



Increased BMI and Waist Circumference are Related to Increased DNA Damage in Women with Overweight and Metabolic Syndrome

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LCT, FVFF, AJTF and CRC designed the study. Authors AJTF, DTP and FVFF performed the statistical analysis. Authors LCT, APCRF, CRC and IOM wrote the protocol and authors LCT, APCRF, FVFF, JLG and CRC wrote the first draft of the manuscript. Authors KCS, DTP, IOM, VSNN and JLG managed the analyses of the study. Authors KCS, LCT and FVFF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To test if the level of oxidative stress is different in women with overweight and with metabolic syndrome.

Study Design: Cross-sectional.

Place and Duration of Study: Endocrinology Clinic of the Botucatu Medical School- UNESP, between March 2013 and March 2014.

Methodology: Eighty women (31.15 ± 7.91 years old) attended at the Endocrinology Clinic of the Botucatu Medical School- UNESP composed this study. According to the body mass index (BMI)

they were divided in 3 groups: Group 1 (G1, n=36 eutrophic); Group 2 (G2, n=21 overweight) and Group 3 (G3, n=23 women with MS-Metabolic syndrome). It was evaluated: dietary intake of macro and micronutrients dietary; antioxidant capacity (HAC) of plasma and levels of malondialdehyde (MDA); carotenoids, retinol and α -tocopherol in peripheral lymphocytes and the comet assay.

Results: Damage to DNA, oxidized purines and the levels of MDA didn't differ between women with overweight and with metabolic syndrome but they are higher than those in the control group. Correlation was positive for BMI and waist circumference (WC) with damage to DNA. Linear regression showed that higher consumption of protein and sodium is related to damage to DNA and both carotenoids and omega- 3 are protectors.

Conclusion: Damage to DNA occurs independent of overweight or obesity and WC could be a predictor for damage to DNA.

Keywords: Body Mass Index (BMI); DNA damage; dietary intake; Metabolic Syndrome (MS).

1. INTRODUCTION

Obesity is a multifactorial condition led by genetic, behavioral, environmental and socioeconomic factors. However, nowadays a sedentary lifestyle and a high caloric intake from sugar and processed foods are also responsible by this epidemic condition [1]. Estimative from World Health Organization demonstrated that in 2016, 39% of adults aged over 18 years were overweight and 13% were obese [2].

A clinical definition of obesity, useful in many contexts, is the body mass index (BMI) ≥ 30.0 kg/m² [3]. However, it is clear that BMI is a poor indicator of body fat rate since it does not differentiates fat or lean mass [4]. Within context, waist circumference (WC) has been shown to predict visceral fat, thus reinforcing the use both BMI and waist circumference in clinical practice to evaluate the risk of metabolic disorders [3].

Metabolic syndrome is a condition usually associated with obesity, and considered a public health problem in many countries since it increases the risk for cardiovascular disease (CVD), type 2 diabetes, hypertension, dyslipidemia and several cancers [5]. Oxidative damage, produced by intracellular reactive oxygen species (ROS), results in modification in DNA base, breaks in single-strand and double-strand, and lesions in apurinic/apyrimidinic, many of them are toxic and/or mutagenic [6]. Therefore, not only ROS are implicated in the etiology of disease, but the result of DNA damage may also be a direct contributor to deleterious biological consequences [7]. A relationship among increased production of ROS, impairment of the antioxidant defense, peroxidative damage to membrane, and processes inflammatory in degenerative disease has been demonstrated [8]. Thus, the evaluation

of biomarkers of oxidative stress can help explore the relation between oxidative damage to macromolecules (such as DNA, lipids, and proteins) and several diseases [9].

In according with some researches, human disorders are proportional to the increase in adipose mass, specially visceral fat [10–12]. Excessive body fat would lead to an increased formation of ROS resulting in oxidative stress [7], however the results are inconclusive. So, the aim of this study was to test if oxidative stress level is different in women with overweight and with metabolic syndrome.

