The Kynurenine Pathway and Polycystic Ovary Syndrome: Inflammation as a Common Denominator

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ABSTRACT: Polycystic ovary syndrome (PCOS) is a complex metabolic disorder commonly seen in females of reproductive age. The pathophysiology of PCOS is multifactorial and includes dysfunction in ovarian steroidogenesis and folliculogenesis, impaired gonadotropin levels, insulin resistance, gut microbiota imbalance, genetic predisposition, and lifestyle preferences. Low-grade inflammatory conditions such as obesity and impaired glucose tolerance are common metabolic disturbances in women with PCOS. A growing body of literature suggests strong evidence rendering PCOS in close proximity with chronic inflammation as documented by high levels of serum white blood cells, C-reactive protein, and various proinflammatory cytokines seen in this condition. Inflammation seems to be the most common metabolic denominator between the kynurenine pathway and PCOS. The association of tryptophan and kynurenine pathway has already been well documented in mood disorders, neurodegenerative diseases, chronic pain conditions, and different inflammatory states. In this manuscript, we describe the influence of sex steroid hormones on different enzymes of the KP; inflammatory nature of PCOS and CRP as a marker of IDO/TDO activity; and the effects of altered gut flora in women with PCOS. This review provides a novel view of the available evidence of tryptophan and downstream metabolites in PCOS in the context of underlying inflammation.

KEYWORDS: Polycystic ovary syndrome, kynurenine pathway, tryptophan, inflammation, metabolic disturbance, obesity, insulin resistance

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Introduction

Polycystic ovary syndrome (PCOS) poses a complex endocrine, metabolic, and polygenic disorder commonly seen in females of reproductive age. The chief findings in women with PCOS include oligo- or amenorrhea, hirsutism, and acne. As per the 2003 Rotterdam consensus, the diagnosis of PCOS encompasses the features of clinical hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology.[1] In the United States, a retrospective study with over 12 million women showed only 1.6% had classic PCOS as per The Rotterdam Criteria with variable geographical distribution throughout the country.[2] However, a more recent study using the same diagnostic criteria reported an overall prevalence of 10% worldwide.[3] Several metabolic conditions, including diabetes mellitus and dyslipidemia, have been well documented in patients with PCOS.[4] The exact pathophysiology of PCOS to date is still in developing stages. The contemporary mechanisms that could explain this condition involve dysfunction in ovarian steroidogenesis and folliculogenesis, impaired gonadotropin levels, and insulin resistance (IR). However, other factors such as gut microbiome,[5] genetic predisposition,[6] and lifestyle modifications[7] have also been implicated. A growing body of evidence suggests that chronic inflammation may play a pivotal role as documented by high levels of serum white blood cells, C-reactive protein (CRP), and various proinflammatory cytokines in women with PCOS.[8,9] A recent in-depth study investigating different metabolites in women with PCOS found significantly elevated levels of tryptophan and tyrosine in the follicular fluid samples.[10] Indeed, the metabolic pathway of tryptophan, and especially the kynurenine pathway (KP), may be an attractive research topic that could help to better understand the relationship between a state of inflammation and PCOS. Therefore, it is the aim of this brief review to present the possible link between tryptophan and downstream metabolites in PCOS in the context of underlying inflammation.

Physiology of Menstrual Cycle and Sex Steroid Hormone Synthesis

The menstrual cycle represents a series of coordinated, succeeding events leading to maturation and release of mature oocyte. The mean duration of menstrual cycle is 28 days and recurs in a regular manner. Factors such as obesity, smoking, and stress have been found to be significantly associated with irregular menstrual cycles in premenopausal women.[11] The first, proliferative or follicular phase of the menstrual cycle begins with the first day of menses and ends with ovulation. The follicular phase is initiated and maintained by the effects of a follicle stimulating hormone (FSH), the production of which is under control of glycoprotein inhibin A and gonadotropin releasing hormone (GnRH).[12] The function of the FSH is to regulate the oocyte selection, folliculogenesis, and

