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Diet-Induced Weight Loss Reduces DNA Damage and Cardiometabolic Risk Factors in Overweight/Obese Women with Polycystic Ovary Syndrome

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Key Words
Diet · DNA damage · Cardiometabolic risk factors · Overweight · Obesity · Polycystic ovary syndrome

Abstract
Aims: We aimed to investigate the impact of following a diet to induce weight loss (500 kcal deficit per day) over DNA damage and cardiometabolic risk factors in women with overweight/obesity diagnosed with polycystic ovary syndrome (PCOS). ***Methods:*** A study was conducted in Natal, RN, Brazil selecting overweight/obese (body mass index ≥25 and <39 kg/m^2) women (18–35 years). The levels of DNA damage were assessed by a single cell gel electrophoresis. Repeated 24 h dietary recall questionnaires, anthropometry, biochemical profile and sex hormones were collected at baseline and after 12 weeks of intervention. ***Results:*** Women exhibiting a decrease in the markers of DNA damage: tail intensity (24.35 ± 5.86 – pre diet vs. 17.15 ± 5.04 – post-diet; $p < 0.001$) and tail moment (20.47 ± 7.85 – pre diet vs. 14.13 ± 6.29 – post-diet; $p < 0.002$). Reduction of calorie intake, weight loss, decreased sexual hormone and cardiometabolic markers such as insulin, homeostasis model assessment of insulin resistance and low-density lipoprotein cholesterol were verified In the multivariate regression analysis, quantitative insulin sensitivity check index and progesterone were responsible for the variation markers in DNA damage before the diet, losing its influence upon diet. ***Conclusion:*** DNA damage and the impact of cardiometabolic risk factors decreased after the intervention in women with PCOS, indicating the relevance of a nutritional approach in this group of patients.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, affecting 6–12% of the referred population [1, 2]. The main features of PCOS include chronic anovulation, hyperandrogenism and altered ovarian morphology [3]. In addition, women with PCOS have an increased prevalence of cardiometabolic risk factors and diabetes [4–6]. Our research group has reported a higher prevalence of central obesity [7], insulin resistance (IR) [8], low-grade chronic inflammation [9], hypertension [10], metabolic syndrome [11] and a worse autonomic cardiac function [12] in women with PCOS compared to healthy women.

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It is well established that obesity, which is present in 30–70% of women with PCOS [13, 14], as well as other cardiometabolic risk factors (e.g. IR) are associated with the generation of reactive oxygen species (ROS), which may increase DNA damage [15, 16]. DNA damage occurs through constant exposure to DNA mutagens of exogenous and/or endogenous origin [17], including cell metabolism and inflammatory responses [18]. Previous studies have reported that women with PCOS present higher DNA damage than healthy ovulatory women, suggesting increased genomic instability [19, 20] and an increased risk for developing cancer in this population [21, 22]. Thus, interventions that decrease obesity and reduce the impact of cardiometabolic risk factors may reduce genomic instability and DNA damage.

From a clinical perspective, a healthy diet with moderate weight reduction (5–10%) bring about a decrease in the number of cardiometabolic risk factors in overweight/obese women, which may reduce genomic instability and DNA damage [23, 24]. The Androgen Excess and PCOS Society states that lifestyle modification, including a healthy diet with moderate weight reduction, should be the first-line therapy for overweight/obese women with PCOS [25, 26]. This recommendation is supported by previous investigations, which reported that reducing caloric diet in the setting of adequate nutritional intake and healthy food choices reduces the impact of several cardiometabolic risk factors in overweight/obese women with PCOS [6, 27, 28]. However, to the best of our knowledge, no previous study has investigated the effects of a diet on DNA damage in women with PCOS. Therefore, this study aims at assessing the effects of diet on DNA damage and cardiometabolic risk factors of overweight/obese women with PCOS. Our initial hypothesis is that diet can play a role in improving the standard of food intake reducing weight and in bringing about a decrease in the number of cardiometabolic risk factors, which in turn would reduce the DNA damage.

