Association between Nitrite / Nitrate and Metabolic Risk in Blacks

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Abstract

Background: The black population paradoxically has a high cardiovascular risk with decreased rates of metabolic syndrome. The objective is analyze the levels of nitric oxide metabolites (nitrite/nitrate) and their applicability as a biomarker of cardiometabolic risk in blacks.

Methods and findings: We measured several laboratory parameters in 202 Black Brazilians. The 50th percentile (50P) of the nitrite/nitrate (NOx) was 122.3 µmol/L, and was chosen as the cutoff point, then the subjects were divided into two groups (i.e., superior or lower percentile). Anthropometric assessment showed that most of the study population had an average Body Mass Index classified as grade 1 obesity, altered waist circumference and pre-hypertension. The NOx values <122.3 µmol/L were associated with higher body mass index (p=0.01), waist circumference (p=0.03) and hip circumference (p=0.04). As to biochemical criteria, the NOx was significantly correlated to blood glucose levels (p=0.04), triglycerides (p=0.04), albumin (p=0.03), uric acid (p=0.01) and urea (p=0.05). In inflammatory and oxidative stress assessment, only protein carbonylation (p<0.01) was associated with the NOx, in which the damage was greater in subjects who had NOx values lower than the 50% percentile.

Conclusion: NOx was significantly associated with obesity markers, and obese individuals have lower levels of nitric oxide. Thus, NOx is a good predictor of cardiometabolic risk in blacks.

Keywords: Nitric oxide, Negroid race, Oxidative stress, Cardiovascular, Afro-descendants

Introduction

Outside of Africa, Brazil is the country that has the largest population of blacks. This population is known to be more socially and economically vulnerable than other Brazilians, with a lower life expectancy and susceptibility to diseases and health problems [1]. Blacks have a higher cardiovascular risk, even at a younger age, [2] however, paradoxically, they have lower rates of metabolic syndrome (MetS) [3]. The reason for this can diminshed the influence of some components of the metabolic syndrome in risk prediction [4].

Nitric oxide is a free radical that is implicated in a wide variety of physiological and pathological conditions [5]. It has an important role as vasodilatador, vasoprotective, bronchodilatador and inflammatory mediator [6-8].

Its metabolites, nitrite/nitrate (NOx), have emerged for the purpose of monitoring the health status of patients with cardiovascular diseases and appears to be promisingly used in the clinical field for the assessment of the pathological state [9]. However, there are no studies that evaluate the NOx levels in the black population, which is a population at high cardiometabolic risk and with peculiar health characteristics. Thus, this study

aims to analyze the levels of nitric oxide metabolites and its applicability as a cardiometabolic biomarker in a population of Brazilian blacks.

Materials and Methods

The study was approved by the Ethics Research Committee/ National Research Ethics Commission protocol number 977.827, according to the Helsinki Declaration.

Recruitment of participants

We recruited and enrolled 202 self-declaring blacks (i.e. blacks and browns) as volunteers in southern Brazil (Uruguaiana/ Rio Grande do Sul). All of the subjects signed an informed consent. The only ones to be excluded were those who present hemoglobinopathies and who are under 18 years of age.

Anthropometric measurements

The participants completed an examination with standardized measurements of weight, height, waist circumference (WC) (measured in average distance between the last rib and iliac crest around the navel) and hip circumference (HC) (measured at the maximum extension of the buttocks). From these measurements we calculated each participant's BMI, waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR). Body mass index (BMI) was calculated by dividing the weight (kg) with height (m²) and the classification was based on standard clinical definitions: normal weight 18.5-24.9 kg/m²; overweight 25.0-29.9 kg/m² and obese ≥ 30 kg/m². The guidelines of the International Diabetes Federation (IDF) were used for the diagnosis of Metabolic Syndrome (MetS) [10].

Sample preparation and measures

Venous blood was collected from all subjects after an overnight fast into tubes with EDTA or no anticoagulants. The samples were immediately centrifuged at 1500 rpm for 10 minutes at 4°C. The serum was utilized for biochemical analysis and Total Antioxidant Capacity (TAC), Total Oxidant Status (TOS), Ischemia Modified Albumin (IMA) and NOx. Erythrocytes were used for activity superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Plasma EDTA was used for the measurement of lipid peroxidation and protein carbonylation.