sex steroid (testosterone, estrogen, and progesterone) hormone production. Of these, estrogen (more specifically, 17β estradiol) represents the main hormone in the first phase of the menstrual cycle, which creates an optimal environment for oocytes by stimulating the growth of the uterine endometrium, stroma, glands, and spiral arteries. In the end of the follicular phase, the peak of 17β estradiol stimulates the production of FSH and luteinizing hormone (LH) (collectively called the LH surge), which then triggers the ovulation. At that point, luteal or secretory phase starts and lasts until the first day of menses. LH stimulates the production of progesterone, the primary regulatory hormone in this phase, to regulate the proliferation endometrial endothelial cells in order to maintain an environment suitable for eventual implantation. The synthesis of 17β estradiol and progesterone near the end of the luteal phase occurs in the corpus luteum. Should the fertilized ovum be implanted, the corpus luteum regresses, and the levels of the 2 hormones decrease rapidly. The production of biologically active sex steroid hormones commences with the conversion of cholesterol to pregnenolone by the cholesterol side-chain cleavage enzyme (P450scc).[13] Pregnenolone transforms into progesterone via the enzyme 3β-hydroxysteroid dehydrogenase. Pregnenolone and progesterone are then metabolized by the 17α-hydroxylase and 17,20-lyase (desmolase) enzymes to dehydroepiandrosterone (DHEA) and androstenedione, respectively. Androstenedione is synthesized in the theca cells under the stimulation of LH and becomes a substrate for the subsequent production of estradiol by FSH. In addition, androstenedione and estrone (aromatized from androstenedione) serve to produce testosterone and estradiol, respectively, via 17β-hydroxysteroid dehydrogenase.

Tryptophan Metabolism and the Kynurenine Pathway

Tryptophan is 1 out of 9 essential amino acids, the bioavailability of which rests exclusively on dietary intake. Tryptophan can be found in protein-rich (meat, seafood, vegetables, and nuts) foods and the daily dietary requirement is around 4.0 mg/kg.[14] When introduced into the gastrointestinal system, around 95% of ingested Trp is metabolized by the kynurenine pathway, 90% of which occurs in the liver via Trp 2,3-dioxygenase (TDO), and 5% to 10% extrahepatically via immune system activation through the effects of indoleamine 2,3-dioxygenase (IDO).[15] Other pathways in the tryptophan metabolism involve tryptophan transformation into tryptamine and indole derivates via tryptophanase, as well as serotonin production via Trp hydroxylase 1.[16] The KP poses an extensive metabolic scheme that ultimately serves to generate nicotinamide adenine dinucleotide (NAD^+). However, the KP yields metabolites that also have a role in mood disorders,[17] neurodegenerative diseases,[18,19] and chronic pain conditions.[20] The catabolic pathway (ie, the KP) of tryptophan commences with the activation of the aforementioned IDO 1 (IDO-1), 2 (IDO-2), and TDO enzymes. In mice, the search for tissue distribution of IDO showed active expression in non-immune (caput of epididymis, prostate gland, placental syncytiotrophoblasts, and different eye structures) and immune (antigen presenting cells [APCs] such as macrophages and dendritic cells) tissues.[21] Such diverse location of this enzyme was proposed to account for its different biological functions, including the suppression of infection and regulation of immunological response. The expression of IDO-1 is regulated under the influence of proinflammatory cytokines including tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), and interleukin 1β (IL-1β), and 6 (IL-6).[22] The stimuli pertinent to enzymatic activity of IDO-2 are still being extensively studied; however, contemporary evidence shows a connection between this enzyme and inflammatory cytokines,[23] although conflicting data exists.[24] Relative to IDO-1 contribution in tryptophan catabolism, the role of IDO-2 was shown to be fairly nonexistent.[25] Nevertheless, the upregulated expression of IDO-2 was documented in liver, kidney, and APC.[26] The TDO enzyme is under the influence of 4 regulatory mechanisms in the liver: hormonal (glucocorticoids, glucagon, and estrogens); stabilization by tryptophan against degradation; inhibition by intermediate products of tryptophan metabolism; and activation by haem.[27] The first catabolite in tryptophan metabolism governed by the IDO1/2 and TDO enzymes is kynurenine (KYN), which further yields kynurenic acid (KYNA)[28] (via kynurenine aminotransferases [KATs]), anthranilic acid (via kynureninase [KYNU])[29] or 3-hydroxykynurenine (3-HK) via kynurenine 3-monooxygenase (KMO).[30] In the next stage of Trp metabolism, both anthranilic acid (through non-specific hydroxylation) and 3-HK (via KYNU) serve as sources for the production of 3-hydroxyanthranilic acid (3HAA). Further downstream, 3-HK acts as a substrate for transamination to produce xanthurenic acid (XA),[31] but may also undergo transformation into 3-hydroxy-L-kynurenamine (3-HKA), a novel metabolite discovered after IFN-γ stimulation of nodal lymphatic endothelial cells and catalyzed by IDO-1 enzyme.[32] 3-HA metabolite is able to undergo condensation and produce cinnabarinic acid, a chemical reaction involved in reactive oxygen species production.[33] More importantly, 3-HA serves to dioxygenate to 2-amino-3-carboxymuconate-6-semialdehyde (ACMS), which then autocyclizes to quinolinic acid (QA).[34,35] The QA represents a crucial mediator in generating NAD^+, thereby providing a source of cellular energy. A graphical description of the aforementioned metabolites and enzymes involved in the kynurenine pathway is shown in Figure 1.

