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Serum concentration of nitric oxide in women smokers and nonsmokers with overweight

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Abstract

Aim: **to evaluate the serum concentration of NO in overweight women, smokers (SG) and nonsmokers (NSG).**

Methods: **blood samples from smokers (n = 20) and nonsmokers (n = 18) were collected to obtain serum, and stored at -80 °C until analysis. NO was assessed by measuring total nitrite, determined by Greiss method. It was adopted as reference 24.4 μmol/L, mean value found in a study with healthy subjects without excess weight. We used the Student t test to compare the means of age and waist circumference, and the Mann-Whitney U test to compare the median of concentrations of nitrite, number of cigarettes/day and Body Mass Index. We adopted a significance level of $p < 0.05$.**

Results: **the median nitrite in SG was 16.53 (2.79 – 69.72) μmol/L, whereas in NSG was 10.85 (1.44 – 43.25) μmol/L ($p = 0.028$). BMI median value to SG and NSG, was respectively 29.50 (25.00 – 38.14) kg/m² and 30.68 (25.10 – 36.98) kg/m² ($p = 0.530$), being classified as overweight. The data showing that the average nitrite was below the estimated value for healthy individuals.**

Conclusion: **the results indicate a decrease of NO metabolites in women with excess weight, independently of being smoker. Despite the significant difference found between groups, these women had values well below the reference value of NO for healthy women. Therefore, it seems that smoking does not interfere in nitrite levels in patients already compromised by obesity.**

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Key words: *Smoking. Nitric oxide. Overweight. Inflammation. Addiction.*

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CONCENTRACIÓN SÉRICA DE ÓXIDO NÍTRICO EN MUJERES FUMADORAS Y NO FUMADORAS CON SOBREPESO

Resumen

Objetivo: **evaluar la concentración sérica de NO en las mujeres con sobrepeso, fumadoras (SG) y no fumadoras (GSN).**

Método: **se recogieron muestras de sangre de las fumadoras (n = 20) y no fumadoras (n = 18) para obtener el suero, y se almacenaron a -80 °C hasta su análisis. NO se evaluó mediante la medición total de nitrito, determinado por el método Greiss. Fue adoptado como referencia de 24,4 μmol/L, valor medio que se encuentra en un estudio con sujetos sanos sin exceso de peso. Se utilizó la prueba t de Student para comparar las medias de edad y la circunferencia de la cintura, así como la prueba de Mann-Whitney para comparar la mediana de las concentraciones de nitrito, número de cigarrillos/día y el Índice de Masa Corporal. Hemos adoptado un nivel de significación de $p < 0,05$.**

Resultados: **la mediana de SG nitrito fue 16,53 (2,79-69,72) mol/L, mientras que en NSG fue 10,85 (1,44-43,25) μmol/L ($p = 0,028$). El IMC valor de la mediana de SG y NSG fue, respectivamente, 29,50 (25,00-38,14) kg/m² y 30,68 (25,10-36,98) kg/m² ($p = 0,530$), siendo clasificado como sobrepeso. Los datos muestran que el nitrito promedio estuvo por debajo del valor estimado para individuos sanos.**

Conclusiones: **los resultados indican una disminución de los metabolitos NO en las mujeres con exceso de peso, independientemente de si son fumadoras o no. A pesar de la diferencia significativa entre los grupos, estas mujeres tenían valores muy por debajo del valor de referencia del NO para las mujeres sanas. Por lo tanto, parece que el fumar no interfiere en los niveles de nitritos en pacientes que ya están comprometidas por la obesidad.**

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Palabras clave: *Fumar. Óxido nítrico. Sobrepeso. Inflamación. Adicción.*

Introduction

Nitric oxide (NO) is a radical free, gaseous, inorganic, which is one of the most important mediators of intracellular and extracellular inflammatory processes. It is produced from L-arginine by a reaction mediated by class of enzymes NO synthase, which may be beneficial or harmful to the body depending on its concentration[1,2].

NO is involved in vascular relaxation having a major role in protecting blood vessels and as a modulator in several essential biological processes. Its effect is present particularly in situations of oxidative stress, generation of oxygen intermediates and deficient antioxidant system[2,3]. Certain substances can alter and improve the malefic effects of NO as the nicotine[4].

