Austin Journal of Nutrition & Metabolism

Open Access

Austin Publishing Group

Research Article

Antioxidant Defense Negatively Correlates with Oxidative Damage, Blood Pressure, Anthropometric and Lipid Profile in Hypertensive Women. What is the Lesson?

de Almeida A[1], Ambrosi PAC[1], Zanini D[2], Mânica A[3], Corralo V[4], Antônio de Sá C[4] and Cardoso AM[2]*

[1]Federal University of Fronteira Sul - Campus Chapecó, Medicine Course, Santa Catarina, Brazil

[2]Graduate Program in Biomedical Sciences, Medicine Course, Federal University of Fronteira Sul - Campus Chapecó, Santa Catarina, Brazil

[3]Health Science Department Organization, Community University of the Region of Chapecó (UNOCHAPECÓ), Chapecó, SC, Brazil

[4]Graduate Program in Health Sciences, Community University of the Region of Chapecó (UNOCHAPECÓ), Chapecó, SC, Brazil

***Corresponding author:** Andréia Machado Cardoso, Federal University of Fronteira Sul, Campus Chapecó, Rodovia SC 484 - Km 02, Fronteira Sul, Brazil

Received: November 11, 2021; **Accepted:** December 03, 2021; **Published:** December 10, 2021

Abstract

Objective: This work evaluated the antioxidant defenses, anthropometric, oxidative and lipid profile parameters in hypertensive women and analyzed correlations between these variables to understand the impact of antioxidant defense in the development and progression of Systemic Arterial Hypertension (SAH).

Design and Methods: The study included 154 women, 81 in the hypertensive group and 73 in the normotensive group. For the evaluation of oxidative stress parameters were analyzed the activity of the enzymes Superoxide Dismutase (SOD), Catalase (CAT), Glutathione S-Transferase (GST), vitamin C levels, Non-Protein SH groups (NPSH), Reactive Oxygen Species (ROS), Thiobarbituric Acid Reactive Substances (TBARS) and protein carbonylation. Total Cholesterol (TC), High-Density Cholesterol (HDL), Low-Density Cholesterol (LDL) and triglycerides, total height, body weight, Body Mass Index (BMI) and body fat percentage were measured. Student's t-test and Pearson's Correlations were performed.

Results: Hypertensive women had higher body mass, fat percentage, BMI, TC, LDL and triglycerides and lower HDL. GST, SOD, CAT, vitamin C and NPSH were decreased in hypertensive women. Oxidative damage parameters were higher in the hypertensive group. Positive correlations were set in hypertensive women between Systolic Blood Pressure (SBP) and TBARS, SBP and ROS, SBP and LDL, PAS and triglycerides, LDL and TBARS, triglycerides and TBARS, triglycerides and ROS, and triglycerides and GST. Negative correlations were established between SBP and NPSH, LDL and NPSH, and triglycerides and NPSH in hypertensive women.

Conclusion: The results suggest that oxidative stress, antioxidant defense reduction, lipid profile, body fat percentage and blood pressure itself interact with each other, being potential agents that favors the progression of SAH.

Keywords: Systemic arterial hypertension; Oxidative stress; Hemodynamic parameters; Lipid profile

Abbreviations

EDTA: Tetra-Acetic Ethylenediamine Acid; ABESOA: Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica; CAT: Catalase; TC: Total Cholesterol; DNA: Deoxyribonucleic Acid; ACEI: Angiotensin Converting Enzyme; OE: Oxidative Stress; ROS: Reactive Oxygen Species; GPx: Glutathione Peroxidase; NPSHs: Non-Protein Thiol Groups; GSH: Reduced Glutathione; GSTs: Glutathione S-Transferase; SAH: Systemic Arterial Hypertension; HDL: High Density Lipoproteins; BMI: Body Mass Index; ISAK: International Society for the Advancement of Kinanthropometry; LDL: Low Density Lipoproteins; WHO: World Health Organization; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SBC: Sociedade Brasileira de Cardiologia; SOD: Superoxide Dismutase; TBARS: Thiobarbituric Acid Reactive Substances; UFFS: Federal University of Fronteira Sul; UNOCHAPECÓ: Community University of Chapecó Region

Introduction

Systemic Arterial Hypertension (SAH) is a chronic non-communicable disease, of a multifactorial nature, which compromises the body's vasodilator and vasoconstrictor mechanisms. According to the guidelines of the World Health Organization (WHO) and the VII Guideline of the Brazilian Society of Cardiology (SBC), arterial hypertension is determined by Systolic Blood Pressure (SBP) ≥ 140 mmHg and/or Diastolic Blood Pressure (DBP) ≥ 90 mmHg in adults who do not use antihypertensive medications [1,2].

According to a study published in The Lancet, the number of adults with hypertension increased from 594 million in 1975 to 1.13 billion in 2015, reaching 597 million men and 529 million women [3]. In Brazil, SAH affects 36 million adult individuals, more than 60% of the elderly, contributing to around 50% of deaths related to cardiovascular diseases [2]. This study is relevant, since SAH is the main risk factor for the development of cardiovascular diseases and,

de Almeida et al. © All rights reserved

Citation: de Almeida A, Ambrosi PAC, Zanini D, Mânica A, Corralo V, Antônio de Sá C, et al. Antioxidant Defense Negatively Correlates with Oxidative Damage, Blood Pressure, Anthropometric and Lipid Profile in Hypertensive Women. What is the Lesson?. Austin J Nutr Metab. 2021; 8(4): 1117.

according to the WHO, each year, 1.6 million Deaths are caused by such diseases only in the Americas region [4].