Serotonin and indole pathways

More than 90% of body 5-hydroxytryptamine (5-HT; serotonin) is produced by the enterochromaffin cells (ECs), a specialized type of intestinal enteroendocrine cells. This process involves the enzymatic activity of Trp hydroxylase 1 (TpH1)

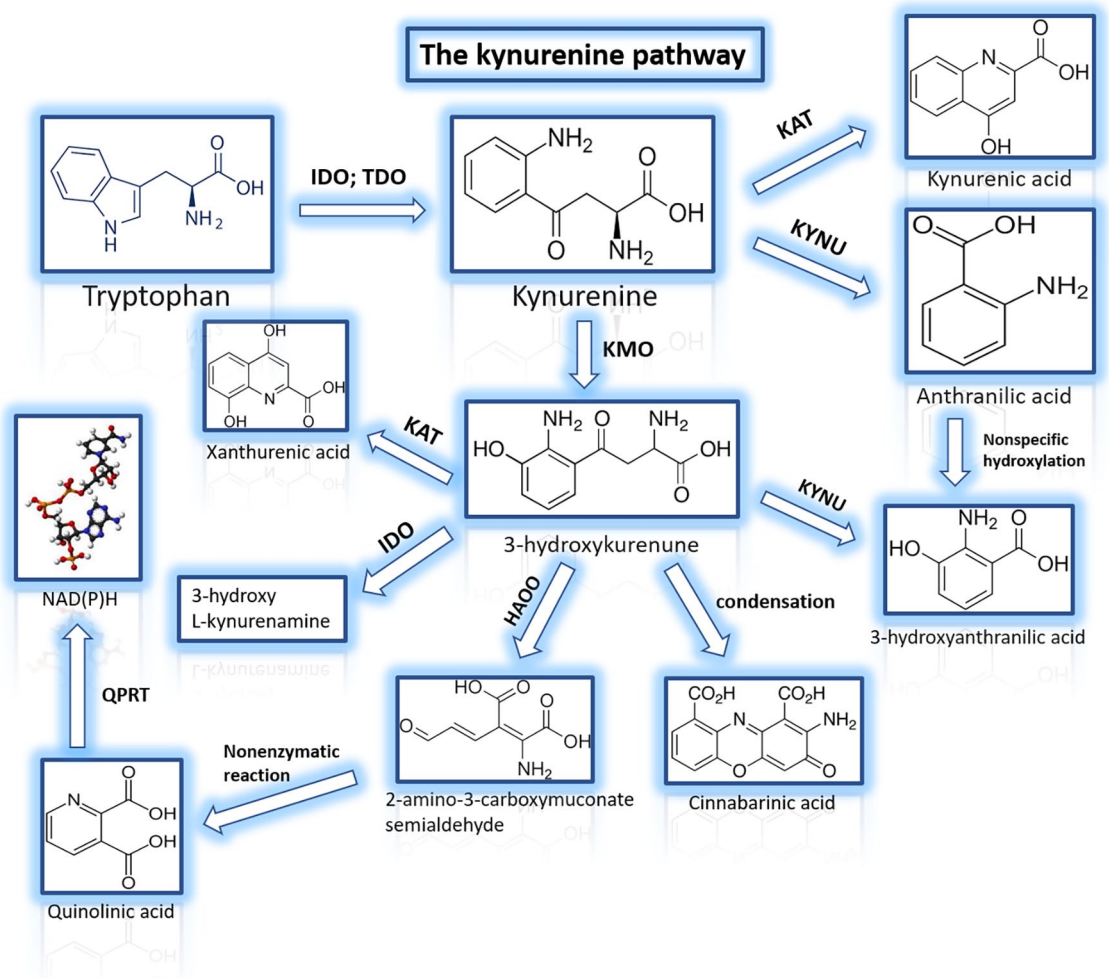

Figure 1. The kynurenine pathway.
Abbreviations: HAOO, 3-hydroxyanthranilate dioxygenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; KYNU, kynureninase; QPRT, quinolinate phosphoribosyltransferase; TDO, Trp 2,3-dioxygenase.