Nicotine stimulates the release of catecholamines, causing injury to the arterial endothelium and promoting atherogenesis. Furthermore, the largest production of free radicals and aromatic compounds cigarette decreases the synthesis of NO in endothelium, impairing relaxation of the arteries revealed an early sign of endothelial dysfunction. Smokers have alterations in vascular biological balance as in vascular tone, favoring vasoconstriction, platelet aggregation and thrombogenesis[5,6].

Tabagism is associated with a number of disorders and also chronic diseases such as hypertension, heart diseases and respiratory diseases. Stopping smoking is an important strategy to reduce morbidity and mortality associated with these tobacco-related diseases[7]. Associated with obesity, the metabolic profile of the smoker can still aggregate other cardiovascular risk factors such as dyslipidemia and insulin resistance[8].

It is known that there is an inverse relation between the use of nicotine and body weight. According to studies, the Body Mass Index (BMI) tends to be lower in smokers compared to nonsmokers, because the use of this substance increases the basal metabolism and alters brain neurotransmitters which contribute to decreased appetite and, consequently, to lower energy intake[7]. However, the lifestyle of smokers contributes to the development of obesity because they are more sedentary and have poor dietary habits. Excess weight is also the key to keep smoking and discourages the search for treatment to stop smoking[9,10], besides being an additional factor that can alter the synthesis and action of NO as well, and inflammatory profile generation[11].

Female individuals have been a focus of study due to factors associated with the development of cardiovascular diseases[12]. During the aging process, changes occur in the metabolic profile resulting in changes in the composition and distribution of adipose tissue, facilitating the weight gain as well as the progression of the atherosclerotic process[13,14]. Several studies suggest that women hormonal changes modulate endothelial cell function through effects on the production or activity of NO. For example, estradiol exerts vasodilation effect, by acting on the stimulation of NO synthesis, as well as the change of progesterone that combined with estrogen can alter the activity of NO[15,16].

Nitrites and nitrates are the stable end-products of the reaction of NO with molecular oxygen. The dosage of NO is complex and therefore its indirect determination by measuring metabolites is widely used in the characterization of inflammatory profile[17].

Until the present moment, there are insufficient reports on the concentration of NO or its metabolites in smokers and nonsmokers who are overweight, requiring more studies about this issue to better understand the inflammatory state associated with the smoking habit. Thus, the aim of this study was to evaluate the serum concentration of NO in female smokers (GT) and overweight nonsmokers women (GNT). Considering, as previously described, the relationship between smoking and body weight, this study also aims to correlate age, BMI, number of cigarettes/day and waist circumference (WC) with NO and, yet, correlate BMI with CC and cigarettes/day and to correlate WC with cigarettes/day.

Methods

This is a study of cross-sectional design with a convenience sample in which 20 smoking women (SG) on treatment for withdrawal at the Center for Tobacco Research and Intervention of the University Hospital of the Federal University of Juiz de Fora were evaluated (CIPITI-HU/UFJF) and 18 non-smoking women (NSG) who initiated treatment for weight loss in Clinical Nutrition Ambulatory/HU-UFJF. The study was carried out with overweight and sedentary women, aged between 20 and 72 and who agreed to participate.

They were invited to sign a consent term and then included in this study. Pregnant women or nursing mothers, severe heart disease, liver disease, chronic or acute, cancer, neurological diseases, psychiatric and Acquired Immunodeficiency Syndrome (AIDS) kidney disease holders were not included in the study. The proposal was approved by the Ethics in Research Committee of the Federal University of Juiz de Fora (Number: 566.857).

In the first day of follow-up, clinical history and anthropometric measurements were assessed in each of the smokers and nonsmokers by trained professionals and students working on both ambulatories. We collected data about smokers' length of smoking, age at initiation of smoking, number of cigarettes smoked per day.

Anthropometric nutritional assessment was performed at the beginning of the treatment of smokers and nonsmokers. Body weight (BW), height, waist

circumference (WC) were measured in a reserved room and with standardized protocols[18]. The BW measurement was performed using electronic digital balance (Welmy®), and the patients were assessed barefoot and wore light clothing. Height was measured with a stadiometer, with the patient standing, barefoot, arms outstretched next to the trunk and heels together, raising their head with eyes fixed on the horizon, touching the vertical rod of the stadiometer. The interpretation of the results followed the cutoffs WC ≥ 80 cm for women, indicating risk of cardiovascular disease measures that exceeded these values. To assess the nutritional status, Body Mass Index (BMI) was calculated by the formula: BMI = weight (kg) / height (m^2). The classification was performed as proposed by World Health Organization[18].