It is known that changes in body composition, such as increases in Body Mass Index (BMI) and obesity, changes in lipid profile and blood glucose, represent a condition that favors the development or aggravation of SAH and its complications [5]. It is possible to identify overweight as a BMI of 25 to 29.9 kg/m^2, and obesity as a BMI greater than or equal to 30 kg/m^2, thus characterizing a condition that has been associated with an increased risk of death by cardiovascular diseases [6]. Similarly, the combination of hypertension and higher values of Total Cholesterol (TC), triglycerides and Low-Density Lipoproteins (LDL) and low values of and High-Density Lipoproteins (HDL) represents an important factor in the increase of cardiovascular risk. A report of 5,376 patients had already demonstrated that a large percentage of hypertensive patients have high cholesterol levels and that the simultaneous occurrence of hypertension and dyslipidemia leads to a cumulative rise in the risk of cardiovascular diseases [7].

The mechanisms that lead to the development of SAH are complex and not yet fully elucidated, but, in the last decades, the evidence of the association between the production of Reactive Oxygen Species (ROS) and arterial hypertension is growing. ROS, produced as intermediaries in the reduction-oxidation reactions, under physiological conditions, are fundamental for cellular functioning. However, the increase in these species leads to a situation called Oxidative Stress (OE). This is established by an imbalance between the ROS formation and the impaired or inefficient action of endogenous and exogenous antioxidants, which can trigger lesions in the various cellular components, initiating several pathological processes [8,9]. Thus, it is of fundamental importance to evaluate the mechanisms related to oxidative damage in hypertensive patients.

The excess of reactive species is countered by antioxidants, produced by the body or ingested through the diet [10]. These antioxidants are classified in enzymatic and non- enzymatic systems. The enzymatic system is the first antioxidant defender in the body, stopping the excess of the superoxide radical anion and hydrogen peroxide. This system has several enzymes, like Glutathione Peroxidase (GPx), Glutathione S-Transferase (GST), Catalase (CAT) and Superoxide Dismutase (SOD) [10]. In non-enzymatic defense, glutathione, which constitutes the most important human Non-Protein SH (NPSH) group and vitamin C stand out. Glutathione can be found in its reduced (GSH) or oxidized (GSSG) form [11]. The NPSH dosage is a good parameter to estimate the body's glutathione content, since it is, in its reduced form, the most abundant thiol group in the intracellular environment [12,13].

Considering the above, this study seeks to evaluate the levels of enzymatic antioxidants (GST, CAT and SOD), non-enzymatic antioxidants (vitamin C and NPSH), parameters of oxidative damage (Substances Reactive To Thiobarbituric Acid (TBARS), ROS and protein carbonylation) and lipid profile in middle-aged hypertensive women, comparing them with normotensive women, to elucidate the relationship between the disease and the development or progression of oxidative stress. Furthermore, it proposes to evaluate and discuss the influence of anthropometric data, lipid profile and medications on the progression of SAH and OE.

Methods

Study characterization

An experimental, cross-sectional study of an applied nature and a quantitative approach was carried out. Also called quantitative research of pre-factual causality, in which it evaluates the effect of a variable or set of variables on another variable or set of variables based on controlled events [14].

Participants

The sample of the present study consisted of volunteer women aged between 42 and 68 years with and without hypertension diagnosis. The sample selection happened in a non-probabilistic way by quotas, voluntarily, considering the eligibility criteria, being initially calculated in 150 women, 75 for the hypertensive group and 75 for the normotensive group. The sample size calculation considered an average standard deviation of the main outcome variables of 10 units, with a statistical power of 90% and a significance level of 0.05 (two-tailed distribution). After selection, the sample consisted of 154 women, 81 in the hypertensive group and 73 in the normotensive group.

In the group of hypertensive women were included those who had the diagnosis of hypertension for at least 3 years and in treatment, with SBP lower than 160 and the diastolic blood pressure lower than 120 mmHg [2]. In the normotensive group were included women without hypertension diagnosis and whose SBP and diastolic blood pressure measured in two non-consecutive days and at different times, were equal to or less than 139 and 89 mmHg, respectively. Women who presented comorbidities that represented a clinical contraindication to participate in the study, women who smoked, and those who used any medication that may have influenced the results of the study were excluded, except for antihypertensive drugs for the hypertensive group.

Anthropometric parameters

Total height: The total height of the participants was determined by the distance between the soles of the feet and the vertex of the head using a portable stadiometer (Gofeka®, Criciúma- SC, Brazil) with an accuracy of 0.1 cm.

Body weight: Body weight was determined by the individual's total body mass using a digital scale (Urbano® OS-100, Canoas-RS, Brazil) with an accuracy of 50 g.

Body mass index: The Body Mass Index (BMI) was calculated by dividing the body mass of each participant by the square of their height expressed in meters, as recommended by [15].

Body fat percentage: The body fat percentage was measured by a bioimpedance scale (Tanita® BC-601 Body Composition Analyzer). The participants rested for 10 minutes and after that electrodes were placed on the feet and hands, obtaining the results through a low-frequency electrical current.

Blood pressure assessment

A mercury column sphygmomanometer (Erkamater®-E300, Germany) was used to measure blood pressure. The procedures were performed according to the recommendations in the "7th Brazilian Guideline for Hypertension" [2], with measurements taken at rest

with an automatic blood pressure monitor (Omron® HEM-742INT, China).

Lipid profile

Total serum cholesterol, Low Density Cholesterol (LDL), High Density Cholesterol (HDL) and triglyceride concentration were analyzed using standard enzymatic methods, with Ortho- Clinical Diagnostis reagents in an automatic analyzer (Vitros 950W dry chemistry system; Johnson & Johnson, Rochester, NY, USA).