Fasting glucose, total cholesterol, HDL cholesterol, triglycerides, uric acid, urea and creatinine were performed by use of standard methods on ChemWell T (Labtest Diagnóstica SA, Minas Gerais, Brazil). LDL cholesterol was estimated by Friedwald's formula [11]. NOx levels were determined indirectly at Cobas Mira (Roche Diagnostics, Basel, Switzerland) by quantification of their metabolites (nitrates and nitrites), based on the Griess reaction, by a method previously described and validated by Tatsch et al. [12].

The glomerular filtration rate (GFR) was estimated by the Cockcroft-Gault equation [13]. TAC and TOS levels were calculated by using a new automated measurement technique described by Erel [14,15]. Malondialdehyde (MDA) levels for the measurement of lipid peroxidation were determined by the previously described spectrometric method [16]. Protein carbonyls were measured

by Levine's method [17]. Catalase activity was determined in accordance with the method that was already described [18]. RANSOD and RANSEL kits (RANDOX Laboratories Ltd., UK) were used to determine the activity of SOD and GPX enzymes following the manufacturer's protocol.

Statistical analysis

Data was statistically analyzed by using SPSS 20.0 software and the results are present as a mean ± standard deviation (SD). The NOx cutoff points were determined by the 50th percentile of the sample. The Student's t-test was used to determine the differences between the mean values of these parameters and the NOx cutoff point. For bivariate analysis between categorical variables and the cutoff point, we used the chi-squared test. Multivariate analysis with logistic regression (Backward Wald method) was used to test interfering factors. The statistical significance was assumed at $p<0.05$.

Results

Two hundred two people participated in the research between March and October of 2014. Most of the volunteers were (162, 80.2%) between 18 to 90 years of age. These individuals reported their race/color as black (57.4%) or brown (''pardo'', in Brazil) (42.6%). Demographic, anthropometric and blood pressure characteristics are shown in **Table 1**. Considering the influence of social and environmental factors in hypertension, presented in **Table 2** some lifestyle indicators.

The participants were divided according to their NOx value in percentiles to identify the relationship according to their different metabolic markers. The dosage of the NOx ranged between 28.1

Table 1 Baseline characteristics from the studied population (n:202).

Characteristics	Mean	SD
Age (years)	46.4	14.7
Peso (kg)	78.1	15.2
BMI (kg/m²)	30.1	5.8
WC (cm)	98.9	12.9
HC (cm)	108.6	11.5
WHR	0.91	0.84
WHtR	0.61	0.08
SBP (mmHg)	133.5	24.6
DBP (mmHg)	86.2	17.3

BMI: Body Mass Index, WC: Waist Circumference, HC: Hip Circumference WHR: Waist-Hip Ratio, WHtR: Waist-to-Height Ratio, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, SD: Standard Deviation.

Table 2 Lifestyle indicators and functional features.

	N	%
Marital Status		
Married	95	47.0
Single	65	32.1
Widower	22	10.9
Divorced	20	10.0
Functional Situation		
Worker	103	51.0
Retired	40	19.8
Student	9	4.5
Unemployed/Housewife	50	24.7
Education		
Illiterate	12	5.9
Elementary school	90	44.5
Middle school	20	9.9
Incomplete High school	21	10.4
Complete High school	43	21.3
College	9	4.5
Postgraduate	7	3.5

Figure 1 Presence of components of metabolic syndrome in percentiles NOX.
* indicates significant difference (p=0.016), MetS= Metabolic Syndrome.

and 598.0 µmol/L and the median, which corresponds to the 50th percentile (50p), was 122.3 µmol/L, thus the sample was divided into 101 (50%) subjects with NOx ≥ 50p and 101 (50%) with NOx <50p. To evaluate the influence of NOx and diseases, **Table 3** presents the baseline diseases of the black population.
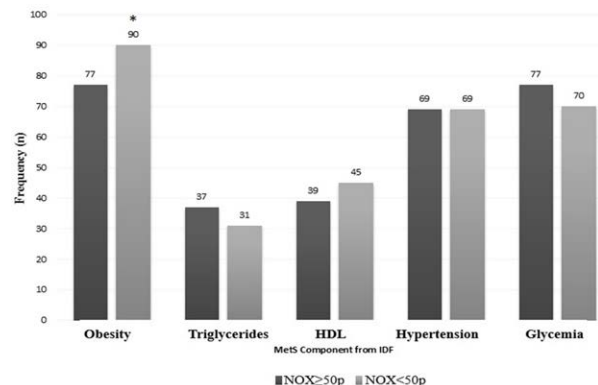
The prevalence of MetS by the IDF criteria was 61% in this population. In the analysis of each MetS criteria individually and their relationship to the percentile 50% of NOx using the chi-square test **(Figure 1)**, only the obesity criterion (given by measuring the WC) was statistically significant with the lower percentile values 50% of NOx (RR=1.71, CI 1.03-2.85, p=0.016).