2. MATERIALS AND METHODS

2.1 Subjects

Cross- sectional Study performed between March 2013 and March 2014 at the Endocrinology Clinic of the Internal Medicine Department, Botucatu Medical School, São Paulo State University (UNESP).

The criteria of selection included: non-consumers more than 60 g/day of alcohol; non-smoking subjects; non-users of statins; non – users of antioxidant supplements during the sixty days before this study; no altered hematological parameters and albumin; no liver and kidney dysfunction; no cancer, diabetes or altered thyroid function.

The subjects studied were part of a group (convenience sample) of participants followed in the Endocrinology clinic. So, based on inclusion criteria, 80 women (mean age of 31.15 ± 7.91 years) composed this study. According to their body mass index (BMI), they were divided into three groups: Group 1 (G1, the control group): 36 women with a BMI between 18.5 and

24.9kg/m²; Group 2 (G2): 21 women with BMI between 25 to 29.9kg/m², classified as women with overweight; and Group 3 (G3): 23 women with MS [Metabolic Syndrome was specified according to the International Diabetes Federation (2005)] [13].

2.2 Anthropometric Analysis

It was measured the body mass of participants (kg), height (cm), and waist circumference (cm). Body mass was measured using a portable scale accurate to 0.1kg (PL 200, Filizola S.A., São Paulo, Brazil). The height was measured with a stadiometer accurate to 0.5cm (Professional Stadiometer Sanny, São Paulo, Brazil). The waist circumference (WC) was measured at the narrowest level between the rib margin and the iliac crest using a non-flexible anthropometric tape precise to 0.1mm (SN-4010, Sanny, São Paulo, Brazil). It was also calculated the subjects' body mass index (BMI = [body weight ÷ (height)²]). All the procedures were done by health professionals previously trained for the data collection.

2.3 Blood Pressure (BP)

The BP was measured at rest in the left superior limb according to recommendation by the American Heart Association, using a digital BP monitor (Digital Omron BP Monitor, Model 11 EM403c, Tokyo, Japan). For each measurement, the subjects rested for 15minutes in the sitting position with their feet supported and kept their arm at the heart level.

2.4 Dietary Intake

Habitual food intake was assessed using three non-consecutive days (two week days and a week-end day) dietary records. The amounts of foods registered by trained professional were converted into grams for the analysis of energy, macro and micronutrient and dietary fiber intake using the Diet Pro®software – version 5.1i. The diet records were analyzed by a single person.

2.5 Plasma Analysis

After 12h overnight fasting, it was obtained the plasma for determination of glucose, triglycerides, total cholesterol and fractions, urea, creatinine, ALT (alanine aminotransferase), AST (aspartate aminotransferase), uric acid and blood counts by using an automatic enzymatic analyzer

system (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China). All the analysis were performed at the Botucatu Medical School- UNESP.

2.5.1 Extraction of lymphocytes

The level of DNA damage (comet assay) was evaluated in the peripheral blood lymphocytes. Blood samples (3ml) were collected into tubes with 3 ml of RPMI 1640 medium (Sigma-Aldrich), placed carefully on 3 ml Histopaque® 1077 (Sigma-Aldrich) and centrifuged at 2500rpm for 30 minutes at 10°C. The lymphocytes layer was removed and mixed with 3ml RPMI 1640 medium and centrifuged again at 1500 rpm for 15 min. After this procedure, the supernatant was discarded and lymphocytes were re-suspended to be used for DNA damage evaluation by comet assay.