Collection and analysis of blood samples

Three tubes of 4 ml of blood were collected, one tube with EDTA as anticoagulant, one with citrate as anticoagulant and one without anticoagulant, by venipuncture of the antecubital vein using the vacuum system. The entire blood collection procedure was performed by trained and qualified professionals and in compliance with all recommended biosafety procedures for this purpose. Immediately after the collections, a portion of citrated whole blood was separated and the rest was centrifuged (15 min. X 3,500 rpm, model KC16R centrifuge, brand Kindly Controle Microprocessado) to obtain plasma and serum. All samples were stored in microtubes at -80ºC for further analysis. The analysis of the samples was carried out after the n had been completed, in a period between three and six months.

Evaluation of oxidative stress parameters

Superoxide dismutase enzyme (SOD) determination: The activity of the SOD enzyme was determined in whole blood according to McCord and Fridovich [16]. And expressed in units of SOD per milligram (SOD/mg) of protein. In this method, the SOD present in the sample competes with the superoxide anion detection system. A unit of SOD is defined as the amount of enzyme that inhibits the oxidation rate of adrenaline by 50%. The oxidation of adrenaline leads to the formation of the colored product, adrenochrome, which is detected by the spectrophotometer. Superoxide dismutase activity was determined by measuring the rate of formation of adrenochrome, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mM, pH 10) and adrenaline (1 mM).

Determination of catalase enzyme (CAT): The determination of CAT activity was performed in whole blood according to a modified method by Nelson and Kiesow [17]. And was expressed in nanomols per milligram (nmol/mg) of protein. This test involves changing the absorbance at 240 nm due to CAT peroxide-dependent hydrogen decomposition. An aliquot (0.02 ml) of blood was homogenized in potassium phosphate buffer, pH 7.0. The spectrophotometric determination was initiated by the addition of 0.07 ml in an aqueous solution of 0.3 mol/l^{-1} of hydrogen peroxide. The change in absorbance at 240 nm was measured for 2 min. CAT activity was calculated using the molar extinction coefficient (0.0436 cm^2μmol) and the results were expressed in nanomols per milligram of protein.

Determination of glutathione s-transferase (GST): GST activity was measured as described by Warholm et al. [18]. The assay mixture contained an adequate amount of sample solution (25 g of protein) and the Bradford method was used to determine the bovine serum albumin content as a protein standard. The activity was expressed through the absorbance Delta.

Determination of vitamin C: vitamin C levels were performed using the modified method by Jacques-Silva et al. [19]. 300ul of the participants' plasma was used in a reaction containing dinitrophenylhydrazine, trichloroacetic acid and sulfuric acid as reagents. The ascorbic acid content was determined at 520 nm and expressed as mg of ascorbic acid /mL of plasma.

Determination of the content of protein and non-protein SH groups: NPSH were measured spectrophotometrically with Ellman's reagent [20]. Aliquots (0.1 mL) of plasma were added to 0.85 mL of 0.3 M phosphate buffer, pH 7.4, and the reaction was read at 412 nm after the addition of 10 mM 2-nitrobenzoic acid (0.05 mL). The data were reported in μmol/mL of plasma.

Determination of reactive oxygen species (ROS): The quantification of ROS serves as an indirect measure of damage to Deoxyribonucleic Acid (DNA). The formation of reactive species in serum was estimated according to Ali, Lebel and Bondy [21]. An aliquot of 50 μL of serum was incubated with 10 μL of 2', 7'-47 Dichlorofluorescein-Diacetate (DCFH-DA, 7 μM). ROS levels were determined using the fluorescence method. The oxidation of DCFH- DA to Dichlorofluorescein (DCF) was measured for the detection of intracellular reactive species. The intensity of DCF fluorescence emission was recorded and 488 to 525 nm excitation 60 min after the addition of DCFH-DA in the medium and the oxidized dichlorofluorescein was determined using a standard curve and the results were expressed in U DCF/mg of protein.

Determination of lipid peroxidation: lipid peroxidation in serum was determined according to Jentzsch et al. [22], Modified. To this, 0.2 ml of serum was added to the reaction mixture containing 1 ml of 1% orthophosphoric acid and 0.25 ml alkaline solution of thiobarbituric acid (final 20 ml), followed by 45 minutes of heating at 95ºC. After cooling, the samples and Malondialdehyde (MDA) standards were read at 532 nm, against the blank of the standard curve. The number of substances reactive to Thiobarbituric Acid (TBARS) was expressed in nmol of MDA/ml of serum.

Determination of protein carbonylation/oxidation: protein oxidation in serum was determined using the method of Levine et al. [23] First, from 1 ml of serum, proteins were precipitated using 0.5 ml of 10% Trichloroacetic Acid (TCA) and centrifuged at 1800 g for 5 min, discarding the supernatant. To this, 0.5 ml of 2, 4-Dinitrophenylhydrazine (DNPH) was added to 10 mmol/1 in 2 mol/1 of HCl and incubated at room temperature for 30 minutes, mixing the samples vigorously every 15 minutes. After incubation, 0.5 ml of 10% TCA was added to the protein precipitate and centrifuged at 1800 g for 5 min. After discarding the supernatant, the precipitate was washed twice with 1 ml of ethanol/ethyl acetate (1:1), centrifuging the supernatant to remove the free DNPH. The precipitate was dissolved in 1.5 ml of protein dissolving solution (2 g of sodium dodecyl sulfate and 50 mg of EDTA in 100 ml of 80 mmol/1 phosphate buffer, pH 8.0) and incubated at 37ºC for 10 min. The color intensity of the supernatant was measured by a spectrophotometer at 370 nm against 2 mol/1 of HCl. The carbonyl content was calculated using the molar extinction coefficient ($21x10^3$ $L{\cdot}mol^{-1}{\cdot}cm^{-1}$), and the results are expressed in nanomols per milligram of protein.

Ethical aspects

This project was submitted to and approved by the Human

Research Ethics Committee of the Federal University of Fronteira Sul, with the following opinion number 1,916,904. All individuals were duly informed about the objectives of the study and the data collection procedures, as well as the relative risks and benefits and, upon agreeing to participate in the study, signed the Free and Informed Consent Term, in two copies, one of which remained with the research participant and the other was maintained by the researchers. Also, the subjects were instructed that they could withdraw their consent and give up participating in the study at any time.