Material and Methods

Subjects and Study Design

Overweight and obese (body mass index (BMI) ≥25 and <39 kg/m^2) women with PCOS, aged from 18 to 35, were eligible for this trial. Thirty-five patients were selected from the Januário Cicco Maternity Hospital of the Federal University of Rio Grande do Norte, Natal, RN, Brazil. The diagnosis of PCOS was made according to the Rotterdam ESHRE-ASRM-sponsored PCOS criteria [3] by 2 independent endocrinologists. Patients diagnosed with other disorders, such as type 2 diabetes, non-classical congenital adrenal hyperplasia, thyroid dysfunction, and hyperprolactinemia as well as patients with renal or hepatic dysfunction or use of medications known to affect reproduction, cardiovascular and/or metabolic function within 90 days of study entry were excluded. This study was approved by the Institutional Ethics Committee and all subjects gave their written informed consent. All patients participated in a 12-week diet intervention program. At baseline and after 12 weeks, the following parameters were analyzed: weight, habitual dietary intake, weight, waist circumference (WC), hormonal and biochemical parameters and DNA damage levels. At study entry, all women were oriented to maintain their habits, especially regarding the physical activity (none of them was involved in a regular physical activity program).

Dietary Intervention

Each participant's energy needs were calculated and adjusted to generate a 500-kcal deficit per day [29, 30]. Dietary characteristics included 15% energy from protein, 60% from carbohydrates (8% simple sugar) and 25% from fat (7% saturated fat) with macronutrient composition calculated as a percentage of the total calories and with a consumption of 25 g/day of fibers. An amount of 400 g/day of fruits and vegetables was ingested by patients. Cereals, roots and tubers, milk and dairy products, meat and eggs, with a low supply of saturated fats, cholesterol and refined grains, were recommended [31, 32]. Diets were supervised by a dietician and delivered together with a list of healthy food, seasonal shopping lists, meal plans, and recipes. Every 2 weeks, patients returned to measure body weight and, if necessary, adjustments were made to the diet to improve compliance. Energy and macronutrient intake were evaluated using 24 h dietary recalls (2R24h) prior to the diet intervention and post-diet. Food intake based on the information obtained with the 24hR was calculated using software Virtual Nutri Plus®, version 2014.

Anthropometric

Patients were submitted to a clinical examination before and after the dietary intervention period. The following anthropometric parameters were assessed: height (m), weight (kg), and WC (cm). WC was measured after expiration, with tape not extensible at the midpoint between the tenth rib and the iliac crest. BMI was calculated as current weight (kg) divided by squared height (m), according to World Health Organization [33].

Biochemical and Hormonal Parameters

Peripheral blood samples were obtained after overnight fasting. The metabolic profile included fasting glucose, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. All biochemical assays were determined using commercial kits (Diagnostic Labtest – SA®, São Paulo, Brazil) by the colorimetric method/enzyme in equipment Bioplus 2000 (Bioplus®, Barueri, São Paulo State, Brazil). LDL-cholesterol was calculated following Friedewald's formula [34]: LDL = total cholesterol – HDL-C – (triglycerides/5). The results are expressed as mg/dl.

Follicle stimulating hormone (FSH), progesterone, testosterone, sex hormone binding globulin (SHBG), and insulin were assessed by chemiluminescence (Immulite 1000®, Diagnostic Products Corporation, Los Angeles, Calif., USA). IR was calculated by the homeostasis model assessment of IR (HOMA-IR): fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5 and the quantitative insulin sensitivity check index (QUICKI), QUICKI = 1/(log insulin (μIU/ml) + log glycemia (mg/dl) [35].