2.5.2 Comet assay

The comet assay was an adaptation of the protocols described by Singh and collaborators [14] and Tice and collaborators [15]. Clean slides were briefly dipped into a container with standard melting point agarose (Sigma-Aldrich) diluted in 1.5% (300 mg/20 mL) PBS buffer (Sigma-Aldrich) (free of Ca²⁺ and Mg²⁺). After this procedure, the slides was dried at room temperature. In the next day 10µl of lymphocytes was added to 120µl of low melting point agarose, diluted in 0.5% (100mg/20 mL) PBS buffer (Sigma-Aldrich) (free of Ca²⁺ and Mg²⁺). This suspension was placed on two previously prepared and identified slides and then overlaid with covers lips (24 x 60 mm) and placed at 4°C for 10 minutes to solidify the agarose.

After this period, covers lips were removed and slides were placed in containers, with ice-cold solution of freshly prepared lysis and (2.5 M NaCl, 100 mM EDTA, 10mM Tris, Triton X-100 and 1% DMSO), where they remained in the dark for a period of 24 hours at 4°C. In order to increase the specificity of the assay, two sheets per individual were treated with endonuclease III enzymes (endo III) and formamidopirimidina-DNA glycosylase (FPG) (BioLabs® Inc, Ipswich, MA, USA) capable of detecting pyrimidines and oxidized purines, respectively [16]. After cell lysis, the slides were placed in a container containing PBS (Ca²⁺ and Mg²⁺ free) for 5 minutes and then transferred to a flask containing Flare 1x (40mM Hepes, 0.1M KCl,

bovine serum albumin (BSA) buffer 0.2 mg/mL and 0.5 mM EDTA, pH 8, (sigma-aldrich)) for 5 minutes. This procedure was repeated three times.

After being placed in a moist chamber, the slides were treated with 50 mL buffer (950 µL Milli-Q H₂O, 40mL Flare 10x and 10mL BSA, control) or 50mL endo III (1:1000 dilution) or 50mL FPG (dilution 1: 1000), covered with a cover slip and incubated for 45 minutes at 37°C. Then the slides were placed in a refrigerator for 10 minutes to solidify the agarose. After this period, the covers lips were carefully removed and the slides transferred to the electrophoresis tank, filled with cold, freshly prepared alkaline buffer (1mM EDTA and 300mM NaOH, pH > 13). After a period of 40 minutes, to unwind DNA, electrophoresis was performed at 25V and 300mA for 30 minutes. After this step, the plates were placed for 15 minutes in a neutralization solution (0.4M Tris, pH 7.5), fixed with 100% ethanol and allowed to dry at room temperature.

At the moment of analysis, the slides were stained with 70µL solution of SYBR Gold (2:10 000; Invitrogen, USA), covered with a cover slip and nucleotides viewed with a fluorescence microscope (400X magnification) coupled within image analysis system (Comet Assay II, Perceptive Instruments, UK). 50 nucleotides per slide were analyzed. The tail intensity (intensity of DNA in the tail) was used as a parameter for assessing levels of DNA damage. The test was performed in duplicate and analyzed blind with coded slides.

2.5.3 Plasma Hydrophilic Antioxidant Capacity (HAC)

The hydrophilic antioxidant capacity in plasma was determined fluorometrically, as described by Beretta et al. (2006) [17] using a VICTOR X2 reader (Perkin Elmer, Boston, MA). The antioxidant activity was quantified by comparing the area under the curve relating to the oxidation kinetics of the suspension phosphatidylcholine (PC), which was used as reference biological matrix. The peroxy radical 2',2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) was used as an initiator of the reaction. The results represent the percent inhibition (4,4 difluoro-5-(4-phenyl 1-3 butadiene)-4-bora-3,4-diaza-s-indacene) (BODIPY) 581/591 plasma with respect to the control sample of BODIPY 581/591 PC liposome. All analyses were performed in triplicate. The results are reported as percentage of protection.

2.5.4 Plasma antioxidants levels

Carotenoids, retinol and α-tocopherol were measured in 100µL of plasma by reversed-phase high performance liquid chromatography (HPLC; Waters Alliance 2695 Separation Module, Waters, Wilmington, MA, USA). The column used was C30 (Waters Alliance, YMC carotenoid: 4.6 x 150mm; 3.0µm). The measurements were performed as previously described by Yeum and collaborators [18].