Statistical analysis

First, the data were submitted to the Shapiro-Wilk test to assess normality. Bearing in mind that the data follow a normal distribution, comparisons between groups (hypertensive and normotensive) were performed using Student's *t*-test. Correlations were performed using Pearson's correlation test. The data are presented as mean and standard deviation. For all analyzes, the statistical program GraphPad® Prism version 8.0 was used. The analyzes were considered significant when $p < 0.05$.

Results

Anthropometric data

In the study, data from 81 hypertensive and 73 normotensive women were analyzed. All study participants were at menopause and without physical exercise for at least 6 months. The anthropometric data of the participants are shown in Table 1. The ages were statistically equal in the group of hypertensive women when compared to the group of normotensive women (53.30 ± 7.31 normotensive women versus 55.60 ± 8.43 hypertensive women). Likewise, height measurements were the same in the hypertensive group when compared to the normotensive group (1.62 ± 5.12 normotensive *vs.* 1.64 ± 4.46 hypertensive). Body mass measurements were higher in the hypertensive group when compared to the normotensive group (62.30 ± 8.81 normotensives *vs.* 66.44 ± 7.22 hypertensive, $p < 0.05$). Similarly, body fat indexes were higher in the hypertensive group when compared to the normotensive group (28.68 ± 3.19 normotensive *vs.* 31.12 ± 4.55 hypertensive, $p < 0.05$), as well as the BMI results (25.19 ± 4.98 normotensives *vs.* 27.95 ± 5.87 hypertensives, $p < 0.05$). The measurements of systolic and diastolic blood pressure were higher in the hypertensive group when compared to the normotensive group (118 ± 15.20 normotensive *vs.* 129 ± 16.19 hypertensive, $p < 0.05$) and (79 ± 10.13 normotensive *vs.* 88 ± 13.66 hypertensive women, $p < 0.05$), respectively.

Analysis of the lipid profile

The results of the analysis of the lipid profile data of the study participants are shown in Table 2. Compared with normotensive women, hypertensive women had higher levels of TC (158.35 ± 27.11 normotensives *vs.* 167.81 ± 36.22 hypertensives, $p < 0.05$). LDL levels were also higher in the hypertensive group when compared to the normotensive group (93.18 ± 15.20 normotensive *vs.* 111.45 ± 16.66 hypertensives, $p < 0.05$). The HDL levels found in the hypertensive group were significantly reduced when compared to the normotensive group (47.77 ± 7.43 normotensive *vs.* 39.22 ± 8.45 hypertensive, $p < 0.05$). Triglyceride levels were higher in the hypertensive group when compared to the normotensive group (123.10 ± 33.98 normotensive *vs.* 153.78 ± 41.29 hypertensives, $p < 0.05$).

Table 1: General characteristics of hypertensive and normotensive participants in the study.

	Normotensive (n = 73)	Hypertensive (n = 81)
Age (years)	53.30 ± 7.31	55.60 ± 8.43
Stature (m)	1.62 ± 5.12	1.64 ± 6.46
Body mass (kg)	65.30 ± 15.81	72.44 ± 17.22*
Body fat (%)	28.68 ± 5.19	31.12 ± 6.55*
BMI (kg/m²)	25.19 ± 4.98	27.95± 5.87*
Systolic blood pressure (mmHg)	118 ± 15.20	129 ± 16.19*
Dyastolic blood pressure (mmHg)	79 ± 10.13	88 ± 13.66*

1 Data are presented with mean and standard deviation. The statistical analysis used was the student's t-test. The "*" indicates a difference between the groups, considering $p < 0.05$.

Table 2: Lipid profile analyzed in the study participants.

	Normotensive	Hypertensive
Total cholesterol (mg/dL)	158.35 ± 27.11	167.81 ± 36.22*
LDL (mg/dL)	93.18 ± 15.20	111.45 ± 16.66*
HDL (mg/dL)	47.77 ± 7.43	39.22 ± 8.45*
Triglycerides (mg/dL)	123.10 ± 33.98	153.78 ± 41.29*

1 Data are presented with mean and standard deviation. The statistical analysis used was the student's t-test. The "*" indicates a difference between the groups, considering $p < 0.05$.

Table 3: Use of antihypertensive medications.

Medication	Number of users	Percentage (%)
Diuretics	65	71.42
ACE inhibitors	61	67.03
Direct vasodilators	19	20.87
Beta blockers	12	13.18
Angiotensin receptor antagonists	10	10.98
Calcium channel antagonists	8	8.79

Use of antihypertensive medications

Antihypertensive drugs used by participants in the hypertensive group are described in Table 3. Among hypertensive women, 65 (71.42%) use diuretics, 61 (67.03%) use ACEIs, 19 (20.87%) use direct vasodilators, 12 (13.18%) use beta-blockers, 10 (10.98%) use angiotensin receptor antagonists and 8 (8.79%) use calcium channel antagonists.