DNA Damage Analysis

DNA damage was analyzed for single-cell gel electrophoresis according to Gontijo and Ticer [36]. A volume of 10 ml peripheral blood was mixed with 100 µl of 0.5% low melting point agarose at 37°C, layered on slides precoated with normal melting point agarose and immediately covered with a coverslip. The slides were allowed to cool at 4°C for 5 min to solidify the agarose. The slides were gently removed and immersed in a freshly prepared solution of ice-cold lysis (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% dimethyl sulfoxide added immediately before use) to lyse cells and to allow the DNA to unfold. After 1 h in the dark at 4°C, slides were briefly rinsed in phosphate-buffered saline and placed in a horizontal electrophoresis unit, filled with fresh alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA pH >13). Electrophoresis was conducted at 4°C for 20 min at 25 V and 300 mA. The slides were neutralized in a buffer (0.4 M Tris pH 7.5). The dry analytical microscope slides were stained with ethidium bromide (20 g/ml in distilled H_2O; 50 liter/blade), covered with a coverslip before analysis in a Zeiss fluorescence microscope. The microscope was connected to a device charge coupling and a personal computer for analysis system (Comet Assay II Perceptive Instruments, UK) to determine the extent of DNA damage. Results were expressed as a percentage of DNA in the tail (tail DNA fraction divided by the amount of DNA in the cell, multiplied by 100 – intensity tail – tail%), tail moment (tail product and the mean distance of migration in tail arbitrary units), and the tail length (distance from the medium or the perimeter of the head estimated to last visible comet tail signal (m)) [37]. Hundred cells randomly selected (50 of each of the 2 replicate slides) were marked by a blood sample.

Statistical Analyses

Results are presented as mean, SD, and 95% CI or as median and interquartile range (25th–75th percentiles). Data were tested for normality using the Shapiro–Wilk test. Comparisons between baselines and post-intervention were performed using paired t test and Wilcoxon signed-rank test for parametric and nonparametric analyses, respectively.

In order to evaluate the relationship between components of the diet, risk metabolic factors and variation of DNA damage, correlation tests of Pearson and Spearman were respectively conducted for assessing the parametric and nonparametric variables. In addition, the associations of DNA damage and risk metabolic factors with PCOS were measured by multivariate linear regression with variables with p values <0.20 in the previous association tests. The model was considered significant when it presented all the explanatory variables with $p < 0.05$.

The effect size (EZ) was calculated using Cohen's d: small effect = 0.2; medium effect = 0.5; large effect = 0.8 [38]. Data were analyzed using SPSS® (Statistical Package for the Social Sciences) version 20.0 for Windows (SPSS, Inc., Chicago, Ill., USA). For the post hoc power analysis, the G*Power 3.0.10 for Windows was performed.

Results

Figure 1 shows the patient's recruitment flow diagram. Twenty-two volunteers (26 ± 6.0 years of age, 1.56 ± 6 6 cm height) completed the study.

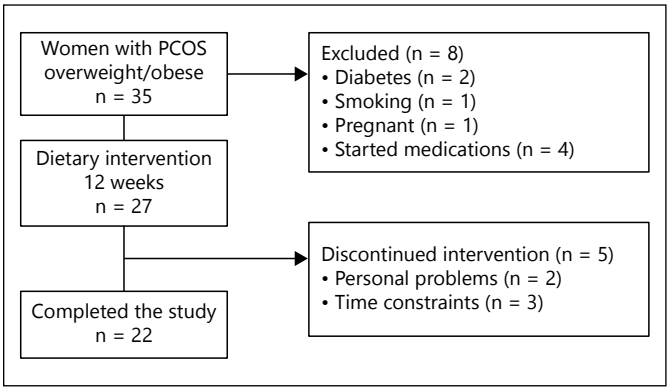

Fig. 1. Flow diagram schematically representing the design of this study.