that hydroxylates tryptophan to 5-hydroxytryptophan (5-HTP), which is then decarboxylated into serotonin. Within the gastrointestinal tract, 5-HT was found relevant in numerous physiologic processes such as intestinal absorption, secretion, and motility.[36] In the CNS, 5-HT is synthesized in the serotonergic neurons of the raphe nuclei through the activity of Trp hydroxylase 2 (TpH2). Finally, 5-HT undergoes a 2-step metabolism process to produce 5-hydroxyindolacetic acid (5-HIAA) via monoamine oxidase as well as melatonin involving acetylation (via arylalkylamine N-acetyltransferase; AANAT) and methylation (via acetylserotonin O-methyltransferase; ASMT).[37,38] While 5-HIAA serves as a biomarker for certain neurological disorders (depression, schizophrenia) and as a credible measurement for the amount of serotonin in the brain, melatonin is associated with regulation of circadian rhythm and immune responses.[39,40] The biosynthesis of indole commences with hydroxylation of tryptophan via the enzyme tryptophanase (TnaA), which was found to be expressed by more than 85 species of Gram-positive and Gram-negative bacteria.[41] Only bacteria encoding the TnaA gene in their chromosome are able to produce indole, which is not the case with any known eukaryotic cell. The available evidence supports the role of indole in attenuating inflammation in the gut through the stimulation of IL-10 (anti-inflammatory) and inhibition of TNF-α and IL-8 (proinflammatory) mediators.[42] In addition, other tryptophan metabolites derived from the gastrointestinal microbiota include indole-3-aldehyde (*Lactobacillus* species) and 3-indolepropionic acid (*Clostridium sporogenes*), and these derivatives maintain microbial homeostasis and exhibit neuroprotective effects, respectively.[43,44] The synthesis of final indolamine metabolite, tryptamine, commences with decarboxylation of tryptophan via aromatic-L-amino acid decarboxylase (AADC). This monoamine alkaloid was found to play an important role as neuromodulator and antioxidant agent.[45,46]

NF-κB signaling and inflammation

The nuclear factor-kB (NF-κB) represents a family of inducible transcription factors relevant in different inflammatory and immune responses. The activation of these transcription factors

involves the canonical and noncanonical pathways.[47,48] In the canonical NK-κB pathway, ligand binds to different cytokine receptors, T-cell and B-cell receptors, TNF receptor, and pattern recognition receptors leading to the recruitment of multisubunit IkB kinase (IKK) complex and ubiquitin-dependent degradation of IkBα.[49,50] The central signaling molecule for the non-canonical pathway is NF-κB-inducing kinase (NIK), which mediates the phosphorylation and ubiquitination of the NF-κB2 precursor protein, p100.[51,52] Bacterial lipopolysaccharide (LPS) activates toll-like receptor 4 (TLR4), one of the cell surface TLR receptors, which stimulates downstream NF-κB signaling pathways upregulating the expression of inflammatory cytokines. Once activated, TLRs form homodimers and subsequently recruit an adapter called myeloid differentiation primary response 88 (MyD88).[53] The death domain of MyD88 recruits IL-1 receptor-associated kinase (IRAK), which then autophosphorilates and recruits TNF receptor-associated factor 6 (TRAF) with subsequent activation of downstream kinases, including NIK and mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1).[54,55] Activated NIK or MEKK1 can individually activate the IkB kinases, leading to phosphorylation and nuclear translocation of NF-κB and initiation of gene transcription. Put into perspective, a study evaluated the relationship between the NF-κB signaling and IDO-1 in an acute colitis experimental model.[56] In IDO-1 knock-out mice, a decreased number of inflammatory cells in peripheral blood and colon coincided with inhibited TLR signaling, suggesting that IDO-1 played an important role in producing inflammatory responses in this model of colitis.

