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Nutritional Intervention and Its Impact on the Inflammatory Process in Women with Polycystic Ovarian Syndrome

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Abstract

Introduction: We aimed to investigate the impact of a diet in the inflammatory profile of overweight and/or obese women diagnosed with Polycystic Ovary Syndrome (PCOS).

Methods: A study was conducted in Natal, Brazil selecting overweight/obese (BMI ≥25 and <39 kg/m^2) women (18-35 years old).). The inflammatory markers were dosed by chemiluminescence without immulitis equipment. Dietary recall questionnaires, repeated 24h. were collected at baseline and after twelve weeks of the intervention.

Results: The Anthropometric indicators and clinical signs of Polycystic Ovarian Syndrome showed that women with PCOS 45% (n= 10) were overweight and 54.6% (n= 12) were obese. Regarding cardiovascular risk, 86.4% presented a risk of metabolic disorders and consequently an increase in cardiovascular disease After a 12-week intervention there was the improvement in the lipid profile weight, anthropometric, hormonal, and biochemical parameters. The Weight decreased 3.5% (~2 kg) was associated with significant reduction in BMI (28.9 ± 5.2 Kg/m^2), FSH (2.4 ± 0.9 mUI/mL), testosterone (129 ± 41 ng/dL), SHBG (115 ± 81.4 mmol/L) and a levels and ease in progesterone (1.89 ± 0.76). It was also noted a reduction of plasma insulin (5.5 ± 1.4 µUI/mL), HOMA-IR 0.9 ± 0.3 mol x µUI), QUICK (0.38 ± 0.02 and LDL -cholesterol levels (86 ± 0.01 mg/dL) as shown in table 4. The Profile of Inflammatory markers in the table 5 in women with polycystic ovarian syndrome. showed a decrease in hs-PCR (39.7 ± 7mg/L), TNF-α (5.3 ± 1.1 pg/mL) and IL-6 (2.3 ± 0.2 pg/mL).

Conclusion: Inflammatory cytokines and inflammation indicators levels decreased after intervention in women with PCOS indicating the relevance of a nutritional approach in this group of patients.

Keywords: Diet; Inflammation; Polycystic Ovary Syndrome; Obesity; Overweight

Introduction

Polycystic Ovary Syndrome (PCOS) is a common hormone disorder. Women suffering from this disease have problems with fertility mostly because of hormonal imbalance [1]. Patients often demonstrate elevated levels of androgens in blood. Although Stein and Leventhal are regarded as the first investigators of Polycystic Ovary Syndrome (PCOS), it was Vallisneri, an Italian medical scientist, physician and naturalist, who in 1721 described a married, infertile woman with shiny ovaries with a white surface and the size of ovaries as pige on eggs [1,2]. Polycystic Ovary Syndrome (PCOS), a common endocrine metabolic disorder with a prevalence estimated of 6-12% reported among those of reproductive age, is characterized by Polycystic Ovary Syndrome (PCOS), oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism, insulin resistance, infertility, reduced

psychological and emotional well-being, negative self-image, and impaired physical, sexual, social and cognitive functioning [3-11]. In 1990 were established the criteria of the National Institute of Health (NIH), followed by ESHRE/ASRM (European Society of Human Reproduction and Embryology/American Society of Reproduction Medicine) criteria established in Rotterdam in 2003. The diagnosis of PCOS requires two out of the following three criteria: oligo-anovulation, clinical or biochemical androgen excess, ultrasound polycystic morphology of at least one ovary (at least 12 follicles measuring 2-9 mm in diameter or ovarian volume greater than 10 cm^3). In the last years, specific diagnostic criteria were developed to allow the diagnosis of PCOS.

Another remarkable feature in women with PCOS is a chronic low-grade inflammation that has emerged as a major factor to the pathogenesis of PCOS, contributing to the promotion of IR and arterogenesis. The inflammatory state is mainly evidenced by producing inflammatory cytokines such as TNF α, IL - 6 and acute phase proteins as well as CRP-us, obesity and IR is common in patients with PCOS which contribute to insulin resistance and other inflammation risk factors in the long term [12-14]. The association between the pro-inflammatory state and cardiometabolic, and Adipose tissue is an endocrine organ able to secrete various substances that interfere with the metabolism of carbohydrates and lipids. Through these metabolic changes in overweight and obese women with PCOS numerous epidemiological studies support the hypothesis that lifestyle change as starting a severe dietary reeducation helps metabolic and inflammatory balance in these women. Inserting in the diet an increase of the consumption of fruits and vegetables, with diets rich in fibers and with low fat content lead to a reduction in the risk factors of chronic diseases [12-14] besides being efficient in reducing the inflammatory process [15,16]. Systematic reviews that sought to clarify this "ideal" diet composition in PCOS identified that weight reduction, achieved by a hypocaloric diet associated with a healthy eating pattern, is able to reduce adipose mass and improve cardiometabolic alterations, regardless of the proportions of the macronutrients used in the diet, in the majority of the studies evaluated [17-20]. Therefore, this study aims to assess the effects of a healthy diet on inflammation risk factors of overweight/obese women with PCOS. Our initial hypothesis is that a diet can improve the standard of food intake, reduce weight and inflammation risk factors and inflammation.