Dietary intake baseline and post-diet of the patients is shown in table 1. After a 12-week intervention, the self-reported 24-hour caloric intake was significantly reduced by 675 ± 440 kcal/day, with reduced consumption of carbohydrates (106.9 ± 25.6 g/day), proteins (20 ± 5.6 g/day), total fat (25.7 ± 5.6 g/day), consumption of saturated fats (21.1 ± 2.7 g/day), cholesterol level (147.2 ± 90.3 g/day), and sodium (792 ± 671.4 mg/day). Increased consumption of fiber (5.6 ± 9.5 g/day) and mono (3.6 ± 2.4) and polyunsaturated fats (2.4 ± 4.4 g/day) was also verified. The levels of vitamins A, C and E were adjusted to the reference values recommended by dietary reference intakes [29].

Table 2 shows the effect of diet intervention on anthropometric, hormonal, and biochemical parameters. The weight loss of 3.5% (~2 kg) was related with significant reduction in BMI, FSH, testosterone and SHBG levels and increase in progesterone. A reduction of fasting blood glucose and plasma insulin, HOMA-IR and an increase in QUICK were also noted. However, there were no significant changes in WC, triglyceride and HDL-cholesterol levels.

A decrease in DNA damage markers was observed after the diet intervention (fig. 2). Tail intensity (24.3 ± 5.9 vs. 17.1 ± 5.0%) and tail moment (20.5 ± 7.8 vs. 14.1 ± 6.3 UA) decreased after intervention ($p < 0.01$) with a large ES (Cohen's d >0.8), while tail length showed a trend to decrease (208.4 ± 45.9 vs. 185.7 ± 40.5 µm; $p = 0.07$).

A post hoc statistical power analysis (2-tailed) for the difference between baseline vs. post-intervention was conducted to determine the achieved power of the Wilcoxon signed-rank test, based on the investigated sample size (n = 22) for the DNA damage parameters. In this line, tail intensity and tail moment, as parameters of DNA damage, presented improvements after intervention.

Table 1. Dietary intake assessed before and at the end of the total 12-week dietary intervention in overweight and obese patients with polycystic ovary syndrome

Nutrient	PCOS women (n = 22)	Baseline (week 0)	Post-diet (week 12)	p value*
Total energy, kcal/day		2,076.2±503.8	1,401.2±264	0.001
Daily intakes (% energy), AMDR				
Protein	10–35	15.2±1.3	29.1±3	0.001
Carbohydrate	45–65	60.2±2.6	50.2±4.4	0.001
Total fat	20–35	24.6±2.1	20.7±1.9	0.001
Daily intakes (RDA/AI), days				
Protein, g	46	82.7±7.3	62.7±6.36	0.001
Carbohydrate, g	130	284.6±18.25	177.7±37.9	0.001
Total dietaryfiber, g	25	17.5±7.86	19.1±8.04	0.322
Total fat, g	–	67.7±23.6	42.3±17.22	0.001
Saturated fat, g	–	19±6.7	12.71±2.24	0.001
Monounsaturated fat, g	–	11.1±3.3	14.7±5.32	0.003
Polyunsaturated fat, g	–	8.9±1.4	11.2±5.20	0.001
Cholesterol, mg	–	294.8±164.3	147.6±50.9	0.001
Sodium, mg	2,300	2,073.2±977.3	1,281.3±426.7	0.001
Vitamin A (RAE), μg	700	581.1±776	707.9±755	0.884
Vitamin C, mg	75	867±863	903.8±717.2	0.862
Vitamin E (AT), mg	15	9.3±2.7	15.2±3.7	0.001
Folate, μg	400	81.3±112	88.8±47	0.016

AMDR = Acceptable macronutrient distribution; RAE = retinol activity equivalents; AT = mg d-α-tocopherol; AI = range.

Data are means ± SD. * t paired test.

Fonte: Institute of Medicine/Food and Nutrition Board, 2005.