Sex Steroid Hormones and the KP

A study conducted in 1951 by Sprince et al[57] for the first time demonstrated an impaired tryptophan metabolism in pregnant women through the excretion of excessive amounts of XA. Brown and the colleagues showed how administration of pyridoxine in pregnant women significantly reduced the urinary excretion of XA, but did not restore kynurenine, hydroxykynurenine, or pyridone to nonpregnancy levels.[58] Moreover, the impaired pattern of tryptophan metabolism suggested that endocrine factors aside from vitamin B6 deficiency may also play a role.[58] In vitamin B6-deficient rats, hormones of the anterior pituitary (follicle stimulating hormone, luteinizing hormone, and prolactin) and ovaries (estrogen and progesterone) were shown to maintain pregnancy in 25% and 80% to 100% animals, respectively, highlighting both pituitary and ovarian hormonal inadequacies in such animal models.[59] It was observed that women receiving Enovid-E (norethynodrel and mestranol) excreted significantly higher amounts of urine XA in comparison to controls following administration of tryptophan loading dose.[60] This finding was reversible with the administration of pyridoxine hydrochloride. Another significant observation was that estradiol and ethinylestradiol, albeit not mestranol, inhibited the vitamin B6-dependent kynurenine aminotransferase, regardless the increased concentrations of pyridoxal phosphate.[61] The observed reactions were a result of the competitive binding nature of pyridoxal phosphate–kynurenine complex and estradiol for the surface of this enzyme. It was documented that estrone sulfate contributed to compromised tryptophan metabolism, more through direct inhibitory effects on kynureninase than by inducing vitamin B6 deficiency.[62] A follow-up study compared a low-dose estrogen oral contraceptive pill with that of Enovid-E and found decreased level of excreted XA, suggesting the dose of estrogen and the duration of its administration determine the severity of disrupted tryptophan metabolism.[63] The anti-inflammatory properties of progesterone have been observed in LPS-induced embryonic resorption in mice.[64] This finding was reinforced when de Bie et al[65] explored the interactions between gonadal hormones and the KP. The authors administered increasing (100 nM, 10 μM, and 100 μM) concentrations of progesterone in IFN-γ-stimulated and unstimulated (control group) primary human macrophages. In the experimental group, the administration of 100 μM of progesterone was able to attenuate the KYN/TRP increase, suggesting that high levels of this hormone exhibit suppressing effects on IDO-1 enzyme. Another finding was that increased KYNA production did not appear to be progesterone dose dependent. Similarly, a positive trend between progesterone and KYNA was noticed in women taking combined oral contraceptive pills, likely in the setting of reduced estradiol concentrations.[66] The literature search has provided scattered information about the relationship between androgen hormones and the KP. Nevertheless, a half-century old study provided an insight that testosterone propionate administration in rats over a period of 14 days decreased the enzymatic activity of TDO.[67] It was postulated that endogenously produced androgens may act as estrogen antagonists with regards to the TDO enzyme activity. A more recent study showed that mice receiving supratherapeutic dose of subcutaneous nandrolene decanoate (androgen/anabolic steroid) for 28 days exhibited anhedonia and depressive-like behavior.[68] The authors were able to directly observe an increased KYN/TRP ratio in the striatum, hippocampus, and prefrontal cortex in the treatment group and suggested the upregulated activity of IDO enzyme as a crucial step in this process. Moreover, the administration of 1-methyl-tryptophan (1-MT), a competitive IDO inhibitor, reversed the observed metabolic changes.

Metabolic Dysfunction in PCOS and the KP

As mentioned earlier in the manuscript, PCOS has been associated with different metabolic comorbidities including diabetes mellitus, dyslipidemia, but also with obesity. A systematic review and meta-analysis directly analyzed the serum levels of proinflammatory markers in women with PCOS.[69] It was shown that an increase in C-reactive protein (CRP), but not IL-6 and TNF-α, was seen in women with this condition