Enzymatic antioxidants

All enzymatic antioxidants analyzed in the study were decreased in hypertensive women, compared to normotensive women GST activity was lower in the hypertensive group when compared to normotensive (1.88 ± 0.2 normotensive *vs.* 1.70 ± 0.2 hypertensive, $p < 0.05$), as well as the activity of SOD (25.00 ± 4.0 normotensive *vs.* 18.00 ± 3.1 hypertensive, $p < 0.05$) and CAT (20.00 ± 4.0 normotensive *vs.* 15.00 ± 3.2 hypertensive women, $p < 0.05$)

Non-enzymatic antioxidants

Similar to enzymatic antioxidants, the analyzed non-enzymatic antioxidants were lower in the hypertensive group, when compared with the normotensive group the level of Vitamin C was lower in the group of hypertensive women when compared to normotensive

Table 4: Correlations between studied variables.

	r value	
	Normotensive	Hypertensive
SPA x TBARS	0.3643	0.5231*
SPA x ROS	0.2399	0.4354*
SPA x carbonil	0.3321	0.3925
SPA x vit C	-0.2187	0.3188
SPA x NPSH	-0.4361	-0.5118*
SPA x CAT	0.4611	0.3277
SPA x SOD	0.4719	0.4196
SPA x GST	0.3755	0.3688
SPA x LDL	0.4676*	0.6188*
SPA x Triglycerides	0.4276	0.7133*
LDL x TBARS	0.3754	0.5284*
LDL x ROS	0.2544	0.3122
LDL x carbonil	0.3912	0.3283
LDL x vit C	-0.1654	-0.2966
LDL x NPSH	-0.3687	-0.4556*
LDL x CAT	0.3218	0.2084
LDL x SOD	0.1865	0.2767
LDL x GST	0.3295	0.3376
Triglycerides x TBARS	0.5444*	0.6989*
Triglycerides x ROS	0.3266	0.4975*
Triglycerides x carbonil	0.2244	0.3987
Triglycerides x vit C	-0.2811	-0.2388
Triglycerides x NPSH	-0.4509*	-0.7600*
Triglycerides x CAT	0.3222	0.3080
Triglycerides x SOD	0.2608	0.2906
Triglycerides x GST	0.4387	0.4866*

Pearson's correlation test was used. The "*" indicates a significant analysis, considering p <0.05.

women (456.20 ± 31.40 normotensive *vs.* 399.10 ± 45.72 hypertensive women, p <0.05) as well as the level of NPSH (1.18 ± 0.21 normotensive *vs.* 0.88 ± 0.30 hypertensive, p <0.05).

Oxidative damage parameters

Hypertensive women demonstrated increased levels of oxidative damage when compared to normotensive ones, based on the analysis of the damage parameters carried out in this study the levels of TBARs were higher in the hypertensive group when compared to normotensive individuals (3.10 ± 1.1 normotensives *vs.* 3.95 ± 0.9 hypertensives, p <0.05). Similarly, hypertensive women also had higher levels of protein carbonylation than normotensive (4.60 ± 1.3 normotensives *vs.* 5.20 ± 1.1 hypertensives, p <0.05), as well as for EROS (4745.00 ± 172.00 normotensive *vs.* 5262.00 ± 132.80 hypertensives, p <0.05).

Correlations between the studied variables

The correlations between the data evaluated are shown in Table 4. In the hypertensive group, there was a positive correlation between PAS and TBARS (r = 0.5231), PAS and EROS (r = 0.4354), PAS and triglycerides (r = 0.7133) and a negative correlation between PAS and NPSH (r = -0.5118). In both groups, there was a positive correlation between PAS and LDL (r = 0.4676 in the normotensive group and r = 0.6188 in the hypertensive group). In the hypertensive group, there was a positive correlation between LDL and TBARS (r = 0.5284) and a negative correlation between LDL and NPSH (r = -0.4556). In both groups, there was a positive correlation between triglycerides and TBARS (r = 0.5444 in the normotensive group and r = 0.6989 in the hypertensive group). In the hypertensive group, there was a positive correlation between triglycerides and EROS (r = 0.4975). In both groups, there was a negative correlation between triglycerides and NPSH (r = -0.4509 in the normotensive group and r = 0.7676 in the hypertensive group). Finally, there was a positive correlation in the hypertensive group between triglycerides and GST (r = 0.4866). All r values presented here are statistically significant (p <0.05).

Discussion

The increase in the production of EROS significantly helps structural and functional changes present in hypertension [24]. Some clinical trials have already succeeded in proving the increase in these species together with the decrease in antioxidant levels [25,26]. However, this study is pioneering in analyzing and correlating anthropometric parameters (BMI), lipid profile (cholesterol and triglycerides), and oxidative stress parameters (EROS levels, endogenous and exogenous antioxidant enzymes, damage to lipids and proteins) in hypertensive patients.

When analyzing the general characteristics of the patients, our study observed that there were differences in all parameters. The hypertensive group had greater weight, greater body mass, greater waist circumference, and higher BMI values compared to the normotensive group. Park; Lee; Kim also found that weight and body mass was higher in hypertensive individuals compared to normotensive ones, and epidemiological studies have shown that the risk of hypertension is significantly increased with greater body mass [27-29]. From the observations made by these studies and ours, it can be concluded that body mass and body fat tend to be higher in hypertensive women compared to normotensive women, contributing to an additional factor in the development of hypertension and its complications.

The average TC level of hypertensive participants was higher than that of normotensive patients. Our results point to positive correlations between levels of PAS and triglycerides and levels of PAS and LDL, corroborating the hypothesis of the coexistence of hypertension and dyslipidemia, which is currently defined as "lipitension" [30]. Dyslipidemia increased serum triglycerides and cholesterol, causes damage to the endothelium, at the same time that such damage is also increased by hypertension, through shear stress and oxidative stress, which consequently will generate less vascular relaxation, increased permeability to lipoproteins, increased collagen and fibronectin synthesis. All of these mechanisms combined promote endothelial dysfunction [30]. Thus, these results help to affirm the possibility that dyslipidemia, in addition to being related to the development of SAH, can also assist in its progression.