Material and Methods

Subjects

Overweight and obese (body mass index ≥25 and <39 kg/m^2) women with PCOS, aged from 18 to 35 years old, 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.

Inclusion and Exclusion criteria

The diagnosis of PCOS was made according to the Rotterdam ESHRE-ASRM-sponsored PCOS criteria [3] by two 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 twelve weeks the following parameters were analyzed: weight, waist circumference, habitual dietary intake, hormonal, biochemical and inflammatory markers. 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).

Diet evaluation

Each participant's energy needs were calculated and adjusted to generate a 500-kcal deficit per day [21,22]. 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 25g/day of fibers. An amount of 400g/day of fruits and vegetables was ingested by patients. Cereals, roots and tubers, milk and dairy products, meat and eggs, with low supply of saturated fats, cholesterol and refined grains, were recommended [22,23]. Diets were supervised by a dietician and delivered together with a list of healthy food, seasonal shopping lists, meal plans, and recipes. Every two 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 a 24h dietary recall (24hR) prior to the diet intervention and post-diet. Food intake based on the information obtained with the 24hR was calculated using the software Virtual Nutri Plus®, version 2014.

Anthropometric measurements

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 Waist Circumference (WC) (cm). WC was measured after expiration, with tape knot extensible at the midpoint between the tenth rib and the iliac crest. Body Mass Index (BMI) was calculated as current weight (kg) divided by squared height (m), according to World Health Organization [23].

Blood sample collection

Blood samples to tests were obtained from the participants under fasting conditions into tubes without Ethylenediaminetetracetic Acid (EDTA). Blood was immediately placed on ice or in a refrigerator, and the samples were centrifuged at 3500 rpm for 10 min at 4 C within 15 min of collection. Plasma was then immediately stored in dark tubes (Eppendorf) under conditions of 80 C to minimize artificial oxidation.

Hormonal and biochemical 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: 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, LA). Insulin Resistance (IR) was calculated by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR): fasting insulin (μIU/ml) x fasting glucose (mmol/l)/22.5 and the quantitative insulin sensitivity check index (QUICKI), QUICKI=1/ (log insulin (μIU/mL) +log glycemia (mg/dL), IL-2, IL6, IL-18, TNF-α e PCR-us were assessed by chemiluminescence (Immulite 1000®) [24,25].

Statistical Analyses

The results are presented as mean, SD, and 95% Confidence Interval (CI) or as median and interquartile range (25^{th}-75^{th} percentiles). The data was tested for normality using the Shapiro-Wilk test. Comparisons between baselines post-intervention were performed using paired t-test and Wilcoxon signed-rank test for parametric and nonparametric analyses, respectively. In addition, the effect size was calculated using Cohen's d: small effect = 0.2; medium effect= 0.5; large effect= 0.8 [26]. Data were analyzed using SPSS® (Statistical Package for the Social Sciences) version 20.0 for Windows® (SPSS, Inc., Chicago, IL). For the post hoc power analysis, the G*Power 3.0.10 for Windows was performed.

In order to identify metabolic risk factors that explain the variation in DNA damage before and after intervention, a multivariate linear regression analysis was performed. Correlation tests of Pearson and Spearman were respectively applied for parametric and nonparametric variables. A multivariate analysis was performed with variables with p values <0.20 in the previous association tests. The model was considered significant when it was presented all the explanatory variables with p<0.05.

Results

In the figure 1 and 2 shows the patient's recruitment flow diagram and experimental design of nutritional monitoring of women with polycystic ovary syndrome. The diagram clarifies the final sample number of the study, identifying the losses during the study. The Anthropometric indicators and clinical signs of Polycystic Ovarian Syndrome showed that women with PCOS 45% (n= 10) were overweight and 54.6% (n= 12) were obese. Regarding cardiovascular risk, 86.4% [19] presented a risk of metabolic disorders and consequently an increase in cardiovascular disease. The dietary intervention shows the evaluation of dietary intake before and at the end of the 12-week dietary intervention in overweight and obese women with polycystic ovary syndrome. The dietary intake of the volunteers, before and after the diet. After a 12-week intervention, self-reported 24-hour caloric intake was significantly reduced by 675, with a reduction in carbohydrate intake, protein, total fat, saturated fat, cholesterol levels, and sodium There was also an increase in fiber intake and mono and polyunsaturated fats. The levels of vitamins A, C and E were adjusted to the reference values recommended by Dietary Reference (DRI) (TRUMBO et al., 2002)**.** The profile clinical, biochemical, hormonal and metabolic markers of women with PCOS show decrease statistically significant differences and healthy control in weight, BMI, FSH, SHBG, Testosterone, Progesterone, Insulin basal, HOMA, QUICK and LDL cholesterol (p> 0.05) all results was describing to research group to Lemos TMAM et al., 2016.