when compared to controls. These results were unchanged when the studies with mismatches in obesity were excluded from the analysis, suggesting that CRP elevations in PCOS were independent of obesity. However, the available literature shows somewhat conflicting information as to the amount of adipose tissue in PCOS patients that correlates both conversely (IL-6 and CRP)[70,71] and inversely (TNF and adiponectin)[72,73] with different cytokines.[74] For example, IL-6 that regulates the hepatic synthesis of CRP, was found to be elevated in obese women with PCOS when compared to non-obese peers. On the other side, the growth of visceral adipose tissue was found to upregulate the production of TNF-α, which in turn stimulated the expression of IL-6 in adipocytes.[75] Ovaries from PCOS patients have an increased number of inflammatory cells (lymphocytes and macrophages) suggesting persistent pro-inflammatory state, whereas obese PCOS patients were found to have pronounced pro-inflammatory levels in granulosa cells.[76-78] Collectively, a high prevalence of obesity among patients with PCOS poses a significant contributing factor for inducing and maintaining a proinflammatory state. Until recently, there has been little information with regard to the relationship between plasma CRP and the KP. Millischer et al[79] tackled this issue by showing how experimental endotoxemia with intravenous administration of LPS induced the enzyme branches of the KP. Post injection (3-6 hours) the authors demonstrated an increased activity of KMO/KYNU and KAT leading to depletion of both tryptophan and kynurenine.[79,80] More relevant to the present review, the activity of IDO-1/TDO2 enzymes was also upregulated at 24 to 48 hours, which coincided with elevated KYN/TRP ratio and peak serum CRP levels. It is noteworthy that plasma CRP levels significantly correlate with other inflammatory markers such as IL-6 and TNF-α and therefore credibly reflect both peripheral and central inflammatory activity.[81] A large study provided evidence supporting higher prevalence of overweight and obesity in women with PCOS, independent of age and geographic region.[82] The roots of obesity lie in excessive caloric intake, still the serum levels of TRP have been shown to be low in obese individuals.[83] Indeed, obese juveniles and adults both had increased KYN/TRP ratio as a direct consequence of upregulated activity of the IDO, but not the TDO enzyme.[84] With the increased proliferation of the adipose tissue, the population of proinflammatory (M1) macrophages increases.[85] It was found that, in response to IFN-γ, obese individuals had upregulated expression of IDO-1, KYNU, KAT II (CCBL2), but not KMO, in primary adipocytes.[86] Further evidence suggested the KMO expression occurred in the residual adipocyte M1 macrophages. In vivo study with isolated rat pancreatic islets of Langerhans provided a first piece of evidence regarding the insulin-releasing activity of tryptophan metabolites.[87] Indeed, the addition of quinoline derivatives of kynurenine metabolites, in particular quinaldic acid (QA) and 8-hydroxyquinaldic acid, to incubation medium resulted in increased output of insulin. However, administration of rising concentrations of QA more potently inhibited proinsulin synthesis, without affecting the non-insulin proteins.[88] To this effect, QA (metabolite of KYNA) and 8-hydroxyquinaldic acid (metabolite of XA) promote insulin release and suppress the glucose-induced proinsulin synthesis. In women with PCOS, diminished insulin responsiveness was found to be secondary to limited availability or impaired mobilization of glucose transporter type 4 (GLUT4) in adipocytes,[89] and granulosa-lutein cells.[90] What is more, metabolic pathways such as tryptophan-kynurenine (TRP-KYN) and kynurenine-nicotinamide adenine dinucleotide (KYN-NAD^+) have been proposed as relevant in the pathogenesis of IR.[91] The idea behind this observation was that chronic inflammation and stress are both involved in TRP-KYN metabolism as well as in pathogenesis of IR and diabetes mellitus. The 2 stressors were also linked to deficiency of pyridoxal 5′-phosphate (P5P), an active form of vitamin B6, and cofactor for kynureninase, which caused the 3-HK metabolic shift from the production of NAD^+ to the formation of XA and KYNA.[91,92] However, a metabolomic study analyzing follicular fluid samples from PCOS women found increased levels of P5P as well as D-glutamic acid, which would suggest preserved NAD + production in this patient population.[93]

GUT Microbiome in PCOS and the KP

Any loss of commensal associations in microbial dysbiosis, defined as an imbalance in the microbial population, has the potential to induce a profound inflammatory state.[94] In vivo studies have shown that germ-free (GF) animals with no intestinal microbiome had a decreased kynurenine/tryptophan ratio, a finding reversible with the restoration of the physiologic gut flora.[95] The involvement of tryptophan metabolic pathways and IDO enzyme have been suggested in a number of gastrointestinal disorders including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).[96-98] From a microbial perspective, bacterial strain such as adhesive-invasive *Escherichia coli* (AIEC) LF82 significantly upregulated the IDO-1 enzyme in an in vitro IBD model of dysbiosis.[99] Women with PCOS exhibit reduction in both the gut microbiome species richness (α diversity) and have changes in the composition of the microbial community (β diversity).[100] The gut microbial composition was compared between lean women with PCOS and healthy lean women and suggested similar diversity between the 2 groups.[101] The study showed an inverse relationship between androgen levels and gut microbial diversity, implying that this altered gut microenvironment would be an important pathophysiological PCOS factor unrelated to obesity or insulin resistance. Yang et al conducted a study with an objective to determine whether intestinal flora dysbiosis may have an impact on insulin resistance and induce PCOS.[102] Compared with controls, women with PCOS had significantly higher insulin resistance suggesting

the importance of metabolic syndrome in PCOS. This study also showed that treatment-naïve PCOS patients had higher intestinal concentration of *Bacteroidetes*. However, with continuous antibiotic treatment cocktail, the reduction of *Bacteroidetes* also coincided with improved phenotype and insulin resistance. The gut microbiota was deemed capable of inducing changes in the KP by altering the degradation rate and availability of gut tryptophan. Studies with germ-free (GF) animals have shown that KP activity and KYN/TRP ratio were reduced secondary to scarcity of physiologic microbiota. The restoration of the normal microbiota was seen to revert the circulating tryptophan levels and the KYN/TRP ratio to baseline values.[95] Secondary to the reduced kynurenine pathway, the GF animals also have an immature immune system. Moreover, these animals were found to have a reduced expression of TLRs, the vital defense mechanism of the innate immune system.[103] Activation of TLRs has been associated with induced downstream tryptophan degradation through the KP via IFN-γ dependent or independent mechanism of IDO-1 induction.[22,95] A study by Qi et al[104] showed that transplantation of fecal microbiota from women with PCOS to healthy recipient mice induced development of typical features of PCOS including IR and hormone imbalances.