Regarding treatment, all women in the group of hypertensive women in the study undergo drug treatment for SAH. Many of them also use more than one associated drug. Studies demonstrate that both in monotherapy and combined use, most classes of drugs

used in SAH are significantly efficient [31]. However, the use of some classes of antihypertensive drugs can affect the parameters of the lipid profile [32], which can help explain dyslipidemia found in the patients of the present study. Thiazide diuretics, especially when used in higher than usual doses, can cause a significant increase in LDL and triglyceride levels. However, a meta-analysis carried out recently shows that further studies are needed to support this claim and clinical judgment should be used for its use in medical practice [33]. Some beta-blockers, such as those commonly used propranolol and atenolol, have demonstrated the potential to significantly increase triglyceride, CT, and LDL levels and significantly decrease HDL levels [34]. The other classes of antihypertensive drugs seem to produce a neutral or slightly positive effect on the lipid profile of users [35]. In addition, the combination of two or more drugs in the treatment seems to only add up to the individual effects of the drugs used [36].

It is known that hypertension and oxidative stress have an important causal relationship, in addition to contributing to the accentuation of each other, ultimately promoting the expansion of SAH and EO [8,37]. Our study supports this information because it found a positive association between oxidative damage parameters such as TBARS and EROS and blood pressure, demonstrating that the higher the pressure, the greater the damage to lipids and the higher the EROS levels.

The group of hypertensive women had the activities of enzymatic antioxidants (GST, CAT, and SOD) decreased when compared to the subset of normotensive women. It was demonstrated by Rodrigo et al. [38], a strong relationship between blood pressure and oxidative stress, corroborating our findings. Other studies have also shown high amounts of EROS in hypertensive patients [39-41]. The literature presents studies with vascular smooth muscle cells and arteries of humans and hypertensive rats where the production of EROS is increased and the antioxidant activity is reduced [42]. It is suggested, therefore, that an increase in systemic blood pressure not only increases the amount of EROS but also contributes to a decrease in the number of enzymatic antioxidants.

Non-enzymatic antioxidants were also decreased in the group of hypertensive women compared to normotensive women. Several clinical trials have performed the acute infusion of vitamin C in individuals with endothelial dysfunction and cardiovascular risk and have found significant benefits for cardiovascular health [43]. Also, vitamin C has been shown to improve nitric oxide activity by increasing the intracellular concentrations of tetrahydrobiopterin, a cofactor for nitric oxide synthase in the endothelium [44]. These results demonstrate vitamin C deficiency and a shortage of non-enzymatic antioxidants in hypertensive, suggesting a relationship with PAS.

The NPSH are direct representatives of plasma GSH levels, our study demonstrated that the group of hypertensive women had lower total NPSH levels when compared to the normotensive group, with a negative correlation between the increase in SBP and NPSH levels. These findings confirm data found in other studies, which also demonstrated the relationship between hypertension and lower levels of GSH, in different age and ethnic groups [44-46]. This reduction can be justified by the oxidation of GSH in GSSG mediated by ROS, decreasing plasma GSH levels, and by the detoxification processes carried out by GPx, also transforming GSH into GSSG [45].

Our results also showed that all parameters of oxidative damage studied were altered in groups of hypertensive women. TBARS levels were significantly higher in the hypertensive group when compared with normotensive ones, with a positive correlation between SBP and TBARS, suggesting a possible causal relationship between these parameters. Other studies have shown similar results, verifying the increase in TBARS in hypertensive individuals and supporting our findings [47,48]. The increase in lipid peroxidation suggests a relationship between higher blood pressure and peroxidative damage [47], in addition to suggesting an imbalance between antioxidant and oxidizing factors in hypertensive women [48].

The levels of protein carbonylation measured in our study proved to be higher in the group of hypertensive women when compared to normotensive women, as verified by Yıldırım et al. [49] and Yavuzer et al. [47] in similar research. In addition to demonstrating an OE state, the increase in carbonylated proteins can lead to a reduction or loss of the biological function of proteins [50].

The ROS levels evaluated were also significantly higher in the hypertensive group compared to the normotensive group, as also demonstrated by Kerr et al. [51], Romero and Reckelhoff [52] and Chen et al. [53].

Thus, our study adds further evidence that the increase in systemic blood pressure promotes an increase in ROS. ROS act in important mechanisms in the circulatory system, such as in the modulation of inflammatory processes, contractility, and endothelial functions, and in pathological conditions, they can contribute to the increase in vascular damage and the progression of chronic diseases, such as hypertension [54,55].

Our study demonstrated a negative correlation between increased LDL levels and NPSH, and a positive correlation between increased LDL and TBARS levels, which means that patients with increased LDL had a lower amount of the non-enzymatic antioxidant NPSH and a greater amount of oxidative damage in lipids, contributing to the hypothesis of a relationship between increased levels of lipid parameters and the presence of SAH, through an increase in oxidative stress, the progression of atherosclerosis and endothelial dysfunction [56]. There was also a negative correlation between the increase in triglycerides and NPSH and an also negative correlation between the increase in triglycerides and GST, in addition to positive correlations with the increase in EROS and TBARS, demonstrating that patients with an increased level of triglycerides also had a smaller amount of non-enzymatic and enzymatic antioxidants consecutively.

Women with increased levels of EROS also demonstrated higher levels of lipid peroxidation, corroborating the relationship explained above. It is known that oxidative changes in lipoproteins, as well as hypertension, are one of the factors that cause diseases such as atherosclerosis, through atherogenesis [57], therefore the relationship between lipid profile and oxidative parameters becomes relevant.

Conclusion

Hypertension is associated with high levels of triglycerides, cholesterol, and BMI, promotes an increase in EROS, and this increase cannot be compensated by enzymatic and non-enzymatic

antioxidants, which are reduced. It is not yet known whether such a decrease is due to the increase in oxidizing agents that end up depleting antioxidants, or if a decrease in their production occurs. Consequently, the excess of these reactive species promotes lipid peroxidation (TBARS), DNA damage, and protein carbonylation.

