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Research Article

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# Elabela, Nitric Oxide and Apelin in Women with Polycystic Ovary Syndrome

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## Abstract

*Polycystic ovary syndrome (PCOS) is a major health issue amid fertile-aged women in all society. Therefore, we investigated levels of Elabela (ELA), Apelin (APLN), and Nitric Oxide (NO) in women with and without PCOS, and to identify whether there is any association between ELA, APLN, NO and metabolic parameters. 27 PCOS and 30 control subjects were included. ELA, APLN and NO levels were analyzed by using enzyme linked immunosorbent assay. Both groups demonstrated no significant difference in terms of age and Body mass index (BMI), Insulin, HOMA-IR, Ferriman–Gallwey score, and free testosterone were significantly higher ($p < 0.05$), whereas high-density lipoprotein (HDL), and estradiol (E2) levels were lower in women with the PCOS than that of controls. Lower ELA levels [(28.6 versus 39.4) ng/ml, $p < 0.05$], and lower NO [(194.8 versus 322.5) µmol/L, $p < 0.001$], and higher APLN levels [(478.7 versus 388.4) pg/ml, $p < 0.05$] were reported in PCOS patients compared to controls. ELA and NO are significantly down regulated while APLN ($p < 0.05$) is significantly up regulated ($p < 0.05$) in PCOS patients compared to controls. Based on the findings of this study, decreased ELA and NO, increased APLN levels may be considered as potential regulators of glucose and fat metabolism in PCOS patients. It was also assumed that early measurement of ELA, APLN and NO in PCOS cases might help to avoid the aggravation of PCOS.*

**Keywords:** Apelin, Elabela, Nitric Oxide, Polycystic Ovary Syndrome

## Introduction

Polycystic ovary syndrome (PCOS) is a metabolic abnormality ailment, affecting at least 6% to 10% of women of reproductive age, and the most common cause of infertility in women [1]. Women with PCOS commonly present hyperandrogenism, menstrual irregularities and insulin resistance (IR) [2]. Furthermore, oligovulation or anovulation is a common sign of PCOS [3]. This disease is also associated with other metabolic abnormalities such as impaired glucose tolerance, diabetes, metabolic syndrome (MetS), and cardiovascular problems [4]. It has been also reported that abnormal neurotransmitters (especially dopamine), intercellular adhesion molecule (ICAM)-1, monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-α, IL-6, lipid profiles, c-peptide, prolactin, anti-Müllerian hormone (AMH), sex hormone binding globulin (SHBG) cortisol, follicle-stimulating hormone (FSH), thyroid hormones, estradiol, testosterone blood profile (leukocytosis, neutrophilia, and platelet aggregation) levels are linked with PCOS manifestation [5-12].

Beside above parameters, nitric oxide (NO) is also one of the most important parameters in women with PCOS [13]. Since it has been reported that there was endothelial dysfunction in women with PCOS [14]. However, there are contradictory reports on the level of NO in PCOS [13]. Some researchers have reported decreased NO levels in patients with PCOS, while some researchers have reported the opposite outcomes, or not changed [15-20]. However, meta-analyses indicated that decreased serum or plasma NO levels are associated with PCOS [13].

Furthermore, adipocytokines are also associated with PCOS by changing normal metabolic profile [21]. In this regard, numerous adipocytokines have been studied. For example, it has been

reported that chemerin, leptin and adiponectin levels are elevated in women with PCOS compared with non-PCOS controls [22]. Based on these findings these researchers suggested that elevated these parameters in women with PCOS may contribute to the etiology and the development of PCOS and may be correlated with IR, metabolic disorder [23,24].

Apelin is also one of new adipocytokines, which is a mitogenic factor for the endothelial cells as the endogenous ligand of the G protein-coupled receptor APJ [25]. This molecule and the APJ receptor are widely expressed in various biological tissues, including gastrointestinal tract, placenta, lung, heart, bone, brain and liver [26]. Also, this molecule is a key regulator in glucose and lipid metabolism and is associated with IR [27].

Some study reported that apelin increased in cases of PCOS compared to control [28,29] while some study found that serum apelin levels are lower in women with PCOS than in controls [30,31]. That is there are also contradictory reports on the level of apelin in case of PCOS.