Lifestyle Modifications in PCOS and the KP

Recommendations from the international evidence-based guideline for the management of PCOS provided high-quality evidence for healthy lifestyle behaviors including healthy eating habits and regular physical activity.[105] A diet rich in saturated fat was shown to positively correlate with LPS levels and TLR4 gene expression in obese women, and these findings were even more pronounced in the presence of PCOS.[106] The same dietary habits produced increased activity of NF-κB alongside increased upregulated TNF-α mRNA levels and circulating plasma CRP, the findings independent of the presence of obesity.[107] Moreover, 2 hours following a saturated fat diet was associated with peak reactive oxygen species (ROS) generation from mononuclear and polymorphonuclear cells, with significantly greater responses in women with concomitant obesity and PCOS versus obesity only.[108] A systematic review and meta-analysis further evaluated different exercise activities and dietary co-interventions on metabolic, cardiorespiratory, and reproductive outcomes in women with PCOS.[109] The analysis demonstrated that exercise training improved cardiometabolic outcomes in this population. A recent study by Cussotto et al[110] aimed to investigate alterations in different routes of TRP metabolism and their relationship with systemic inflammation in obese patients versus control group. Obese subjects had lower circulating plasma levels of TRP and increased KYN/TRP ratio, suggestive of IDO activation. The markers of systemic inflammation, high-sensitive CRP (hsCRP) and high-sensitive IL-6 (hsIL-6) were both increased in obese subjects and correlated positively with KYN/TRP ratio in the whole population, consistent with inflammatory-driven IDO activation. A study by Halama et al[111] further investigated whether moderate exercise produced any effect on the metabolic homeostasis in women with or without PCOS with respect to IR. The amino acid profile prior to exercise differed between the PCOS and control group; however, there were no differences in tryptophan levels between the groups at baseline. Furthermore, the authors of the same study administered lipid infusion to stimulate acute IR, which showed alterations in the levels of plasma acetylcarnitines, lysophospholipides, and tryptophan. The state of induced IR correlated with a significant decline in tryptophan levels in both PCOS and control groups. These observations highlight the crosstalk between lipid metabolism and tryptophan levels, and validate the findings of previously reported study.[112] Collectively, there is evidence that tryptophan stimulates functional pancreatic β cells to secrete insulin, which in turn activates hepatic fatty acid synthetase and adipose tissue lipoprotein lipase.[112-115]

Discussion

The role of tryptophan in neurodegenerative diseases, mood disorders, and chronic pain conditions has been discussed in the past; however, to the best of our knowledge, this is the first review to explore the link between the known PCOS variables including sex steroid hormones, chronic inflammation, gut microbiome, and lifestyle modifications with the downstream TRP metabolites. The available literature indicates that non-pregnant women taking combined OCP (norethynodrel and mestranol) had enhanced urinary excretion of XA, reversible with pyridoxine (25 mg 4 times daily for 2 days). From a hormonal perspective, the pooled data shows that estrone sulfate (natural estrogen, E_1S) inhibits kynureninase more than it induces vitamin B6 deficiency, while beta-estradiol (natural estrogen) and ethinylestradiol (synthetic estrogen) inhibit kynurenine aminotransferase in a competitive and non-competitive fashion, respectively. The search for an endocrine marker specific for PCOS identified elevated levels of E_1 and E_2 (≈100% higher) and E_1S (≈182% higher, $P = .003$).[116] E_1S represents an abundant source of circulating estrogen that can metabolize into E_1 by sulfatase, whereas E_1 serves as a substrate for the production of E_1S by sulfotransferase.[117,118] Moreover, high levels of progesterone (100 μM) were found to exhibit anti-inflammatory properties through IDO-1 inhibition and this was also documented in women taking OCPs, likely in the setting of reduced estradiol. It was previously documented that progesterone deficiency was strongly associated with LH abnormalities and androgen levels, and has been hypothesized as the primary hormonal abnormality in PCOS.[119] Finally, the use of supratherapeutic (10 mg/kg/day for 28 days) dose of nandrolone decanoate, an androgen/anabolic steroid with a significant androgenic and progestogenic activity, was shown to upregulate the IDO-1 enzyme. It is to be noted that nandrolene has similar chemical appearance as testosterone, but it