Table 2. Baseline and post-diet anthropometric, hormones and metabolic parameters in overweight and obese patients with polycystic ovary syndrome

PCOS women (n = 22)	Baseline (week 0)	Post-diet (week 12)	p value[a]
Anthropometric			
Body weight, kg	73.3±17.0	71.2±16.4	0.001
BMI, kg/m^2	29.8±6.1	28.9±5.8	0.001
WC, cm	95.4±15.8	93.2±13.2	0.13
Hormones			
FSH, mUI/ml	4.0±1.3	2.4±0.9	0.001
Progesterone, ng/ml	0.37 (0.26–0.58)	1.89 (1.13–2.42)	0.001
SHBG, nmol/l	158 (130–187)	115 (99–122)	0.001
Testosterone, ng/dl	140 (113.2–207.7)	129 (81.4–169.2)	0.001
Metabolic			
Fasting glucose, mg/dl	79±9.6	74.6±8.7	0.027
Fasting insulin, μIU/ml	11.3 (8.07–15.07)	5.5 (4.1–7.6)	0.001
HOMA-IR, mol × μU/l	1.9 (1.3–3.3)	0.9 (0.7–1.3)	0.001
QUICK	0.34± 0.03	0.38±0.02	0.001
Triglycerides, mg/dl	123 (63–164)	94 (47.5–135)	0.385
HDL-cholesterol, mg/dl	43.7±13.7	46.2±15	0.068
LDL-cholesterol, mg/dl	89 (70–104)	86 (50–50)	0.001

Data are expressed mean ± SDs or median (25th–75th percentile).

[a] Statistical comparisons were performed by Student t test paired for means and by Wilcoxon test for medians.

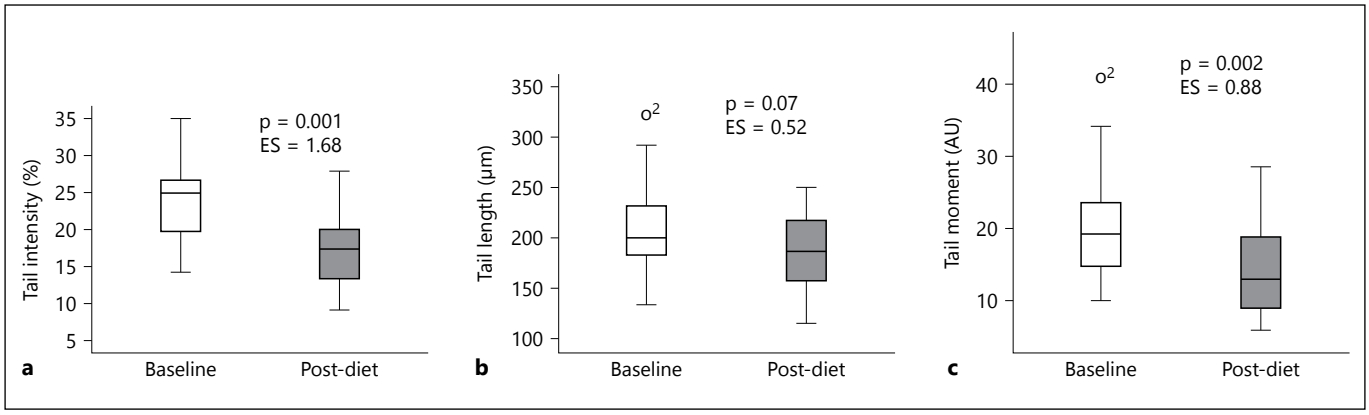

Fig. 2. Variation of DNA damage markers: tail intensity (**a**), tail length (**b**) and tail moment (**c**) in overweight and obese patients with PCOS (n = 22). Boxplots show median, upper and lower quartiles and minimum and maximum data. The ES was calculated by Cohen's d.