The NOx percentiles were crossed with the anthropometric markers, and **Table 4** shows that blacks with lower levels of NOx (below the 50th percentile) had significantly higher BMI, WC and HC. The biochemical, inflammatory and oxidative parameters were analyzed and compared with the percentile of nitric oxide metabolites and the results are shown in **Table 5**. The NOx ≥ 50p group had higher levels of glucose (p=0.049), triglycerides (p=0.046), albumin (p=0.034), Uric acid (p=0.019) and urea (p=0.052) than the NOx<50p group. The analysis of logistic regression showed that the variables waist circumference and carbonyl levels were related with the NOx ≥ 50p group independently of sex, age and all other variables tested.

Discussion

As shown in **Table 1**, the studied black population consists of adults in whom obesity criteria are outside the normal range. The average body mass index (BMI) was 30.1kg/m², which characterizes obesity degree I. The obesity rate for blacks is known to be higher than in whites. One study showed that the prevalence of obesity among blacks and whites was 23.3% and 11.2% respectively, while the overweight category included 76.2% of blacks and 67.2% of whites [19].

The mean values of waist circumference (98.9 cm) are above the

limits recommended by the IDF abdominal adiposity, indicating a large deposit of abdominal fat. Increased waist circumference values have been associated with increasing age, particularly in a study of predominantly Brazilian black women [20]. When stratifying the waist circumference measures by sex, the average WC for men was 95.8 ± 12.4 cm and 99.8 ± 13.0 cm (p=0.08) for women which, was not significant, then it is a homogeneous population. Besides, the model of logistic regression showed that waist circumference was variable independently associated with NOx ≥ 50p group, indicating that elevated levels of nitric oxide has a close relationship with obesity indicators.

The average value of WHR=0.91 indicates cardiometabolic risk. The values of WHR=0.85 for men and WHR=0.90 for women as the cutoff point were considered compatible with specificity between high and moderate and useful as predictors of hypertension [21]. WHR by gender was 0.94 ± 0.10 cm for men and 0.90 ± 0.08 cm for women (p=0.08), thus highlighting the increase of this anthropometric relationship in this population, regardless of gender.

The average blood pressure was 133 × 86 mmHg, which was classified as borderline according to the Brazilian Society of Hypertension [22]. A population-based study in our state (Rio Grande do Sul) with nine hundred and eighteen adults showed a 33.7% prevalence of hypertension, and 49.8% of such a population was unaware of it [23]. This is an important finding, since hypertension is a non-communicable chronic disease with high morbidity and mortality, usually asymptomatic, and the lack of knowledge about it is a challenge, both in Brazil and in the world, for the control of blood pressure [23] [24].

The general data so far shows a population in cardiovascular risk, as seen in the average levels of blood pressure and anthropometrically checked. A study shows that 79% of hypertensive men and 65% of hypertensive women have cardiovascular events as a direct result of overweight, indicating a linear relationship between BMI and blood pressure [25]. It is estimated that a 1% reduction in the BMI would also result in a significant reduction in diabetes, cardiovascular diseases and

Table 3 Baseline diseases and differences by percentile value of 50% NOx.

Disease	N (%)	NOx ≥ 50p N (%)	NOx <50p N (%)	Odds Ratio (IC 95%)
Hypertension	108 (53.5)	57 (52.8)	51 (47.2)	1.2 (0.7-2.2)
Diabetes	32 (15.8)	15 (46.9)	17 (53.1)	0.8 (0.4-1.8)
Dyslipidemia	45 (22.2)	29 (64.4)a	16 (35.6)	2.0 (1.0-4.0)
Acute Myocardial Infarction	6 (3.0)	2 (33.3)	4 (66.7)	0.4 (0.1-2.7)
Angina	50 (24.7)	25 (50.0)	25 (50.0)	1.0 (0.5-1.9)
Stroke	8 (3.9)	6 (75.0)	2 (25.0)	3.1 (0.6-15.9)
Smoke	38 (18.8)	20 (52.6)	18 (47.4)	1.1 (0.6-2.3)
Use of alcohol	52 (25.7)	27 (50.0)	27 (50.0)	1.0 (0.5-1.9)
Sedentary	131 (64.8)	62 (47.3)	69 (52.7)	0.7 (0.4-1.3)
a= p<0.05				

Table 4 Obesity and blood pressure criteria compared with percentiles of NOx.