2.6 Statistical Analysis

Groups were compared by One-way ANOVA followed by Tukey's multiple comparison test for symmetrical data. Non-symmetric data were analyzed using a generalized linear model with gamma distribution followed by the Wald adjusted multiple comparison test. Through Pearson's correlation coefficient, variables which were in a significant relationship to the DNA damage and oxidative damage to purines and pyrimidines were determined. A stepwise multiple linear regression model was used to assess which nutrients influence DNA damage, considering DNA damage as continuous response variable and the intake of nutrients as explanatory variables. Data are presented as means and standard deviations. All the tests were performed using SAS for Windows, v9.3. with a significant level at 5%.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Anthropometric data and blood pressure

Women with overweight (G2) and with metabolic syndrome (G3) presented weight, BMI, WC and SBP higher than the control group, with G3 presenting the highest values. DBP was high only in G3. There was no difference for height (Table 1).

3.1.2 Biochemical determinations

Table 2 shows plasma biochemical parameters. Total cholesterol didn't differ among groups and HDL-cholesterol was lower in G3 in comparison to the other groups. LDL-cholesterol and triglycerides presented increased levels in G2 and G3 compared to G1. About glucose levels, G2 and G3 presented higher levels compared to G1, but the highest levels were found in G3 (Table 2).

3.1.3 Macronutrients intake

The dietary intake of macronutrients and micronutrients are presented in Table 3. Except for protein (higher in G3) all the others didn't present difference among the groups. Regarding the micronutrients intake, the consumption of vitamin C and vitamin D was lower in G3 compared to the other groups. All the other micronutrients didn't present difference.

3.1.4 Antioxidants in plasma

The concentrations of antioxidants are presented in Table 4.

Cryptoxanthin and α -carotene were reduced in G3. On the other hand, uric acid and retinol were increased in G3. There was no difference among the groups for lutein, lycopene and α -tocopherol.

3.1.5 Biomarker of oxidative stress and damage to DNA

Table 5 shows the damage to DNA, total antioxidant capacity (TAP) and levels of malondialdehyde (MDA) in each group. About the damage to DNA, it is important to emphasize that both G2 and G3 presented more damage compared to G1. The same pattern was observed in oxidative damage to purines. Damage to pyrimidines was higher according to the nutritional status ($G1 < G2 < G3$). Total antioxidant capacity was increased in G3. MDA levels was higher in G2 and G3 compared to G1.

3.1.6 Correlation among BMI, WC, DNA damage, oxidative damage to purines and oxidative damage to pyrimidines

Fig. 1 shows the correlation between DNA damage and BMI (Fig. 1A) and between DNA damage and WC (Fig. 1D). Both anthropometric variables presented correlation with DNA damage, such as: BMI and oxidative damage to pyrimidines (Fig. 1B), BMI and oxidative damage to purines (Fig. 1C), WC and oxidative damage to pyrimidines (Fig. 1E) and WC and oxidative damage to purines (Fig. 1F). However, the correlation between DNA damage and WC was stronger.

3.1.7 Association between DNA damage, consumption of macro and micronutrients and plasmatic variants

The final linear regression model showed a positive association between the consumption of sodium and protein and DNA damage. On the

other hand, polyunsaturated fat intake and plasma levels of α -carotene were negatively associated with DNA damage (Table 6).

3.2 Discussion

The aim of this study was to evaluate if the oxidative stress is different in women with overweight and with metabolic syndrome. Our results showed that the damage to DNA already occurs in women with overweight. Moreover, it was also demonstrated that increased waist circumference is associated with DNA damage, independent of the level of BMI.