All the mechanisms analyzed in this study, added together, reinforce the importance of oxidative stress in the pathogenesis of systemic arterial hypertension, in addition to suggesting that factors related to oxidative stress, lipid profile, body fat percentage, medications used, and blood pressure itself interact between themselves, in a synergistic mechanism, being able to cooperate for the progression of SAH and increase of oxidative damage. The current challenge is to better understand each factor that causes and contributes to the pathophysiology of SAH and its interrelationships, to optimize prevention and design or adapt future treatments targeting SAH.

Highlights

- Hypertensive women showed higher anthropometric, lipid profile and oxidative stress parameters than normotensive women.
- There is a potential influence of antihypertensive drugs on the increase of lipid profile parameters in hypertensive women.
- Activity of enzymatic antioxidants and levels of non-enzymatic antioxidants are decreased in hypertensive women when compared to normotensive women.
- Hypertensive women have higher oxidative damage to lipids and proteins and increased levels of reactive oxygen species.
- Hypertension, lipid profile parameters and oxidative stress have important correlations, which may contribute to the progression of SAH and increased oxidative damage.

Declaration

Acknowledgments: This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and UFFS (Universidade Federal da Fronteira Sul).

Data availability: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author contribution: AMC, AA and PACA, conceived the idea, AMC, DZ and AM designed the recruitment of the participants and the experimental protocol DZ, AM and VC collected the blood and separated total blood, serum and plasma. CAS, carried out the anthropometric measurements. AMC, DZ and AM, performed the laboratorial experiments and statistical analysis and created the figures. AA and PACA, wrote the paper. PACA, created the graphical abstract AMC, corrected the final version. All authors have read and approve the final version of this manuscript.

References

1. World Health Organization. Technical package for cardiovascular disease management in primary health care: evidence-based treatment protocols (World Health Organization). 2018.
2. Sociedade Brasileira de Cardiologia. Arq Bras Cardiol. 2017; 107.
3. Zhou B, et al. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. The Lancet. 2017; 389: 37-55.
4. Calò L. OPAS/OMS BIREME - Dia Mundial da Hipertensão 2016. OPAS/OMS. 2016.
5. Callo G, Gigante DP, Barros FC, Horta BL. Lifetime overweight and obesity and body composition in adulthood: the 1982 Pelotas (Brazil) birth cohort study. Cad. Saúde Pública. 2016; 32: e00174014.
6. Barroso TA, Marins LB, Alves R, Gonçalves ACS, Barroso SG, Rocha G de S. Association of Central Obesity with The Incidence of Cardiovascular Diseases and Risk Factors. Int. J. Cardiovasc. Sci. 2017; 30: 416-424.
7. Rodrigues CJ de O, Ribeiro HF, Ribeiro AB, Zanella MT, Batista MC. Metab. Cholesterol goal attainment in hypertensive patients: the impact of metabolic syndrome components. Syndr. Relat. Disord. 2012; 10: 195-201.
8. Castro AFC de. Stress oxidativo e Hipertensão Arterial Essencial. Universidade do Porto, Porto. 2010.
9. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. 2015; 97: 55-74.
10. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell. Physiol. Biochem. 2017; 44: 532-553.
11. Huber PC, Almeida WP, Fátima Â de. Glutathione and related enzymes: biological roles and importance in pathological processes. Quím. Nova. 2008; 31: 1170-1179.
12. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed. Pharmacother. Biomedecine Pharmacother. 2003; 57: 145-155.
13. Souza LC, Wilhelm EA, Bortolatto CF, Nogueira CW, Boeira SP, Jesse CR. The protective effect of melatonin against brain oxidative stress and hyperlocomotion in a rat model of mania induced by ouabain. Behav. Brain Res. 2014; 271: 316-324.
14. Rauen F. Roteiros de iniciação científica: os primeiros passos da pesquisa científica desde a concepção até a produção e a apresentação (Palhoça: Editora Unisul). 2015.
15. Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica (Ed). 2016.
16. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 1969; 244: 6049-6055.
17. Nelson DP, Kiesow LA. Enthalpy of decomposition of hydrogen peroxide by catalase at 25 degrees C (with molar extinction coefficients of H 2 O 2 solutions in the UV). Anal. Biochem. 1972; 49: 474-478.
18. Warholm M, Guthenberg C, Bahr C von, Mannervik B. Glutathione transferases from human liver. Methods Enzymol. 1985; 113: 499-504.
19. Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JB. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacol. Toxicol. 2001; 88: 119-125.
20. Ellman GL. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959; 82: 70-77.
21. Ali SF, LeBel CP, Bondy SC. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology. 1992; 13: 637-648.
22. Jentzsch AM, Bachmann H, Fürst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. Free Radic. Biol. Med. 1996; 20: 251-256.
23. Levine RL, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186: 464-478.
24. Zalba G, San José G, Moreno MU, Fortuño MA, Fortuño A, Beaumont FJ, et al. Oxidative Stress in Arterial Hypertension. Hypertens. Dallas Tex 1979. 2001; 38: 1395-1399.
25. Mehta JL, Lopez LM, Chen L, Cox OE. Alterations in nitric oxide synthase

activity, superoxide anion generation, and platelet aggregation in systemic hypertension, and effects of celiprolol. Am. J. Cardiol. 1994; 74: 901-905.

26. Lacy F, O'Connor D, Schmid-Schönbein G. Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. J. Hypertens. 1998; 16: 291-303.

27. Park J, Lee J-S, Kim J. Relationship between dietary sodium, potassium, and calcium, anthropometric indexes, and blood pressure in young and middle aged Korean adults. Nutr. Res. Pract. 2010; 4: 155-162.

28. Nyamdorj R, et al. Comparison of body mass index with waist circumference, waist-to-hip ratio, and waist-to-stature ratio as a predictor of hypertension incidence in Mauritius. J. Hypertens. 2008; 26: 866-870.

29. Williams PT. Increases in Weight and Body Size Increase the Odds for Hypertension During 7 Years of Follow-up. Obes. Silver Spring Md. 2008; 16: 2541-2548.

