, and plasma triglycerides suggested that a portable multi-analyser is a valid alternative for monitoring metabolic disorders and cardiovascular risk factors (Eizerik, 2012; Coqueiro et al., 2014).

Despite these limitations, it is important to highlight that it was the first study to observe and describe the allelic and genotype frequencies of the *OXTR gene* polymorphism (rs2254298) in a population of Brazilian elderly. Which is known for its heterogeneous ethnic and cultural profile, especially in the south of Brazil, and to perform the serum oxytocin dosage in a group above 60 years of age, providing some evidence on how this hormone influences some metabolic variables.

Finally, the results suggest that the Postprandial TGC levels increase as OT levels decrease, being this hormone significantly high in lean elderly. Moreover, the present study showed a lack of association between OXTR polymorphism and cardiovascular and metabolic risk factors. Therefore, it is proposed that further studies are necessary to elucidate the role of this polymorphism in the metabolic and cardiovascular physiology of the elderly and in individuals of other age groups.

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Author statement contributors

C.B.J. Participated in the elaboration, construction of the methodological design, clinical evaluation of the study subjects, statistical analysis and discussion of the results. I.G and M.G.V.G designed the study and managed the literature searches and analyses. C.A.B carried out the genotypes and database. L.S.R was responsible for performing the anthropometry. B.L.C was responsible for the analysis of oxytocin and serum cortisol.

All authors contributed to and have approved the final manuscript.

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