Regarding the anthropometric and biochemical parameters, BMI, waist circumference, diastolic and systolic blood pressure, and glucose were higher in group with metabolic syndrome (G3) compared to overweight group (G2). Several studies found this same result [19–22]. Many chronic diseases are also result from obesity (e.g., metabolic syndrome, diabetes mellitus, liver and cardiovascular diseases, and cancer). Obesity is associated with low-grade chronic systemic inflammation in adipose tissue that promotes pro-inflammatory status exercising a critical role in the pathogenesis of obesity-related disorders [23].

Although medical and epidemiological literature studying the relationship between diet composition and a variety of illnesses such as cardiovascular disease, high blood pressure, and diabetes [24,25], our results show no difference in the intake for the most of macronutrients and micronutrients among the groups. Even being a non expected result, the literature reports some possible explanation for this: a- the patient forgets to report the food consumed (omission errors), as soon reporting foods that have not been consumed; b- obese people tend to underestimate their food intake [26].

Reactive oxygen species (ROS) occur under physiological conditions and in many diseases causing direct or indirect damage in different organs; thus, it is known that oxidative stress (OS) is involved in pathological processes such as obesity, diabetes, cardiovascular disease, and atherogenic processes. It has been reported that obesity may induce systemic OS, a condition associated with an irregular production of adipokines, which contributes to the development of the metabolic syndrome [27]. Interestingly, damage to DNA, oxidative damage to purines and the levels of MDA were the same in

overweight and metabolic syndrome women and higher compared to control group even with no difference in plasma antioxidant levels among the groups. Studies show that not only an increase in fat mass leads to an increased oxidative stress and consequently to oxidative damage, but also metabolic syndrome and type II diabetes usually aggravate oxidative stress and damage [28]. However, different from the literature our data showed that the damage to DNA is the same in overweight and MS women.

Added to this, our results show a correlation between BMI and WC with damage to DNA, oxidative damage to purines and pyrimidines. Considering this, the results suggest that WC could be used as a predictor for oxidative stress and DNA damage in conditions which is not possible to analyze oxidative stress parameters. Corroborating this, although the current classification of obesity is based on the Body Mass Index (BMI), which is the weight (in kilograms) divided by the square of height (in meters), BMI has limitations because it does not distinguish between lean mass and fat; it may overestimate body fat in well-trained body builders and underestimate body fat in older persons. Moreover, BMI does not identify fat distribution. So, it is now well recognized that

abdominal fat is a major risk for obesity-related diseases, contributing to pro-oxidant and pro-inflammatory states, as well as to alterations in glucose and lipid metabolisms [29].

The final linear regression for damage to DNA was negative for α -carotene and polyunsaturated fat acid. The literature shows that carotenoid can prevent oxidative stress and DNA damage [30,31] as well as polyunsaturated fat acid, especially omega-3 from cold water fish [32]. The literature also reports that n-3 polyunsaturated fatty acids increases the levels of HDL cholesterol and decrease LDL cholesterol [33] and protect against autoimmune diseases, type 2 diabetes, rheumatoid arthritis and cancer [34]. On the other hand, the final linear regression for damage to DNA was positive for protein and sodium. Studies show that diets high in sodium may predispose individuals not only to the development of obesity but also to complications such as hypertension [35,36]. Moreover, sodium intake was also positively correlated with oxidative stress in experimental studies; however, the mechanism responsible for this effect is still being studied, but it has been suggested that a high-salt diet stimulates the formation of reactive species through the activation of NADPH oxidase [37].