Blood samples were taken after 12 to 14h overnight fasting and centrifuged, within 30 to 45 min of collection and stored at - 80°C until analysis. These tests were performed at the Center of Cellular Technology and Applied Immunology (IMUNOCET) of the Institute of Biological Sciences/UFJF. We used the Greiss method for determining serum NO, since it is the most frequently used assay in experimental and clinical studies[11,2]. In brief, excess protein was removed by precipitation in acid/salt. To perform the test, 100mL serum samples were transferred to 96 templates subsequently added to equal volume of Griess reagent (1% sulfanilamide, 0.1% N - (1-naphthy) ethylene diamine Hydrochloride 5% H_3PO_4, Sigma, St. Louis, MO, USA). The concentration of NO was determined by comparison with a standard solution of sodium nitrite and from the linear standard curve established by 0–100µmol/L sodium nitrate.

Statistical analysis

Initially the homogeneity of variances and normality were tested. It was adopted as criterion significance level of 5% (α= 0.05). All data were analyzed using the statistical package PASW 18.0.

To compare the mean age and CC between groups, it was performed the Student's t test. To compare the variables, cigarettes / day, BMI and NO between the groups, it was performed the Mann-Whitney U test. The correlation analysis between groups, between BMI, WC and NO was performed using the Spearman test.

Results

The study included 38 women (smokers = 20 and nonsmokers = 18). The average age of participants was 47.63 ± 11.33 years. The SG had a mean age of 52.15 ± 8.58 years and the NSG averaged 42.61 ± 12.11 years. The median value of NO of all participants was 11.36 (1.44 - 69.72) mmol/L, while in the SG was 16.53 (2.79 - 69.72) mmol/L and the NSG was 10.85 (1.44 - 43.25) mmol/L. Importantly, the groups showed homogeneous in relation to the variables analyzed.

In the descriptive analysis of the cigarettes smoked by SG, we found median of 20 (5 - 40) cigarettes/day. To the BMI values of all women, median was 29.98 (25 - 38.14) kg/m^2. The SG presented median value of 29.50 (25 - 38.14) kg/m^2 and the NSG presented median value of 30.68 (25.10 - 36.98) kg/m^2. When analyzing the data for WC, the mean encountered for all participants was 91.23 ± 12.29 cm, the mean for the SG was 91.48 ± 12.76 cm, and the mean of NSG was 90.97 ± 12.11 cm. All descriptive data are presented in table I.

When comparing the study variables between the groups, it was found that there is a statistical difference between the average age of groups (SG = 52.15 ± 8.58 years; NSG = 42.61 ± 12.11 years; p = 0.001). In the comparative analysis of the WC values, it was also found statistically significant differences between groups (SG = 91.48 ± 12.76 cm; NSG = 90.97 ± 12.11 cm; p = 0.001). Comparing the NO values, the SG and the NSG also showed a statistically significant difference (SG = 16.53 (2.79 - 69.72) mmol/L; NSG = 10.85 (1.44 - 43.25) mmol / L; p = 0.028). There was no significant difference when comparing the median

Table I
Descriptive data of age, WC, NO, BMI and number of cigarettes/day of total subjects, and SG and NSG groups

Variables	*All subjects (n=38)*	*SG (n= 20)*	*NSG (n= 18)*
	Mean ± Standard Deviation	*Mean ± Standard Deviation*	*Mean ± Standard Deviation*
Age (years)	47.63 ± 11.33	52.15 ± 8.58	42.61 ± 12.11
WC (cm)	91.23 ± 12.29	91.48 ± 12.76	90.97 ± 12.11
	Median (Min-Max)	*Median (Min-Max)*	*Median (Min-Max)*
NO (µmol/L)	11.36 (1.44 – 69.72)	16.53 (2.79 – 69.72)	10.85 (1.44 – 43.25)
BMI (Kg/m^2)	29.98 (25 – 38.14)	29.50 (25 – 38.14)	30.68 (25.10 – 36.98)
Cigarettes/day	-----	20 (5 – 40)	-----

BMI between the SG and the NSG (29.50 kg/m^2 and 30.68 kg/m^2; p = 0.530), confirming also overweight condition in both groups. All comparative data are presented in table II.