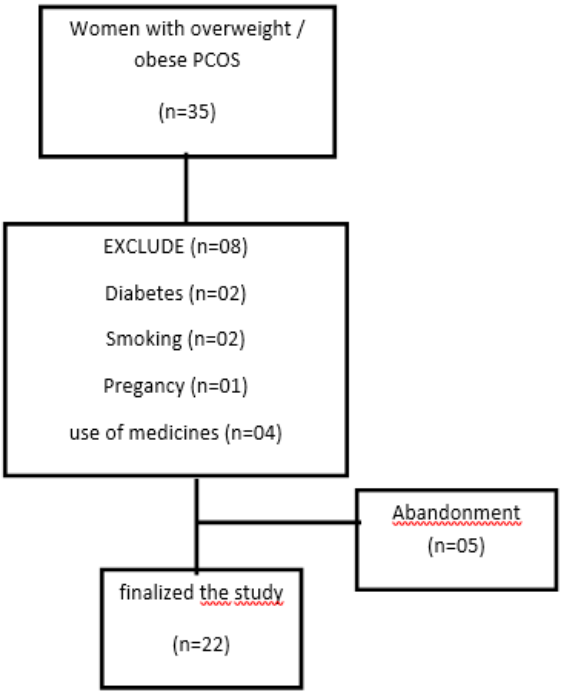

Figure 1: Patient´s Recruitment flow diagram.

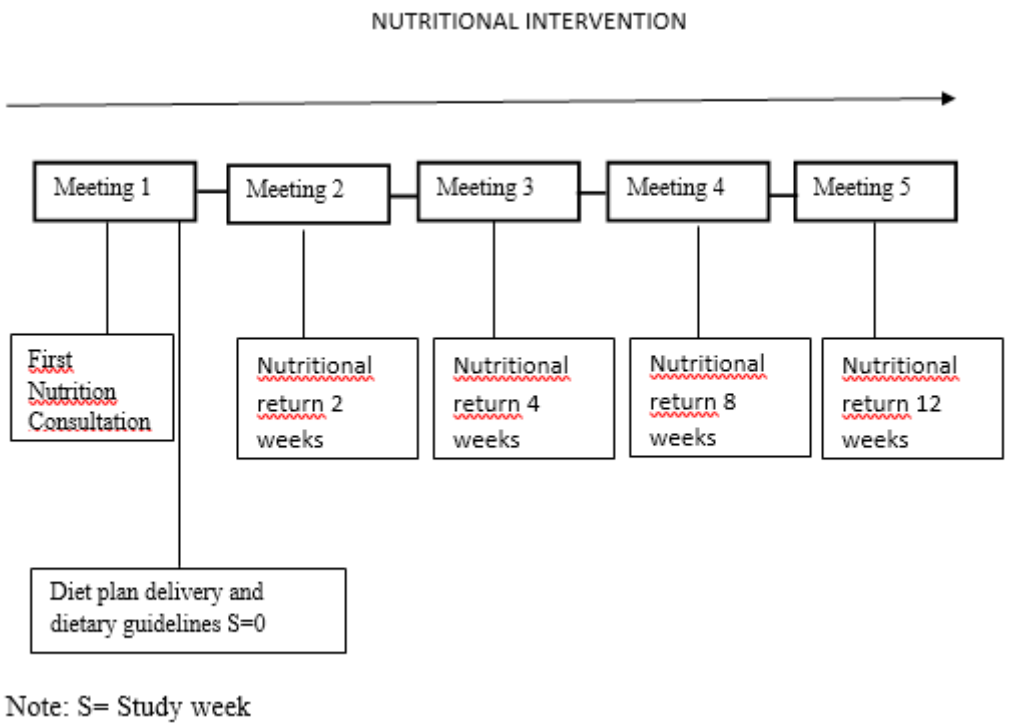

Figure 2: Experimental design of nutritional monitoring of women with polycystic ovary syndrome.

In the table 1 is the unpublished part of this work showing the profile of Inflammatory markers in women with polycystic ovarian syndrome. After dietary intervention we observed a decrease in hs-CRP, TNF-α and IL-6.

