Original Investigation

Vitamin B_3 (niacin), B_6, C, and iron intake are associated with the free androgen index, especially in normoandrogenic polycystic ovary syndrome

Hestiantoro et al. Micronutrients intake associated with PCOS

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Abstract

Objective: Nutritional intake is one of the most common environmental risk factors of polycystic ovary syndrome (PCOS) because it is associated with obesity and insulin resistance. This study aims to determine the relationship between micronutrient intake and androgen levels associated with PCOS.

Material and Methods: This cross-sectional study was performed on 79 PCOS patients, consisted of 50 normoandrogenic (NA) and 29 hyperandrogenic (HA) patients and 66 healthy controls. Dietary intake assessment was performed using a modified 38-item semi-quantitative food frequency questionnaire. Bivariate, correlation, and multivariate analyses were performed to determine the association between study variables, and p-value less than 0.05 was considered as statistically significant difference.

Results: The baseline characteristics in all groups were similar, except for body mass index and hormonal profile, compared to those in the other groups, found to be higher in the hyperandrogenic PCOS group. There was found a significantly negative correlation between the free androgen index and intake of vitamin B1, vitamin B2, niacin, vitamin B6, calcium, and iron in the normoandrogenic PCOS group, while we did not observe it in the hyperandrogenic PCOS group. Multivariate linear regression analysis reveals that the intake of vitamin B_6, vitamin C, niacin, and iron had a significant effect on the free androgen index.

Conclusion: There is an effect of micronutrient intake on androgen levels in women with PCOS. The association was more significant in the normoandrogenic PCOS than in the hyperandrogenic PCOS. These findings reveal an association between micronutrients and androgens and PCOS at a systemic level.

Keywords: Androgens, hyperandrogenism, micronutrients, polycystic ovary syndrome

Introduction

PCOS is a severe health risk for women of all ages, and it has long-term consequences for their health and well-being.[1] There are at least four unique phenotypes of PCOS, three of which are PCOS with classic features of hyperandrogenism, and one phenotype is PCOS with normal androgen levels.[2,3] Various studies have identified clinical, biochemical, and even genetic differences between normoandrogenic and hyperandrogenic.[3,4] However, no studies can reveal the pathophysiological characteristics of PCOS with normal androgen levels. The molecular pathomechanism of PCOS is not well known due to the disorder's diverse character. However, it has been suggested that the interaction between hereditary and environmental factors plays a role in the development and variability of PCOS symptoms.[5,6] Among the aforementioned environmental factors, nutritional intake and physical activity are two of the most important predictors of PCOS risk. Given these facts, many researchers are currently attempting to determine the best nutritional intake pattern to include in a PCOS treatment plan.[7,8,9]

Although several studies have been conducted on the impact of macronutrients in PCOS, few studies have looked at the importance and role of micronutrients. Indeed, some current research suggests that appropriate micronutrient intake could help to reduce PCOS symptoms, including insulin resistance and hyperandrogenism.[7,10] Unfortunately, there has not been much research into the direct link between micronutrient intake and androgen profiles. To address this knowledge gap, we aimed to reveal the relationship between micronutrient intake and androgen levels associated with PCOS, particularly in the normoandrogenic group.

Material and Methods

Study designs and ethical consideration

We performed a cross-sectional study to establish the association between certain micronutrients intake and androgen levels in women with PCOS. The authorization to perform this study was obtained from the Ethical Committee Board with an ethical clearance number of 0449/UN2.F1/ETIK/2018. Before subject recruitment and data collection, study objectives were clearly explained to the subjects, and written consent was provided by each individual who has agreed to participate. Data processing was conducted anonymously and in strict confidence.