	Nox ≥ 50p (mean ± sd)	NOx < 50p (mean ± sd)	p
BMI (kg/m²)	29.08 ± 5.72	31.07 ± 5.75	0.01
WC (cm)	97.03 ± 12.89	100.86 ± 12.79	0.03
HC (cm)	106.89 ± 11.22	110.22 ± 11.59	0.04
WHR	0.91 ± 0.09	0.91 ± 0.07	0.68
SBP (mmHg)	132.60 ± 26.29	134.45 ±2 3.01	0.59
DBP (mmHg)	85.95 ± 18.28	86.50 ± 16.42	0.82

BMI: Body Mass Index, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist-Hip Ratio, WHtR: Waist-to-Height Ratio, SBP: Systolic Blood Pressure, DBP:Diastolic Blood Pressure, SD: Standard Deviation, Ns: Not significant

Table 5 Biochemical, inflammatory and oxidative parameters versus values in NOx percentile among blacks.

Parameter	NOx ≥ 50p (mean ± sd)	NOx < 50p (mean ± sd)	P
Glucose (mg/dL)	131.35 ± 50.56	118.84 ± 38.42	0.04
Total Cholesterol (mg/dL)	184.81 ± 40.17	185.50 ± 44.91	0.90
HDL Cholesterol (mg/dL)	50.91 ± 13.30	49.17 ± 12.36	0.33
LDL Cholesterol (mg/dL)	104.36 ± 14.78	112.55 ± 39.71	0.12
Triglycerides (mg/dL)	150.32 ± 99.67	126.65 ± 63.58	0.04
Albumin (g/dL)	4.08 ± 0.66	3.89 ± 0.56	0.03
GFR (mL/min/1,73m²)	121.43 ± 89.75	122.25 ± 44.44	0.93
Uric acid (mg/dL)	5.71 ± 2.01	5.12 ± 1.27	0.01
Urea (mg/dL)	26.06 ± 15.17	22.67±6.85	0.05
Creatinin (mg/dL)	0.84±0.30	0.79 ± 0.16	0.24
AST (U/L)	27.95 ± 8.18	29.41 ± 12.43	0.35
ALT (U/L)	11.28 ± 4.84	12.46 ± 7.39	0.20
GGT (U/L)	28.34 ± 38.41	34.09 ± 34.60	0.29
CRP (mg/L)	9.07 ± 6.21	9.28 ± 7.17	0.81
TAC (mmol Trolox Equivalent/L)	0.28 ± 0.14	0.29 ± 0.15	0.68
TOS (mmol Trolox Equivalent/L)	83.96 ± 49.24	85.19 ± 50.33	0.86
MDA (µmol MDA/ mL)	28.80 ± 11.30	27.56 ± 12.96	0.50
Carbonyl (nmol/g prot)	5.00 ± 2.12	6.33 ± 2.48	<0.01
CAT (U/mg Hb)	57.52 ± 14.37	54.79 ± 13.72	0.17
SOD (U/mg prot)	0.27 ± 0.98	0.19 ± 0.04	0.39
GPx (U/mg prot)	1725.30 ± 210.08	1746.25 ± 139.78	0.40

GFR: Glomerular Filtration Rate, AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-Glutamyl Transferase, CRP: C-reactive Protein, TAC: Total Antioxidante Capacity, TOS: Total Oxidant Status, MDA:Malondialdehyde, CAT:Catalase, SOD:Superoxide Dismutase, GPx: Glutathione peroxidase

cancer in the population [26]. Cardiovascular diseases account for a large number of hospitalizations and health care costs in Brazil [26]. Some explanations for the higher prevalence of hypertension among blacks include greater sensitivity to alcohol, increased salt sensitivity and the high renal retention of sodium, as well as differences in phenotypes and genotypes [27].