Correlational analyses were performed on age, BMI, number of cigarettes/day and WC with NO and also between BMI and WC, between BMI and cigarettes/day, and between WC and cigarettes/day between the groups. In the correlation analysis between the NO and WC, it was found a positive and statistically significant correlation in SG (r = 0.540; p = 0.014), while in NSG, no significant correlation was found (r = - 0.172, p = 0.494).

In correlation analyses between NO and BMI, NO and cigarettes/day in SG, and NO and age were not statistically significant correlations in both groups. There was also no significant correlation between BMI and cigarettes/day and between WC and cigarettes/day. In the correlation between BMI and WC, we observed a positive and significant correlation in the SG (r = 0.633; p = 0.005). All correlation data are showed in table III.

Discussion

The present study found significant difference in the concentration of NO in smoking and non-smoking women, both overweight. It was noted that 100% of smoking and non-smoking women showed values of NO lower than 24.4 µmol/L found by Ghasemi *et al.* (2008)[19] in a population-based study of healthy women. As demonstrated in previous studies[20,21], these values can be related to potential risk for the development of chronic low-grade inflammation, culminating in vascular changes due to excess body fat, which can be aggravated by smoking, known factor involved in these disorders in different clinical conditions.

In Eid *et al.* (2004) study[22], which evaluated the concentration of endogenous NO in obese and smokers individuals, it was found average NO concentration 26.1 µmol/L, while the median value found in this study group of overweight smokers was 16.53 µmol/L. The authors warn that overweight and smoking affect the concentrations of NO may exacerbate endothelial dysfunction, collaborating with the worsening of the

Table II
Comparison between the mean of age and WC and between the medians of NO and BMI between the SG and NSG groups

	SG		*SNG*		
	N	*Mean ± Standard Deviation*	*N*	*Mean ± Standard Deviation*	*p value*
Age (year)	20	52.15± 8.58	18	42.61± 12.11	0.000*
WC (cm)	20	91.48 ± 12.76	18	90.97 ± 12.11	0.000*
		Median (Min-Max)		*Median (Min-Max)*	
NO (µmol/L)	20	16.53 (2.79 – 69.72)	18	10.85 (1,44 – 43.25)	0.028*
BMI (kg/m^2)	20	29.50 (20 - 37)	18	28.00 (22 - 34)	0.530

BMI - Body Mass Index; NO - metabolites of nitric oxide; WC - waist circumference.
*p <0.05. Comparison smokers vs. non-smokers groups.

Table III
Correlation between age, BMI, WC, cigarettes/day and NO between the SG and NSG groups

	SG		*NSG*	
Correlation variables	*r*	*p*	*r*	*P*
NO - BMI	0,260	0.269	-0.053	0.835
NO - Cigarettes/day	0.182	0.441	-	-
NO - Age	-0.130	0.584	0.022	0.932
NO - WC	0.540	0.014*	-0.172	0.494
BMI - Cigarettes/day	-0.310	0.184	-	-
BMI - WC	0.119	0.616	0.633	0.005**
WC - Cigarettes/day	0.085	0.723	-	-

BMI – Body Mass Index; NO – Metabolites of Nitric Oxide;
WC – Waist Circumference.
* p < 0,005.
** p < 0,001.

inflammatory process. They also reported that high BMI combined with smoking may be determinant for metabolic disorders contributing to atherosclerotic disease, insulin resistance and diabetes. However, in the study of EID *et al*. (2004)[22] reported results were not only obese subjects.

In our study, we compared individuals with excess weight smokers and nonsmokers and different than expected, the average concentrations of NO found in women nonsmokers who are overweight (10.85 µmol/L) was 35.36% less than those found in the group of smokers (16.53 µmol/L). The significant difference ($p = 0.028$) found in NO values between groups confirms the idea that overweight smokers seem to have an increased production of nitric oxide than non-smokers, which may be an effect of nicotine since this seems to increase the levels of leptin, which is known to stimulate the production of nitric oxide[23].

The median value of NO (16,53 µmol/L) found in the SG was higher than that found in the NSG (10,85µmol/L), although below the reference value for healthy individuals, but it seems that this result is related with age in SG (52.1 years). This finding is supported for two studies[19,24], which demonstrated a significant relationship between age and serum NO. In this studies, the authors correlated the concentrations of NO with age in 5 categories (20-29, 30-39, 40-49, 50-59, 60+ years) and they realized that over 50-year women had highest levels of serum NO. It was also reported a possible relationship with the climacteric period, and/or menopause[19,25].