Study population

A total of 145 reproductive age women comprised of 79 PCOS patients and 66 non-PCOS controls were enrolled for the present study. The sample size was calculated according to the sample size formula for the cross-sectional correlation test based on power analysis. The selection of subjects was made according to particular inclusion and exclusion criteria. The inclusion criteria for PCOS subjects were reproductive-age women who have been diagnosed with PCOS according to Rotterdam criteria. The inclusion criteria for subjects in the control group were reproductive-age women with normal menstrual cycles who did not meet the diagnostic criteria of PCOS. Subjects were excluded if they were pregnant or breastfeeding; on medications known to alter metabolic parameters for the past two months, such as anti-dyslipidemic, anti-diabetic, or hormonal medications; had any endocrine abnormalities, such as diabetes, thyroid diseases, hyperprolactinemia, or Cushing diseases.

The study population was further subdivided into hyperandrogenic (HA) and normoandrogenic (NA) groups according to their free androgen index (FAI), with FAI ≥ 5 used as a cut-off for hyperandrogenism.[11] In addition to having FAI < 5, subjects in NA groups had to have normal serum testosterone and sex hormone-binding globulin (SHBG) concentrations, as well as no symptoms of hyperandrogenism such as hirsutism, acne vulgaris, androgenic alopecia, and acanthosis nigricans.[12]

Dietary intake assessment

A dietary intake assessment was performed by well-trained staff using a modified 38-item semi-quantitative food frequency questionnaire (SQ-FFQ). The SQ-FFQ assessed daily intake of foods and beverages in the past three months with six possible responses that ranged from never; 1 – 3 times a month; 1 – 3 times a week; 4 – 6 times a week; once a day; or more than once a day, which can be converted into daily servings of 0, 0.5, 2, 5, 7, and 14 times per week, respectively. The quantity of food consumed, both reported in household measures and grams, was converted and homogenized to grams. The mean frequency of food intake was further multiplied by the portion size, which resulted in an estimated weekly intake. The recorded data were analyzed using Nutrisurvey software to estimate micronutrient intakes, according to Indonesian Food Composition Data.

This SQ-FFQ validation study was conducted among a subsample of 40 participants against 30-days repeated 24-hour dietary recall. The participants were asked to fill in both the SQ-FFQ and dietary recall. Then, the means of nutrient intake obtained from both questionnaires were calculated and compared using paired t-test. According to the statistical analysis, the mean intake of nutrients from SQ-FFQ and dietary recall did not differ significantly; hence, a modified 38-item SQ-FFQ as a dietary assessment tool was proper.

Laboratory evaluation

The peripheral venous sample was collected and centrifuged to separate the serum for the quantitative measurement of testosterone and SHBG concentrations using the enzyme-linked immunosorbent assay (ELISA) method. Serum testosterone and SHBG concentrations were determined using a commercial fluorescence enzyme immunoassay kit according to the manufacturer's instructions: ST AIA–Pack Testosterone (TOSOH, Japan, Cat. No. 0025204) and ST AIA – Pack SHBG (TOSOH Bioscience, Japan), respectively. The assay was of the sandwich-type using a pre-coated 96 well plate and a supply of enzyme-labeled secondary antibodies. The sample required for analysis was 300 µl. The sample cup and test cup were prepared and labelled with ID for each sample before measurement. Then, the sample cup and test cup were inserted into the TOSOH instrument. Free androgen index (FAI) was calculated as total testosterone to SHBG ratio (both in nmol/L) and was reported in percentage (%).

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Services (SPSS) software, version 22.0 (IBM Corps., USA). Kolmogorov-Smirnov test was used to ensure the normality of the data distribution. Descriptive analysis was performed to report the baseline characteristics of our study population, which was presented as mean ± SD (standard deviation) for numerical variables. Bivariate analysis of the data was performed using independent T or Mann-Whitney U test to determine the mean difference between two numerical variables. The correlation between dependent and independent variables was calculated using Pearson's or Spearman's correlation coefficient. Multiple linear regression analysis was used to determine the micronutrients that were significantly and independently associated with FAI. The significance level was set at 95%, with a p-value of 0.05 or less considered statistically significant.