Table 1. Anthropometric data and blood pressure in women control (G1), with –overweight (G2) and with metabolic syndrome (G3)

Variable	G1 (n=36)	G2 (n=21)	G3 (n=23)
Age (years)	27.10 ± 4.7 ^a	33.8 ± 8.5 ^b	35.1 ± 8.6 ^b
Weight (kg)	55.5 ± 5.0 ^a	75.2 ± 6.9 ^b	95.8 ± 21.0 ^c
Height (m)	1.62 ± 0.05 ^a	1.65 ± 0.07 ^a	1.63 ± 0.07 ^a
BMI (kg/m ²)	21.1 ± 1.7 ^a	27.7 ± 1.8 ^b	36.1 ± 7.3 ^c
WC (cm)	70.4 ± 5.5 ^a	89.2 ± 6.6 ^b	109 ± 17 ^c
SBP (mmHg)	109 ± 9 ^a	116 ± 10 ^b	126 ± 12 ^c
DBP (mmHg)	71.7 ± 7.7 ^a	76.2 ± 6.5 ^a	84.3 ± 7.1 ^b

BMI = body mass index, WC = waist circumference, SBP = systolic blood pressure, DBP = diastolic blood pressure. Results are expressed as means and standard deviation. Means followed by different superscript letter indicating whether significant differences among groups (ANOVA followed by Tukey's test at $p < 0.05$)

Table 2. Plasma biochemical profile in control (G1), overweight (G2) and metabolic syndrome (G3) women

Variable	G1 (n=36)	G2 (n=21)	G3 (n=23)
Cholesterol (mg/dL)	184 ± 31 ^a	204 ± 31 ^a	198 ± 25 ^a
HDL-cholesterol (mg/dL)	68.1 ± 16.9 ^a	61.4 ± 15.0 ^a	46.0 ± 10.7 ^b
LDL-cholesterol (mg/dL)	98.6 ± 31.8 ^a	117 ± 36 ^{ab}	122 ± 26 ^b
Triglycerides(mg/dL)	88.4 ± 31.1 ^a	122 ± 63 ^b	149 ± 60 ^b
Glucose (mg/dL)	73.2 ± 4.7 ^a	78.9 ± 6.8 ^b	85.5 ± 7.7 ^c

Results are expressed as means with standard deviation. Means followed by different superscript letter indicating whether significant differences among groups (ANOVA followed by Tukey's test at $p < 0.05$)

Table 3. Dietary intake of macronutrients and micronutrients in control (G1), overweight (G2) and metabolic syndrome (G3) women

Variable	G1 (n=36)	G2 (n=21)	G3 (n=23)
Macronutrients			
Carbohydrate (g)	207 ± 76 ^a	215 ± 81 ^a	208 ± 64 ^a
Cholesterol (mg)	176 ± 90 ^a	184 ± 76 ^a	195 ± 117 ^a
Fiber (g)	14.1 ± 6.7 ^a	12.2 ± 5.0 ^a	11.8 ± 3.8 ^a
Monounsaturated fat (g)	13.4 ± 6.6 ^a	14.3 ± 7.1 ^a	14.1 ± 6.5 ^a
Polyunsaturated fat (g)	9.9 ± 5.2 ^a	9.9 ± 9.0 ^a	8.9 ± 4.0 ^a
Saturated fat (g)	13.1 ± 7.2 ^a	14.3 ± 6.3 ^a	12.7 ± 6.0 ^a
Total fat (g)	59.4 ± 19.4 ^a	65.6 ± 30.7 ^a	57.0 ± 20.9 ^a
Protein (g)	70.7 ± 20.3 ^a	75.3 ± 20.9 ^{ab}	105 ± 133 ^b
Micronutrients			
Calcium (mg)*	637 ± 199 ^{ab}	586 ± 348 ^a	442 ± 284 ^b
Iron (mg)**	37.9 ± 99.2 ^a	36.4 ± 112.1 ^b	11.0 ± 4.6 ^a
Folate (µg)**	152 ± 74 ^a	149 ± 65 ^a	134 ± 38 ^a
Phosphorus (mg) *	802 ± 244 ^a	803 ± 254 ^a	772 ± 173 ^a
Magnesium (mg)*	159 ± 79 ^a	213 ± 199 ^a	135 ± 35 ^a
Potassium (mg) *	1764 ± 635 ^a	2037 ± 1650 ^a	1565 ± 438 ^a
Selenium (µg) **	79.6 ± 82.7 ^a	81.2 ± 79.6 ^a	70.2 ± 29.2 ^a
Sodium (mg) **	1705 ± 667 ^a	2447 ± 1322 ^b	2130 ± 730 ^{ab}
Vitamin A (equiv. retinol)**	794 ± 401 ^a	649 ± 434 ^a	631 ± 486 ^a
Vitamin C (mg)**	148 ± 131 ^a	78.8 ± 47.1 ^b	79.8 ± 53.0 ^b
Vitamin D (µg)**	25.6 ± 49.9 ^a	3.62 ± 8.24 ^a	3.18 ± 5.55 ^b
Vitamin E (mg)**	76.3 ± 243.9 ^{ab}	41.6 ± 139.9 ^a	12.7 ± 6.3 ^b
Zinc (mg)**	10.2 ± 22.4 ^a	16.4 ± 34.3 ^a	17.3 ± 30.0 ^a