Although NO is highly toxic, it has a beneficial characteristic. This molecule is part of the arsenal of the first defense mechanism of the body, acting as antibacterial, antiparasitic and antiviral against invading microorganisms. NO in the cardiovascular system is responsible for maintaining blood flow and control of tissue extravasation, as well as broncho-pulmonary system homeostasis, keeping the airway caliber and balancing the relation ventilation/perfusion[17]. Was reported a reduction in plasma concentrations of NO in smokers associated with smoking in accordance with increase in the concentration of carbon monoxide in exhaled air (COex)[26]. Furthermore, in individuals with greater weight, reduction of NO can be present. In our study, there was no association between number of cigarettes/day and concentration of NO metabolites.

Not only active smokers but also passive smokers is liable to the deleterious effects of nicotine and that over time, may develop serious illnesses resulting in higher morbidity and mortality[27]. For this purpose, it becomes important, the measurement of serum levels of NO in the overall clinical evaluation of smokers, and should not be less important that evaluate the profile of the smoker, the level of nicotine dependence and motivation to quit smoking[28,29]. This dosage could also be a priority for passive smokers, because due daily and intermittent contact with cigarette smoke, probably their pathways of oxidative stress already are changed.

The overweight and smoking status present in the women involved in this study, may have contributed to the decreased levels of nitrites found. These factors increase the production of free radicals, reduce the bioavailability of NO and intensify the inflammatory state. The tobacco use is associated with toxic mechanisms direct of nicotine, such as aggravation of atherosclerosis and increased risk of coronary artery disease[6]. Overweight induces the inflammatory process generated by a sedentary lifestyle and bad alimentary habits, causing the unbalance of blood glucose and altered lipoprotein metabolism, lowering HDL-c, increasing LDL-c and total cholesterol[30].

This strengthens the premise that smoking and being overweight are important factors associated with changes in balance oxidant and antioxidant, pro-inflammatory and anti-inflammatory profile. Resulting in harmful to health, particularly when combined with other factors such as age, sedentary lifestyle, cigarettes/day, pre and/or post-menopause period, which is aggravated for these two groups[22,24,31].

It was also found that the values of BMI and WC confirm that, besides being overweight, these women have their body fat distributed with android profile. Yet, the average age of these women corresponds to the period of climacteric, menopause and post menopause, tending to increase the risk factors for coronary heart disease. Similar results were found in a review of literature[32]. Several other studies reported data demonstrating that women in menopause period had BMI increased, as well as changes in the pattern of body fat distribution, accentuating the WC. However, it is believed that reduction of body weight induced by balanced diet, physical activity and health habits can reverse endothelial dysfunction, decreasing the pro-inflammatory process, improving the NO production[32,33].

The results of this study should be interpreted with regard to its strengths and limitations. The present study design was a cross-sectional study which can only examine an exposure at a particular time and cannot determine causal relations. Furthermore, the small sample size of the groups (SG and NSG) may have contributed to significant differences not found in some results. Other studies with larger samples should be conducted to test this negative relation about the health of the smokers with overweight. Another limitation was that this study did not consider the quantification of COex in the groups to classify the smoking status and whether there were passive smokers among the control group.

Despite some limitations, our results provide evidence that smoking associated with overweight contributes to changes in nitric oxide concentrations in women. Furthermore, this study adds to literature a warning of the health damage caused by smoking combined with being overweight, as there are few studies in the literature correlating overweight women who are smokers and overweight. It is thought that

the deepening of thorough clinical evaluation of individuals who decide to quit smoking and lose weight healthfully increases the outlook of an improvement in overall clinical presentation, reversing damage caused by smoking and reducing risks for developing chronic non-transmissive diseases.

Conclusion

The Nitric Oxide values found for NSG were significantly lower than the SG values. Despite this difference, both groups may be suffering decrease in NO bioavailability, which may be associated with increased risk factors for diseases related to smoking and overweight. The values found of BMI and WC corroborate with the likely damage to the organism, and if no intervention action was performed, such damage will worsen over time. Therefore, it seems that the association of smoking with overweight interferes in the final levels of nitrite.

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Conflict of interest statement

The authors certify that no have affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements). The authors no have too, financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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