Results

A total of 145 reproductive age women consented and were involved in this study, consisted of 79 PCOS patients and 66 control subjects. The total of 79 PCOS patients consisted of 50 normoandrogenic and 29 hyperandrogenic patients. According to WHO Asia Pacific body mass index (BMI) classification, the mean BMI of subjects in the PCOS group was classified as obese 1 (26.35 ± 5.43 kg/m^2). Hyperandrogenic patients also have a slightly higher BMI than normoandrogenic PCOS (27.57 ± 6.03 kg/m2 vs. 25.66 ± 4.99 kg/m2). In comparison, the mean BMI of subjects in the control group was classified as overweight (24.02 ± 3.85 kg/m^2). Subjects in the PCOS group also showed a considerably higher FAI compared to subjects in the control group. The mean FAI of subjects in the PCOS group was 14.72 ±

52.70, which was considered hyperandrogenemia. The average FAI of normoandrogenic PCOS patients also experienced a slight increase compared to the control group (3.81 ± 2.03 % vs. 2.27 ± 1.54 %). Table 1 and Table 2 present the baseline characteristics of the normoandrogenic PCOS, hyperandrogenic PCOS, and control groups.

The mean of various micronutrient intakes in the three groups did not show a significant difference. Table 3 and Table 4 present the mean micronutrient intake of the normoandrogenic PCOS, hyperandrogenic PCOS, and control groups. The FAI in the normoandrogenic PCOS patient group had a significant negative correlation with the intake of vitamins B_1, B_2, B_6, niacin, calcium, and iron. ($r = -0.340$, $p = 0.016$; $r = -0.367$, $p = 0.009$; $r = -0.356$, $p = 0.011$; $r = -0.389$, p 0.005; $r = -0.343$, $p = 0.015$; $r = -0.384$, $p = 0.006$, respectively). Table 5 shows the correlation between FAI and micronutrient intake in normoandrogenic and hyperandrogenic PCOS. Meanwhile, there was no significant correlation of FAI with any micronutrient intake in the hyperandrogenic PCOS group.

Table 6 shows the results of multivariate linear regression within PCOS groups. Multivariate linear regression analysis of PCOS patients, both normoandrogenic and hyperandrogenic, revealed significant negative effect of vitamin B_6 intake to FAI. ($\beta = -1.825$, $p < 0.001$). Meanwhile, intake of vitamin C, iron, and niacin showed a significant positive relationship with FAI in PCOS patients ($\beta = 5.844$, $p < 0.001$; $\beta = 2.381$, $p = 0.020$; $\beta = 2.599$, $p = 0.011$, respectively). There was no significant effect of intake of other micronutrients on FAI.

Discussion

Vitamins, particularly vitamins A and C, act as antioxidants and play an essential role in suppressing chronic inflammation linked to PCOS.[13] However, our study did not demonstrate any significant mean differences of any micronutrient intake in PCOS women compared with normal women. To the best of our knowledge, our study is the first study that compares the intakes of vitamin A, calcium, and iron in women with PCOS and normal women. As for vitamin B_1, vitamin B_2, niacin, vitamin B_6, vitamin C, and folate, Szuczuko et al. have conducted a systematic review study and found out vitamin C intake was lower in women with PCOS than in normal women, while for other micronutrients, no difference was found.[14] Meanwhile, Zaeemzadeh et al. found that the dietary intake of zinc was significantly more low in PCOS women with metabolic syndrome than in control groups.[15] However, our studies did not indicate a lower vitamin C and zinc intake in the PCOS group.