Inflammatory Profile	Before diet (n=27)	End diet (n=20)	p value
hsCRP (mg/L)	105 ± 30	39 ± 7**	0.01
TNF-α (pg/mL)	7.8 ± 1.3	5.3 ± 1.1**	0.01
IL-6 (pg/mL)	6.4 ± 0.7	2.3 ± 0.2**	0.01
IL-2	0.3 ± 0.2	0.59 ± 0.09	0.32
IL-18	1.21 ± 0.71	1.4 ± 0.6	0.01
Values expressed as mean ± standard deviation by the paired or median T test by the Wilcoxon test; - p <0.01			

Table 1: Profile of Inflammatory markers in women with polycystic ovarian syndrome.

Discussion

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders and it affects 6-20% of reproductive-aged women leading to irregular periods, infertility and increased androgen levels causing hirsutism and acne [26,27]. Since the 1990 NIH sponsored conference on PCOS, it has become appreciated that the syndrome encompasses a broader spectrum of signs and symptoms of ovarian dysfunction than those defined by the original diagnostic criteria, such as, Chronic anovulation, Clinical and/or biochemical signs of hyperandrogenism and exclusion of other etiologies, Oligo- or anovulation, polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome [28-30]. Obesity affects most women with PCOS, and they have a higher prevalence of both impaired glucose tolerance and type 2 diabetes. Women with PCOS show increased cardiovascular

risk through a higher incidence of hypertension, an adverse lipid profile, and insulin resistance (IR) [28,29]. The underlying pathophysiology of PCOS is unclear and it appears to be a combination of hyperandrogenism [8], insulin resistance and the factors that cause follicular arrest [9]. Up to 60% of PCOS women have insulin resistance, up to 40% have impaired glucose tolerance and 10% may develop type 2 diabetes by the age of 40 years old [9]. In PCOS, dietary glucose intake in these women induces an inflammatory response and an increase in reactive oxygen species, as well as increased NFκB activation that are independent of obesity and the release of TNFα, IL-6 from circulating MNC and in vitro glucose exposure [7,8]. In addition, these markers of oxidative stress and inflammation are associated with measures of insulin sensitivity and / or glucose-resistant fasting measures of insulin [5,8,9]. Thus, diet-induced inflammation in PCOS culminates in proinflammatory signaling that is known to be involved in the development of insulin resistance and atherogenesis. Our results revealed elevation in the markers of IR, such as the HOMA-IR index and reduction of markers of insulin sensitivity, such as QUICK. This justifies a greater predisposition to the onset of IR and changes in glucose metabolism that are related to the syndrome. Similar results were found by other authors who affirm that IR and compensatory hyperinsulinemia can occur in PCOS, regardless of the presence of obesity [29-32].

Therefore, lifestyle changes in women with PCOS, such as changes in eating habits, physical exercise and therapeutic strategies that promote the correction of the inflammatory and metabolic process, especially insulin through the reduction of fat mass after insertion of a dietary profile for these women [33]. Most studies addressing the state of low-grade chronic inflammation in PCOS have focused on the measurement of circulating C-Reactive Protein (CRP) using high sensitivity assays. CRP is an acute phase marker produced by the liver after stimulation by IL-6, the endocrine cytokine that in this case is produced directly by adipose tissue [23]. CRP levels> 3 mg/L are also predictive of a cardiovascular event compared to the ATP III criteria for the metabolic syndrome, it also plays a functional role promoting the absorption of lipids in foamy macrophages within atherosclerotic plaques. The dietary intake of the volunteers after a 12-week intervention, self-reported 24-hour caloric intake was significantly reduced by 675 (± 440) kcal / day, with a reduction in carbohydrate intake (106.9 ± 25.6 g/day), protein (20 ± 5.6 g/day), total fat (25.7 ± 5.6 g/day), saturated fat (21.1 ± 2.7 g/day), cholesterol levels (147.2 ± 90.3 g/day), and sodium (792 ± 671.4 mg/day). There was also an increase in fiber intake (5.6 ± 9.5 g/day) and mono (3.6 ± 2.4) and polyunsaturated fats (2.4 ± 4.4 g/day). The levels of vitamins A, C and E were adjusted to the reference values recommended by Dietary Reference (DRI) [33].

Some of the recent studies have shown that lifestyle changes in women with PCOS, such as changes in eating habits, exercise, will decrease body mass index and insulin sensitivity, decreases such as serum androgen concentrations and inflammation [8,10]. These findings corroborate the results obtained by Moran *et al*. which showed that 44% of women with PCOS improved these rates with a loss of weight through the hypocaloric diet. An interesting and innovative finding of the present study is that we observed a reduction of our inflammatory markers after weight reduction, we highlight the TNF-α that revealed TNF-α protein expression in both subcutaneous and visceral tissues with adipocyte cell volume in obese people without additional disease.

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