The other newly discovered adipocytokine is elabela (also known as Apela or Toddle). Injection of this molecule increases diuresis and water intake in rats [32]. Elabela is also essential for mouse embryonic angiogenesis [33]. It has been reported that a lack of elabela causes damages to the placenta and the maternal cardiovascular system [34]. The apelinergic system (APJ signaling) activates the PI3K/Akt pathway and NO production in endothelial cells [35]. Inhibition of APJ signaling by *Elabela* knockout results in increased apoptosis in the organs [36]. It has been also reported that elabela- and apelin-APJ induce angiogenesis, promote NO yield to elevate blood flow and reduce oxidative stress [36,37]. As also known, the apelinergic system family members (elabela- and apelin-APJ and NO) is involved in the pathogenesis of glucose intolerance, and some other metabolic diseases [38].

As mentioned above, apelin and NO levels have been studied in women with PCOS, but reports were contradictory in case of PCOS. There is also no elabela study present in women with PCOS currently in the literature. Elabela, apelin-APJ and NO were interacting together in biological system. Therefore, studying any of them alone cannot help to enlight any pathology of diseases without studying those parameters together. Based on these facts, this study was conducted to find out whether blood elabela, apelin and nitric oxide levels are different between PCOS women and healthy women and help to en light contradictory reports on the level of apelin and NO in case of PCOS.

## Materials and Methods

The design of the present study was approved by the non-invasive research ethics committee of Kafkas University Faculty of Medicine (decision date: 03.04.2019, decision no: 5; issue no: 80576354-050-99). 27 women with PCOS were enrolled for this study along with 30 body mass index and age-matched women who had regular menses and no clinical or biochemical hyperandrogenism or PCOS were served as control subjects. None of participated subjects consumed alcohol or smoked. Detailed physical examinations were made for all participants. The diagnosis of PCOS was based on the Rotterdam criteria in the presence of at least two of the following three findings: (1) oligomenorrhea and/or anovulation, (2) biochemical and/or clinical hyperandrogenism, and (3) sonographic findings of polycystic ovaries (multiple cysts >12 in number of 2–9 mm size) [2]. Exclusion Criteria were as follows: Women who had their menarche less than 3 years, women over 45 years old, congenital adrenal hyperplasia, Cushing's syndrome, amenorrhea of menopause, hyperglycemia, thyroid disease (hyperthyroidism, hypothyroidism), hyperprolactinemia, lung failure, renal failure, anemia, dystrophy, metabolic syndrome (MetS), and androgen secreting tumors, heart failure, hypertension and other cardiovascular diseases. Before the three months from the study, none of subjects had taken on hormonal contraceptives, other medications. Fasting glucose, hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), some hormone parameters [free testosterone (fT), follicle stimulating hormone (FSH), estradiol (E2), luteinizing hormone (LH), thyroid stimulating hormone (TSH), free thyroxine levels (free T3 and free T4), insulin], lipid profiles [triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) levels were obtained from the patient file records. Samples were taken in the early follicular phase (day 2–5 of the menstrual cycle) after overnight fasting. Homeostasis model assessment [HOMA-IR, calculated as: fasting glucose × fasting insulin / 22.5] for evaluating insulin resistance are used [39]. Clinical hyperandrogenism was performed by the presence of hirsutism (Ferriman-Gallwey score over 8) [40,41].

### Elabela, Apelin and Nitric Oxide Analyses

ELA, APLN, and NO measurements were analyzed with commercial ELISA kits (22). ELA (Human Elabela, catalogue no: S1508, Peninsula Laboratories International, Inc., San Carlos, USA), APLN (Human APLN, catalogue no: EH2174 Fine Biotech Co., Ltd., Wuhan, China), and NO levels (Human Nitric oxide, catalogue no: 201-12-1511, Sunred Biological Technology Co., Ltd., Shanghai, China) were analyzed according to manufacturer instructions provided in each ELISA kit. Throughout the study period, The Bio-Tek ELX50 (BioTek Instruments, USA) automatic plate-washer was used for plate washes, and ChroMate and Microplate Reader P4300 devices (Awareness Technology Instruments, USA) were used for concentration measurements. ELA, APLN and NO measurement ranges varied 0–100.000 pg/mL 62.5–4.000 pg/mL, and 4–600 µmol/L, respectively.