In another study, Szczuko et al. found plasma levels of vitamin C in PCOS women were higher than in non-PCOS women, while plasma levels of vitamin B in PCOS women were lower than in non-PCOS women.[16] Given that our study did not confirm significant differences in intakes of all micronutrients, including vitamins B and C, we suspected that the effect of vitamin C on PCOS incidence is due to its concentration in serum rather than its intake. The concentration of vitamin C, regardless of the amount of intake, in women with PCOS is related to the organism's response to oxidative stress. During oxidative stress and activation of anti-inflammatory reactions, the concentration of ascorbic acid and cortisol in rat plasma increases but decreases in the adrenal glands.[17,18]

Our study reveals significant correlations between several micronutrient intake (vitamin C, B_6, niacin, iron) and the free androgen index in women with PCOS. We demonstrated that vitamin C intake is positively associated with the free androgen index in women with PCOS. Szczuko et al. also confirmed a positive correlation between plasma vitamin C levels and total testosterone.[16] Vitamin C has antioxidant properties that can suppress chronic inflammation in PCOS. In addition, ascorbic acid is found in large quantities in the pituitary gland, so it is thought to have an important role in the secretion of the hormones follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. Furthermore, studies have shown that treatment with ascorbic acid increases FSH and testosterone levels, but these studies have

not been performed in healthy women.[19] This supports and may explain our study's finding that vitamin C intake affects androgens in women with PCOS.

Our study showed that niacin (B_3) intake had a significant effect on FAI in women with PCOS. This can be explained by studies on PCOS mouse models showing that the metabolite N1-methyl nicotinamide (MNAM), a metabolite of a niacin-derived compound, helps solve the problem of hyperandrogenism and ovarian adenosine 5'monophosphate-activated protein kinase (AMPK) via aldehyde oxidase 1 (AOX1), which plays a role in detoxifying the enzymes that metabolize it.[20,21] Because niacin may activate AMPK,[22] we speculate that decreased AMPK activity due to niacin deficiency is closely related to increased frequency of gonadotropin-releasing hormone (GnRH) pulsatility and increased LH production in the pituitary[23,24] as well as AMPK-dependent steroidogenesis disorders in the ovaries,[25,26] in PCOS subjects. However, other studies have shown that niacin level has a negative correlation with SHBG.[16] The kynurenine pathway uses tryptophan from the food to produce niacin. The kynurenine pathway is found mostly in the liver and, to a lesser extent, in extrahepatic organs.[27] This may explain the stronger correlation between niacin intake and FAI in the normoandrogenic group, as demonstrated in this study.

One possible link between vitamin B_6 and androgens is homocysteine. Several vitamin B and zinc play a role in the elimination of homocysteine from circulation. The re-methylation process involves folate, B_2, B_3, and zinc, while the transsulfuration process involves B6 and zinc. Meanwhile, the study found that blood homocysteine levels were negatively correlated with SHBG concentrations.[28] Therefore, the association of FAI with vitamins B_2, B_3, B_6, folate, and zinc may be mediated by SHBG concentrations.

Iron dysregulation can lead to reduced circulating levels of total testosterone. The association between iron and testosterone was weaker in overweight or obese patients than in normoweight patients.[29] This can support the findings of our study, namely a significant correlation between iron intake and FAI in women with PCOS, and this correlation was found to be weaker in hyperandrogenic PCOS patients who were predominantly overweight or obese.

A meta-analysis by Janjuha et al., who conducted studies in normal populations, stated that they did not find a significant effect of micronutrient supplementation on sex hormones, including vitamin A, vitamin C, iron, and zinc.[12] In contrast to Janjuha et al., our study of women with PCOS confirmed an association between androgens and intake of several types of micronutrients, as described above. Furthermore, regarding the fact that the hyperandrogenic phenotype was associated with insulin resistance, whereas the normoandrogenic phenotype is not,[30] we also reveal a stronger correlation in normoandrogenic PCOS than hyperandrogenic PCOS.

Study Limitations

Our study still has some limitations that need to be considered when interpreting the results. Our study was conducted with small sample size and cross-sectional design. It would be better if a longitudinal study design were carried out to determine the causal relationship between androgens and micronutrients in women with PCOS. It should also be noted that this study examined the intake of micronutrients, not the pre-intake serum levels and post-intake serum levels of micronutrients, which of course, were influenced by absorption, transport, and demand.