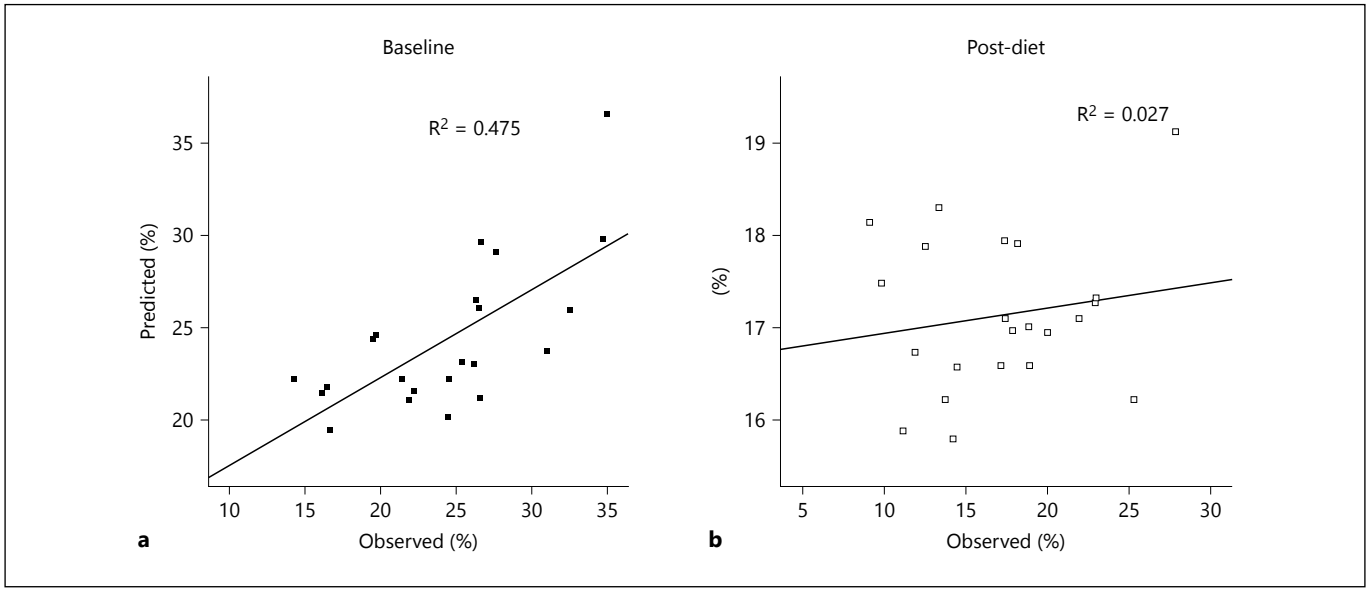

Fig. 3. a, **b** Multiple logistic regression with OR and 95% for overweight and obese patients with PCOS (n = 22), identifying the levels of progesterone and QUICK responsible for the change of DNA damage measured by the tail intensity.

Considering an alpha of 0.05, the achieved power was 0.99 for both tail intensity and tail moment.

In PCOS patients, we found a negative correlation between the change in hormones SHBG (r = –0.48, p = 0.02) and testosterone (r = –0.41, p = 0.04) and DNA damage. The other parameters were not significantly associated with the variation of the damage. It was also not possible to identify a significant association between the components of the diet with DNA damage. Multiple logistic regression identified progesterone level and QUICK that were responsible for 47.5% of the variation of DNA damage measured by tail intensity. The ratios for the corresponding probabilities (95% CI) for QUICK and progesterone were 84.8% (20.17–149) and 6.11% (1.17–11), respectively, before the diet. The same variables did not explain the variation of the DNA damage after the diet. Figure 3 plots the different values of the tail intensity in observed vs. predicted probabilities before and after the diet.

Discussion

The main findings of our study were that a healthy diet intervention with an average weight loss of 3.5% reduced the DNA damage and impact of cardiometabolic risk factors (mainly IR) in overweight/obese women with PCOS. To the best of our knowledge, this is the first report that shows the positive impact of a healthy diet intervention on genomic instability of an adult female population with increased cardiometabolic risk factors (i.e. women with PCOS).