Results are expressed as means with standard deviation. Means followed by different superscript letter indicating whether significant differences among groups (ANOVA followed by Tukey's test at $p < 0.05$). * ANOVA followed by Tukey's multiple comparison test. ** Generalized linear model with gamma distribution followed by Wald multiple comparison test

Table 4. Plasma carotenoids, α-tocopherol, uric acid and retinol of women control (G1), with overweight (G2) and with metabolic syndrome (G3)

Variable	G1 (n=36)	G2 (n=21)	G3 (n=23)
Lutein (µg/dL)	8.35 ± 6.13 ^a	5.13 ± 3.10 ^a	7.72 ± 6.54 ^a
Cryptoxanthin (µg/dL)	18.9 ± 18.4 ^a	10.1 ± 9.2 ^{ab}	7.86 ± 8.45 ^b
α-carotene (µg/dL)	5.75 ± 4.57 ^b	2.63 ± 1.49 ^a	2.76 ± 1.69 ^a
β-carotene (µg/dL)	12.2 ± 11.5 ^a	6.32 ± 3.99 ^{ab}	5.22 ± 3.36 ^b
Lycopene (µg/dL)	5.87 ± 6.94 ^a	6.09 ± 5.26 ^a	6.06 ± 5.86 ^a
α-tocopherol (µg/dL)	491 ± 254 ^a	462 ± 242 ^a	626 ± 255 ^a
Uric acid (mg/dL)	3.75 ± 0.77 ^a	4.39 ± 1.09 ^b	5.32 ± 1.14 ^c
Retinol (µg/dL)	80.2 ± 34.6 ^{ab}	68.4 ± 29.6 ^a	106 ± 77 ^b

Results are expressed as means and standard deviations Means followed by different superscript letter indicating whether significant differences among groups (generalized linear model with gamma distribution followed by Wald multiple comparison test at 5%)

Table 5. DNA damage, total antioxidant capacity (TAP), malondialdehyde (MDA) levels in women control (G1), with overweight (G2) and with metabolic syndrome (G3).

Variable	G1 (n=36)	G2 (n=21)	G3 (n=23)
DNA damage (%) **	49.9 ± 9.0 ^a	76.7 ± 13.3 ^b	76.1 ± 9.9 ^b
Oxidative damage to purines (%)**	60.8 ± 10.3 ^a	81.4 ± 11.3 ^b	76.7 ± 9.9 ^b
Oxidative damage to pyrimidines (%)**	54.2 ± 10.6 ^a	84.8 ± 4.6 ^b	71.7 ± 10.7 ^c
TAP (%) *	37.8 ± 12.4 ^a	44.0 ± 14.4 ^{ab}	51.3 ± 10.1 ^b
MDA (umol/L)*	12.6 ± 8.4 ^a	34.5 ± 18.0 ^b	41.2 ± 20.0 ^b