30. Dalal JJ, Padmanabhan TNC, Jain P, Patil S, Vasnawala H, Gulati A. LIPITENSION: Interplay between dyslipidemia and hypertension. Indian J. Endocrinol. Metab. 2012; 16: 240-245.

31. Paz MA, et al. Treatment efficacy of anti-hypertensive drugs in monotherapy or combination: ATOM systematic review and meta-analysis of randomized clinical trials according to PRISMA statement. Medicine (Baltimore). 2016; 95: e4071.

32. Deshmukh M, Lee HW, McFarlane SI, Whaley-Connell A. Antihypertensive medications and their effects on lipid metabolism. Curr. Diab. Rep. 2008; 8: 214-220.

33. Akhtar F, Khalid F, Wang H, Zhang D, and Gong X. The Effect of Thiazide Diuretics on Blood Lipid Profile in Hypertensive Adults: A Meta-analysis of Randomized Controlled Trials. Cureus. 2018; 10: e2651.

34. Al-Alsadoon LH, Ali TS, Al-Bajari SA, Shafiq AM. Indian J. Public Health Res. Dev. 2020; 11: 1966-1971.

35. Kannel WB, Carter BL. Initial drug therapy for hypertensive patients with hyperlipidemia. Am. Heart J. 1989; 118: 1012-1021.

36. Kasiske BL, Ma JZ, Kalil RS, Louis TA. Effects of antihypertensive therapy on serum lipids. Ann. Intern. Med. 1995; 122: 133-141.

37. Marçal DMO, Oliveira AM. Infarma - Ciênc. Farm. 2012; 23: 8-13.

38. Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bächler JP. Relationship between oxidative stress and essential hypertension. Hypertens. Res. 2007; 30: 1159-1167.

39. Lacy F, Kailasam MT, O'Connor DT, Schmid-Schönbein GW, Parmer RJ. Plasma hydrogen peroxide production in human essential hypertension: role of heredity, gender, and ethnicity. Hypertens. Dallas Tex 1979; 2000; 36: 878-884.

40. Stojiljkovic MP, Lopes HF, Zhang D, Morrow JD, Goodfriend TL, Egan BM. Increasing plasma fatty acids elevates F2-isoprostanes in humans: implications for the cardiovascular risk factor cluster. J. Hypertens. 2002; 20: 1215-1221.

41. Touyz Rhian M. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension. 2004; 44: 248-252.

42. Touyz RM, Schiffrin EL. Increased generation of superoxide by angiotensin II in smooth muscle cells from resistance arteries of hypertensive patients: role of phospholipase D-dependent NAD(P)H oxidase-sensitive pathways. J. Hypertens. 2001; 19: 1245-1254.

43. Juraschek SP, Guallar E, Appel LJ, Miller ER. Effects of vitamin C supplementation on blood pressure: a meta-analysis of randomized controlled trials. Am. J. Clin. Nutr. 2012; 95: 1079-1088.

44. Huang A, Vita JA, Venema RC, Keaney JF. Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. J. Biol. Chem. 2000; 275: 17399-17406.

45. Vasconcelos SML, Goulart MOF, Silva MAM da, Manfredini V, Benfato M da S, Rabelo LA, et al. Markers of Redox Imbalance in the Blood of Hypertensive Patients of a Community in Northeastern Brazil. Arq. Bras. Cardiol. 2011; 97: 141-147.

46. Muda P, Kampus P, Zilmer M, Zilmer K, Kairane C, Ristimäe T, et al. Homocysteine and red blood cell glutathione as indices for middle-aged untreated essential hypertension patients. J. Hypertens. 2003; 21: 2329-2333.

47. Yavuzer S, et al. The role of protein oxidation and DNA damage in elderly hypertension. Aging Clin. Exp. Res. 2016; 28: 625-632.

48. Pawluk H, Pawluk R, Robaczewska J, Kędziora-Kornatowska K, Kędziora J. Biomarkers of antioxidant status and lipid peroxidation in elderly patients with hypertension. Redox Rep. Commun. Free Radic. Res. 2017; 22: 542-546.

49. Yıldırım E, et al. The impact of protein oxidation on sustained and white coat hypertension. Anatol. J. Cardiol. 2017; 17: 210-216.

50. Cakatay U, Kayali R, Uzun H. Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. Clin. Exp. Med. 2008; 8: 51-57.

51. Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF, Hamilton CA. Superoxide Anion Production Is Increased in a Model of Genetic Hypertension. Hypertens. Dallas Tex 1979. 1999; 33: 1353-1358.

52. Romero J. Carlos, Reckelhoff Jane F. Role of Angiotensin and Oxidative Stress in Essential Hypertension. Hypertension. 1999; 34: 943-949.

53. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant Effects of Vitamins C and E Are Associated With Altered Activation of Vascular NADPH Oxidase and Superoxide Dismutase in Stroke-Prone SHR. Hypertens. Dallas Tex 1979. 2001; 38: 606-611.

54. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: implications in human hypertension. Hypertens. Res. 2011; 34: 5-14.

55. Montezano AC, Touyz RM. Reactive oxygen species, vascular Noxs, and hypertension: focus on translational and clinical research. Antioxid. Redox Signal. 2014; 20: 164-182.

56. Thomas F, Bean K, Guize L, Quentzel S, Argyriadis P, Benetos A. Combined effects of systolic blood pressure and serum cholesterol on cardiovascular mortality in young (<55 years) men and women. Eur. Heart J. 2002; 23: 528-535.

57. Ferreira PFC. Modificações oxidativas de lipoproteínas de baixa densidade e seus anticorpos = associação com componentes do transporte reverso de colesterol e com a aterosclerose carotídea em adultos sem doença aterosclerótica estabelecida. Universidade Estadual de Campinas, Campinas. 2011.