Effects of diet, weight loss and nutritional factors associated with DNA damage and/or repair processes are inconclusive in the literature. In this sense, our results support the hypothesis that a proper and balanced diet (even with a discrete loss of weight) exerts a protection role on genome, by enhancing DNA repair and/or preventing cell death and mutations as shown in previous studies [24, 39–41].

Some factors might have influenced the reduced DNA damage and the variation in dietary components observed in our study (e.g. reduction of fat and replacement the saturated fat consumption by polyunsaturated, reducing intake carbohydrates or protein), even without any significant association with reduced DNA damage may have influenced the variation of genotoxicity, since levels of DNA damage were correlated with various dietary fat sources and types of dietary fat [42]. In addition, the reduction in the consumption of simple carbohydrates and the increase in consumption of fiber were previously demonstrated to be related to the maintenance of intestinal microbiota, which helps in preventing cancer [43]. Also, the reduction of DNA damage in this study, might reflect the increase in consumption of antioxidants vitamins A, C and E, present in fruits and vegetables, which are associated with the combat of reactive oxygen species, reducing damage to DNA and increasing DNA repair capacity [44–46]. Such increase in the consumption of antioxidants in our study might have generated an improvement in the oxidative balance of patients, since reduced levels of antioxidants have been reported in PCOS women [47]. Together, these results support a beneficial role of proper nutrient intake pattern in generating genome protection in PCOS.

Previous studies reported that PCOS women have increased susceptibility to oxidation of DNA compared to ovulatory healthy women [19, 20, 48, 49], highlighting the importance of conducting clinical practices addressed to minimize the damaging effects caused by the syndrome. The exact mechanisms related to genomic instability in women with PCOS is uncertain. However, hyperandrogenism, hyperglycemia, and RI with the increased generation of ROS [50, 51] may also be associated with DNA damage, as well as other aspects of genomic instability [52, 53]. Importantly, DNA damage (if not repaired) might result in the initiation of mutagenic and carcinogenic processes [17, 54].

An important finding of our study was the improvement in insulin sensitivity after dietary intervention, since women with PCOS commonly present altered glucose metabolism, increasing the risk of type 2 diabetes [55]. Therefore, the decrease of ~50% of fasting insulin and HOMA-IR observed after dietary intervention was associated with the reduction of DNA damage, which might be related to an attenuated state of oxidative stress, commonly found elevated in PCOS women (regardless of excess weight) [56, 57]. Our results also demonstrated an improvement in the hormonal profile, with reduction of hyperandrogenism, as reported in other studies [58, 59] and even a positive correlation with the reduction of DNA damage. Such a model can be explained by the direct relationship between androgen levels, and increased expression of oxidative protein has been described [60]. In addition, our multivariate model demonstrates, before nutritional intervention, an influence of the level of progesterone and fast in the variation of DNA damage. This influence was reduced after the treatment, suggesting that reducing these risk factors can generate a protective effect to the genome.

Finally, the weight loss associated with a hypocaloric diet adopted by the volunteers could have also contributed to the reduction in DNA damage presented in our results. Overweight, consequently arising from meals rich in fat and high carbohydrate content, acts as a stress factor to promote the proliferation of adipocyte causing overproduction of ROS. Therefore, controlling consumption of nutrients might have resulted in lower ROS production, allowing for a balance in the metabolism and conferring genomic protection [61].

The absence of a control group is the main limitation of our study. Thus, further investigations including a control group are needed.

In conclusion, a healthy diet intervention, even with a modest weight loss, reduced the DNA damage and the impact of cardiometabolic risk factors in overweight/obese women with PCOS, suggesting a genomic protection. These findings reinforce the importance of a healthy diet in clinical management of women with PCOS.

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Disclosure statement

The authors have nothing to disclose.

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