Conclusion

This study observed an association between the free androgen index and intake of some of the micronutrients such as vitamin B_6, vitamin C, niacin, and iron. These findings suggest a role for micronutrients in the incidence of PCOS and androgens at the systemic level. We suggest future studies could be done with a larger number of samples, measuring nutrient levels in plasma and focusing on its association with the increased role of homocysteine in PCOS.

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Table 1. The characteristics of overall study population (control vs. PCOS)

Characteristics	Control (n=66)	PCOS (n=79)	p
Age (years)	30.00 ± 5.52	29.23 ± 3.66	0.440
Height (m)	1.56 ± 0.04	1.58 ± 0.00	0.158
Weight (kg)	59.14 ± 10.25	66.17 ± 14.74	0.005*
BMI (kg/m^2)	24.02 ± 3.85	26.35 ± 5.43	0.006*
Testosterone level (ng/mL)	0.45 ± 0.37	0.99 ± 2.49	< 0.001*
Total testosterone (nmol/L)	1.57 ± 1.28	3.46 ± 8.63	< 0.001*
SHBG (nmol/L)	167.25 ± 740.44	49.77 ± 65.86	< 0.001*
Free androgen index (%)	2.27 ± 1.54	14.72 ± 52.70	< 0.001*

Continuous variables are shown as means ± standard deviations. Independent t-test was performed for the control vs. PCOS. BMI body mass index, FAI Free androgen index, SHBG Sex hormone-binding globulin. *Indicates statistical significance at the level P-value<0.05

Table 2. The characteristics of overall study population (control vs. normoadrogenic PCOS vs. hyperandrogenic PCOS)

Characteristics	Control (n=66)	Normoandrogenic (n=50)	Hyperandrogenic (n=29)	p
Age (years)	30.00 ± 5.52	29.56 ± 3.53	28.64 ± 3.89	0.506
Height (m)	1.56 ± 0.04	1.57 ± 0.59	1.59 ± 0.65	0.263
Weight (kg)	59.14 ± 10.25	64.06 ± 13.52	69.93 ± 16.28	0.004*
BMI (kg/m^2)	24.02 ± 3.85	25.66 ± 4.99	27.57 ± 6.03	0.008*
Testosterone level (ng/mL)	0.45 ± 0.37	0.52 ± 0.35	1.85 ± 4.03	< 0.001*
Total testosterone (nmol/L)	1.57 ± 1.28	1.80 ± 1.22	6.41 ± 14.00	< 0.001*
SHBG (nmol/L)	167.25 ± 740.44	62.90 ± 77.94	26.31 ± 21.34	< 0.001*
Free androgen index (%)	2.27 ± 1.54	3.81 ± 2.03	34.19 ± 85.44	< 0.001*

Continuous variables are shown as means ± standard deviations. ANOVA was performed for the control vs. normoandrogenic PCOS vs. hyperandrogenic PCOS. BMI body mass index, FAI Free androgen index, SHBG Sex hormone-binding globulin. *Indicates statistical significance at the level P-value<0.05

Table 3. Micronutrients' intake in control and overall PCOS subjects			
Micronutrient	**Control (n=66)**	**PCOS (n=79)**	**p**
Vitamin A (mg)	1035.23 ± 1153.58	1114.67 ± 1332.35	0.694
Vitamin B_1 (mg)	0.51 ± 0.35	0.53 ± 0.40	0.946
Vitamin B_2 (mg)	1.06 ± 0.74	1.11 ± 0.86	0.949
Vitamin B_6 (mg)	0.92 ± 0.65	0.95 ± 0.68	0.883
Vitamin C (mg)	6.17 ± 7.02	6.39 ± 7.19	0.789
Calcium (mg)	381.97 ± 255.58	350.77 ± 264.11	0.347
Zinc (mg)	9.64 ± 7.09	9.95 ± 7.06	0.871
Iron (mg)	5.72 ± 4.19	5.57 ± 4.11	0.816
Niacin (mg)	10.85 ± 8.70	11.24 ± 9.76	0.970
Folic acid (mg)	136.57 ± 134.32	150.07 ± 144.07	0.512
Continuous variables are shown as means ± standard deviations. Independent t-test was performed for the control vs. PCOS. *p < 0.05 indicates statistical significance at the 0.05 level			