The marital status of individuals influences self-care and family dynamics [28]. In the health profile, studies related to self-care, healthier lifestyles and better quality of life in married subjects nearly signify the majority of our population (46.8%). More than half of the population (50.2%) are illiterate or have not finished elementary school, thus indicating a low level of education. The low educational level of most of the sample may be related to the results of the functional situation presented by the participants with the monthly income. The average income was 1.5 times the minimum wages (R$788.00; approximately $255.00, ranging from 1 to 10 minimum salaries. Lower income and education are commonly associated with poor access to health, bad quality of care, higher mortality, and, then, higher rates of disease. We observed a high prevalence of hypertension (53.5%) in this sample of the black population, and 31.6% it already had a cardiovascular event. Previous Brazilian studies have found a hypertensive prevalence of 19.8% in Rio Grande do Sul state [29], and prevalence of hypertensive prevalence of 29.9% associated with obesity and black women in Bahia state [30]. In the United States, a study found the prevalence of hypertension among blacks of 40% and whites 22%, indicating great disproportionality [3]. Although we attempted to verify that the NOx value was related to the presence of previous diseases by bivariate chi-square analysis, only dyslipidemia were significantly associated with NOx ≥ 50p (p=0.041). Blacks generally have lower rates of dyslipidemia in comparison to whites, but, in compensation, have higher rates of obesity, hypertension and diabetes [31].

The low bioavailability of NOx can be associated with the reaction of nitric oxide and oxygen with the formation of peroxynitrite, commonly because of inflammatory characteristics and oxidative stress in obesity [32]. Considering the physiological role of NO in the decrease of circulating NOx has detrimental effects, which result in cellular dysfunction and can cause insulin resistance, hypertension, pancreatic β cell dysfunction and diabetes [33].

As shown in Table 5, for glucose values in both percentiles, there is an increased value over that which would be considered suitable for fasting glucose until 100 mg/dL according to the criteria IDF, [10] and only 15.8% of the population had a diagnosis of diabetes. We suggest that these results strongly indicate the possibility of insulin resistance (IR) or prediabetes, whereas a failure in β pancreatic cells or insulin sensitivity may be causing the hyperglycemia that was presented by studied subjects.

An increase in NOx levels and hyperglycemia in patients with diabetes mellitus type II has been observed. The association between diabetes and oxidative stress (i.e., the formation of peroxynitrite) may explain why, even with high levels of NO, vascular resistance and, consequently, endothelial dysfunction can increase [34].

Although the triglyceride values differ, both percentiles are below or near normal value are less than 150 mg/dL. Triglyceride levels in blacks are usually lower, [35] even when other risk factors such as diabetes mellitus type II, cardiovascular disease and IR are present. Namely the absence of increased triglycerides does not imply the absence of cardiovascular risk in this population.

The albumin level decreased and had become associated with the NOx lower than the 50th percentile, although it was still slightly above the normal value (3.5 mg/dL). The hypoalbuminemia may result from liver or kidney disease, could have been associated with increased oxidative stress [36], or is likely to be a link between hyperglycemia and the renal profile presented by the participants. Obese individuals usually have decreased renal blood flow [37], which corroborates the results of urea and uric acid decreased in participants with lower NOx than the 50th percentile, who have shown an association with worse anthropometric indicators; however, the GFR presented for both NOx values indicates the preservation of renal function.

The damage to proteins evidenced by carbonyl was significantly higher in individuals with lower NOx (p<0.01; indirectly proportional). Proteins are immediate targets of oxidative stress and, when oxidation occurs in their side chains, carbonyl groups are produced. These carbonyl groups can be used as protein damage biomarkers, and its accumulation has been observed in several pathologies [38]. The affinity of the superoxide anion with nitric oxide is greater than with superoxide dismutase. This reaction and the formation of peroxynitrite contributed to decreased bioavailability of NO, hence, a lower NOx value, which explains our findings in carbonylation.

This study has some limitations. Diet can affect the levels of circulating nitric oxide metabolites, and the subjects enrolled in our study were not on a specific diet before blood collection. This context is important since ingestion of some functional foods and nutraceuticals have antioxidant effect and may also have beneficial effects on the cardiovascular risk [39,40]. Even, there was a great variety of individuals and results due to the absence of rigorous exclusion criteria (e.g., the presence of previous diseases).

Conclusions

The relationships that are shown between the percentiles of nitrite/nitrate and biochemical parameters, such as inflammatory and oxidative stress, suggest that NOx is a good predictor of cardiometabolic risk, that is strongly associated with biochemical markers such as glucose and triglycerides, as well as measures to obesity that, in itself, represents an increased risk for other diseases.

Furthermore, the NOx dosage is easily adapted to laboratory testing and can be routinely programmed for automatic analyzers, thus requiring very little sample preparation and allowing it to be used in research as well as diagnostic laboratories. It is, also useful in predicting metabolic risk and guiding good treatment measures.

Funding

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