### Statistical Analysis

Data were analyzed by statistical package for the social sciences (SPSS) version 22 software (SPSS Inc., Chicago, IL, USA). Normality of distribution was determined by Kolmogorov-Smirnov/Shapiro-Wilk's tests) techniques. Student's t-test was applied for comparisons if variables were normally distributed, whereas the Mann– Whitney U test was used for comparisons. The multiple linear regression analysis was used to compare the glucose, lipid profiles, HOMA-IR, ELA, APLN and NO analyses.

Data are expressed as mean ± standard deviation. <0.05 *p*-values were considered significant for all the analyses.

## Results

Demographic and some biochemical and hormonal characteristics of women with and without PCOS are shown in Table 1. There is no difference between groups in the term of age and BMI. Insulin, HOMA-IR, FG scores, and fT were significantly higher ($p < 0.05$), whereas HDL, and E2 levels were lower in women with the PCOS than in controls. ELA (28.6±2.9 ng/mL versus 39.4± 3.6 ng/mL, $p < 0.005$) (Fig. 1), and NO concentrations were lower in women with PCOS (194.8± 16.9 µmol/L than in controls (322.5 ± 26.8 µmol/L, $p < 0.001$), (Fig. 2). On the other hand it was found that APLN was higher ($p < 0.05$) in women with PCOS (478.7±40.1 pg/mL) than in controls (388.4 ± 32.3 pg/ml), (Fig. 3). ELA in groups were negatively correlated with fT and LH levels ($r$ = -0.289 and r = -0.354, respectively, $p < 0.001$) while APLN in groups were positively correlated with fT and LH levels. It was also found that ELA and NO concentrations were positively correlated with TG, fT, HOMA-IR, FG scores, and insulin concentrations while APLN concentrations were negatively correlated with TG, fT, HOMA-IR, FG scores, and insulin concentrations.

**Table 1:** Comparison of Demographic, Biochemical and Hormonal Characteristics of Women With and Without PCOS

| Parameters | Control (n:30) | PCOS (n:27) | *p* |
|---|---|---|---|
| Age (y) | 27.4 ± 3.7 | 25.8 ±2.9 | 0.110 |
| BMI (kg/m$^2$) | 23.4 ± 4.7 | 22.9 ± 4.2 | 0.160 |
| Glucose (mg/dL) | 94.6 ± 6.2 | 88.4 ± 5.1 | 0.174 |
| Hemoglobin (g/dL) | 13.9 ± 0.1 | 14.1 ± 0.8 | 0.306 |
| Glycated hemoglobin (%) | 5.2 ± 0.3 | 5.4 ± 0.2 | 0.082 |
| ALT (IU/L) | 19.8 ± 2.7 | 24.3 ± 3.9 | 0.211 |
| AST (IU/L) | 22.7 ± 5.2 | 23.3 ± 4.1 | 0.670 |
| fT(pg/ml) | 4.6 ±.6 | 1.9 ± .2 | 0.001 |
| FSH (mIU/mL) | 5.9 ± 1.1 | 6.1 ± 1.4 | 0.910 |
| E2 (pg/mL) | 66.3 ± 9.7 | 53.4 ± 10.6 | 0.005 |
| LH (mIU/mL) | 8.8 ± 7.2 | 9.3 ± 6.6 | 0.442 |
| TSH (mIU/mL) | 1.89±.21 | 1.61±.22 | 0.794 |
| fT3 (pg/mL) | 2.89 ±0.37 | 3.77 ±0.52 | 0.833 |
| fT4 (pg/mL) | 1.67±0.11 | 1.59±0.11 | 0.931 |
| Insulin (µIU/mL) | 3.2 ± 1.3 | 5.4 ± 1.9 | 0.001 |
| HOMA-IR | 3.1 | 1.7 | 0.001 |
| TG (mg/dL) | 63.2 ± 19.6 | 77.4 ± 24.9 | 0.042 |
| Cholesterol (mg/dL) | 172.8± 25.3 | 196.1± 23.4 | 0.821 |
| LDL (mg/dL) | 109.7 ± 22.9 | 129.2 ± 311 | 0.623 |
| HDL (mg/dL) | 55.9 ± 9.8 | 38.7 ± 7.9 | 0.005 |
| FG score | 2 | 9 | 0.001 |

**ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **BMI**= Body mass index; **E2**= estradiol, **FG**= Ferriman–Gallwey score; **FSH**= follicle stimulating hormone; **fT**=free testosterone; **fT3-fT4**=free thyroxine levels; **HDL**= high-density lipoprotein cholesterol; **HOMA-IR**= homeostatic model assessment of insulin resistance; **LDL**= low-density lipoprotein cholesterol; **LH**= luteinizing hormone; **PCOS**= polycystic ovary syndrome; **TC**= total cholesterol; **TG**= triglycerides; **TSH**= thyroid stimulating hormone

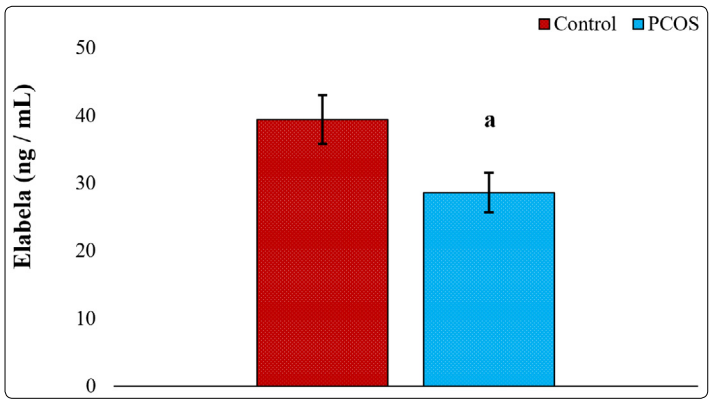


**Figure 1:** Comparison of Elabela Concentration in Women with Polycystic Ovary Syndrome. PCOS = Polycystic Ovary Syndrome. a: $p < 0.005$

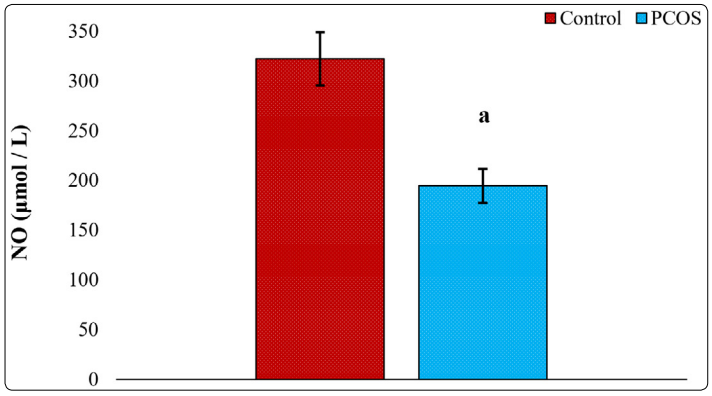


**Figure 2:** Comparison of Nitric Oxide Concentration in Women with Polycystic Ovary Syndrome. PCOS = Polycystic Ovary Syndrome. a: $p < 0.001$

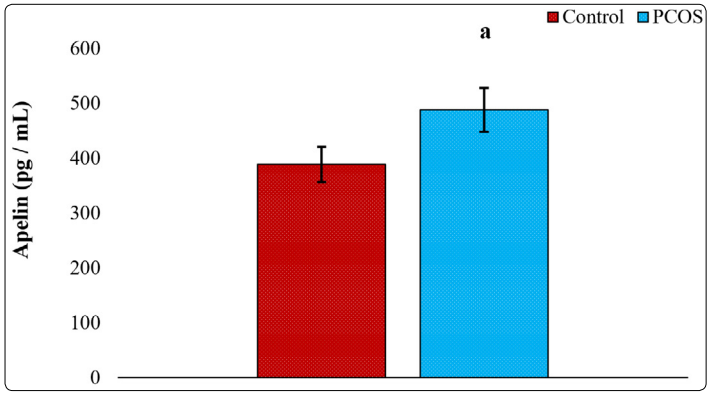


**Figure 3:** Comparison of Apelin Concentration in Women with Polycystic Ovary Syndrome. PCOS = Polycystic Ovary Syndrome. a: $p < 0.05$