Table 4. Micronutrients` intake in control, normoandrogenic PCOS, and hyperandrogenic PCOS subjects				
Micronutrient	**Control (n=66)**	**Normoandrogenic (n=50)**	**Hyperandrogenic (n=29)**	**p**
Vitamin A (mg)	1035.23 ± 1153.58	881.54 ± 937.27	1530.97 ± 1785.45	0.517
Vitamin B_1 (mg)	0.51 ± 0.35	0.50 ± 0.37	0.57 ± 0.44	0.691
Vitamin B_2 (mg)	1.06 ± 0.74	1.03 ± 0.78	1.24 ± 0.99	0.610
Vitamin B_6 (mg)	0.92 ± 0.65	0.92 ± 0.63	1.01 + 0.77	0.914
Vitamin C (mg)	6.17 ± 7.02	6.16 ± 7.10	6.80 ± 7.44	0.655
Calcium (mg)	381.97 ± 255.58	349.45 ± 292.37	353.11 ± 209.25	0.431
Zinc (mg)	9.64 ± 7.09	9.19 ± 6.24	11.29 ± 8.28	0.421
Iron (mg)	5.72 ± 4.19	5.23 ± 3.79	6.18 ± 4.64	0.522
Niacin (mg)	10.85 ± 8.70	10.67 ± 8.11	12.24 ± 12.28	0.970
Folic acid (mg)	136.57 ± 134.32	132.36 ± 120.20	181.87 ± 177.03	0.512
Continuous variables are shown as means ± standard deviations. ANOVA was performed for the control vs. normoandrogenic PCOS vs. hyperandrogenic PCOS. * p < 0.05 indicates statistical significance at the 0.05 level				

Table 5. Correlation analysis between micronutrients intake and FAI in PCOS groups				
Characteristics	**Normoandrogenic PCOS (n=50)**		**Hyperandrogenic PCOS (n=29)**	
	r	**p**	**r**	**p**
Vitamin A (mg)	- 0.255	0.074	- 0.097	0.617
Vitamin B_1 (mg)	- 0.340	0.016*	0.104	0.592
Vitamin B_2 (mg)	- 0.367	0.009*	- 0.016	0.934
Vitamin B_6 (mg)	- 0.356	0.011*	0.102	0.600
Vitamin C (mg)	- 0.199	0.165	0.117	0.547
Calcium (mg)	- 0.389	0.005*	0.012	0.952
Zinc (mg)	- 0.277	0.052	0.070	0.717
Iron (mg)	- 0.343	0.015*	0.126	0.515
Niacin (mg)	- 0.384	0.006*	0.156	0.419
Folic acid (mg)	- 0.271	0.057	0.106	0.585
Pearson correlation analysis was performed between micronutrients intake and Free Androgen Index (FAI). r is the Pearson correlation coefficient which shows the strength of the association between micronutrients intake and androgen levels. *p < 0.05 indicates statistical significance at the 0.05 level				

Table 6. The results of multivariate linear regression within PCOS groups				
Variables	**Unstandardized β ± SE β**	**Standardized β**	**p**	**95% CI range for ExpB**
Vitamin B6	-140.473 ± 38.183	-1.825	< 0.001*	(- 216.465) – (- 64.481)
Vitamin C	6.022 ± 1.030	5.844	< 0.001*	3.968 – 8.075
Iron	9.694 ± 4.071	2.381	0.020*	1.583 – 17.806
Niacin	4.131 ± 1.589	2.599	0.011*	0.965 – 7.298
Multivariate linear regression was performed. *p < 0.05 indicates statistical significance at the 0.05 level				