Results are expressed as mean and standard deviation Means followed by different superscript letter indicating whether significant differences among groups. ** Generalized linear model with gamma distribution followed by Wald multiple comparison tests. * ANOVA followed by Tukey's multiple comparison tests ($p < 0.05$). TAP= total antioxidant capacity, MDA = malondialdehyde

Table 6. Final linear regression model relating DNA damage with consumption of macro and micronutrients and plasmatic variants

Variable	Estimate	EP	P – value
Intercept	57.67351	4.83736	< 0.0001
α-carotene (µg/dL)	-1.13616	0.45101	0.0139
Polyunsaturated fat (g)	-1.16142	0.31356	0.0004
Protein (g)	0.05391	0.02171	0.0153
Sodium (mg)	0.00898	0.00206	< 0.0001

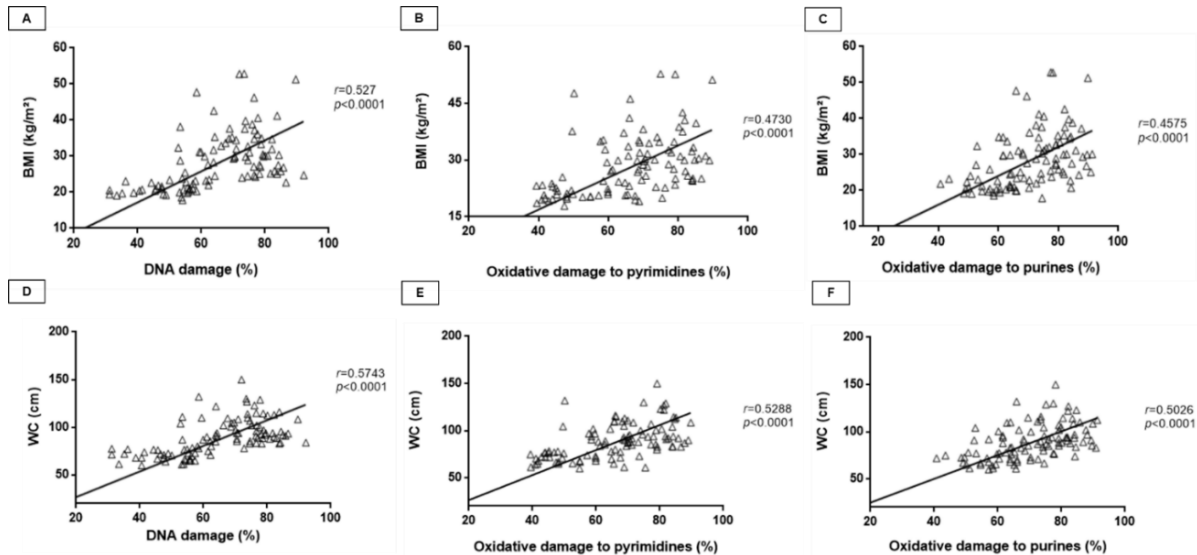


Fig. 1. Correlation between BMI and DNA damage (A); BMI and oxidative damage to pyrimidines (B), BMI and oxidative damage to purines (C), WC and DNA damage (D), WC and oxidative damage to pyrimidines (E), WC and oxidative damage to purines (F). BMI- body mass index (kg/m²); WC- waist circumference (cm). Pearson Correlation between the variables

4. CONCLUSION

In summary, this paper brings important finds: increased BMI is associated with metabolic syndrome, higher BMI and waist circumference is associated with damage to DNA and oxidative stress but damage to DNA is the same in overweight and MS women, and high intake of protein and sodium increases damage to DNA. So, it is possible to conclude that increased BMI and waist circumference is related to increased damage to DNA but is not different between overweight and metabolic syndrome women. It is also suggested that WC could be used as predictor of damage to DNA.

CONSENT AND ETHICAL APPROVAL

The Ethics Committee on Human Research from the same university approved the study protocol (Protocol 3788-2011) and the written

informed consent was obtained from all participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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