## Discussion

This study shows that there may be possible associations among ELA, APLN and NO in women with PCOS. The apelinergic system (ELA and APLN activate the apelin (APJ) receptor) is a crucial regulator of blood glucose concentration via its actions on the pancreas, skeletal muscle and adipose tissue [36,38,42]. In this study it was found that ELA was significantly lower while APLN

was significantly higher in women with PCOS compared with control. It has been previously reported that the ELA and APLN had some beneficial effects in case of obesity with insulin resistance and cardiovascular disease [36,38,43]. Decreased ELA levels in women with PCOS might be associated with increased insulin concentration (insulin resistance). This observation in women with PCOS was in agreement with results reported by Zhang and his coworkers who found that ELA levels decreased in patient with type 2 diabetes [44]. Thus, the reduction of ELA could be a new predictor for polycystic ovary syndrome. The reduction of ELA may be a result of its decreased synthesis in the pancreas, liver, kidneys, lung, spleen and testes where are widely expressed in those tissues [45]. These results indicated that insulin and ELA might be closely associated with each other to control blood sugar tightly. Furthermore, ELA might provide a convenient mechanism for glucose utilization, glucose uptake, and improved insulin resistance.

In this study, APLN levels were higher in women with PCOS. However, alterations in apelin concentrations in relation to PCOS patients have been reported in studies with conflicting results [28-31]. As indicated above some researchers reported that APLN level significant increase in women with PCOS [28,29] while some researchers found a significant decrease in apelin level in PCOS patients [30,31]. Our research results are consistent with researcher who found that APLN level were significantly increased in case of PCOS. PCOS [28,29] Increased APLN levels in women with PCOS might be associated to control insulin concentration. Since in this study, insulin level in women with PCOS was significantly higher than in control. High insulin levels in women with PCOS might induce apelin concentration as reported in this research. Insulin promotes APLN concentration, which then higher concentrations of APLN may compensate for insulin deficiency and overcome insulin resistance. APLN and APJ also regulate insulin secretion in pancreatic islets [46]. It has been also found that APLN increases the glucose utilization of skeletal and adipose tissues in insulin-resistant mice [46,47]. That's why maybe our glucose concentrations in this research were not statistically different in both groups. Supporting this notion, improvements in glucose infusion rate and insulin sensitivity were reported on APLN infusion to healthy overweight volunteers under euglycemic-hyperinsulinemic clamp.

As reported earlier, low-dose intraventricular injections of APLN decreases blood glucose and significantly improve glucose utilization, a process that is dependent on the NO pathway [48]. As mentioned above there is also link between ELA, APLN and NO [42,48]. Therefore, in this study we also investigated the fate of NO in case of PCOS patients. We found that NO concentrations were decreased in PCOS patients. However, previous NO reports in PCOS patients contradict each other. Some studies have reported lower NO levels in PCOS patients while some studies have found higher NO levels in PCOS patients. Meanwhile some of other studies recorded no significant difference in NO levels in women with PCOS compared with controls [13,15-20].

These data agree with the observations of researchers who reported that NO levels were decreased in PCOS patients compared with controls [15-18]. Women with PCOS have insulin resistance, which is linked with endothelial dysfunction and loss of NO biological activity [49]. NO is produced mainly by the vascular endothelium and other cell types [50]. As known, insulin resistance is associated with endothelial dysfunction [49]. Damaged endothelial cells cannot release enough NO to circulation. Earlier reports had indicated that expression of eNOS and iNOS were decreased in PCOS women [15]. Also, NO is produced via the NADPH-dependent oxidation of l-arginine by the enzyme, nitric oxide synthase (NOS) [51]. NO production can be diminished in the decrease of expression of eNOS and iNOS. Therefore, NO level might be found lower in this research in case of PCOS. Also reported that the level of insulin has a significant negative correlation with NO production [52]. Also our results demonstrated that apelin was positively correlated with the parameters of IR (HOMA-IR). Therefore, it might be possible that elevating apelin concentration in women with PCOS subjects might be related to a defensive response, which may represent ability for adaptation to IR [45].

## Conclusion

These results suggest that ELA and APLN regulate glucose metabolism by modulating insulin and can therefore influence PCOS disease by stimulating mitochondrial biosynthesis, fatty acid oxidation, and endothelial NO synthase via the adenosine monophosphate (AMP)-activated protein kinase (AMPK) and the serine/threonine protein kinase PKB (also known as Akt) pathways, resulting in increased glucose uptake in biological tissues. Therefore, changes in the decreased ELA and NO levels and increased APLN level in blood could be a new biomarker for predicting PCOS disease and its complications, and also these parameters might be helpful in the management of patients with PCOS.

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