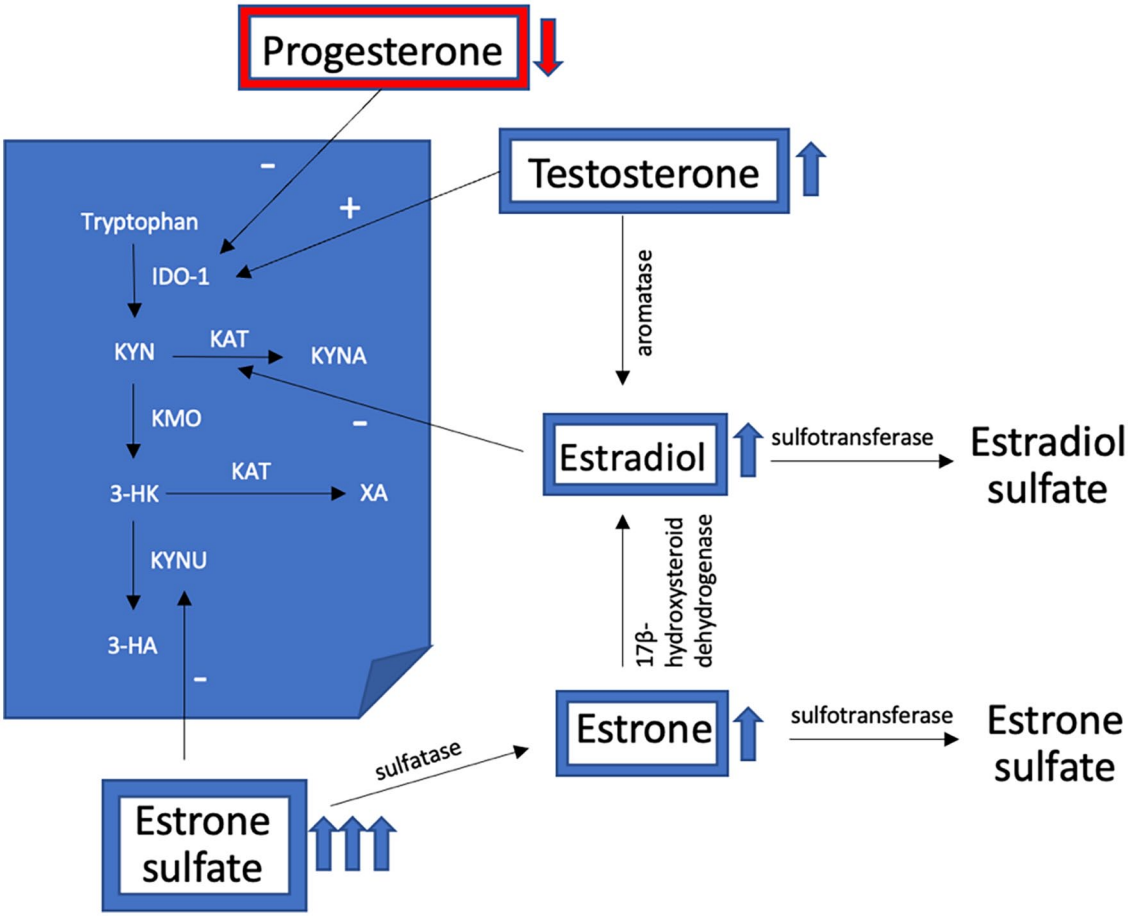

Figure 2. Schematic description of sex steroid hormones and their relationship with the KP enzymes relevant to the present review.
Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; IDO-1, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; KYN, kynurenine; KYNA, kynurenic acid; KYNU, kynureninase; XA, xanthurenic acid.

binds to androgen receptors with greater affinity. The interplay between different sex steroid hormones in PCOS and their effects on different enzymes of the KP is shown in Figure 2.

A growing body of literature suggests strong evidence rendering PCOS in close proximity with chronic inflammation. Low-grade inflammatory conditions such as obesity and impaired glucose tolerance are common metabolic disturbances in women with PCOS. The excess body fat (ie, obesity) maintains an optimal environment for the activation of immune cells (eg, macrophages) and the production of proinflammatory cytokines including IL-6, IL-1β, and TNF-α that also give rise to IR in adipocytes. Continued consumption of excess dietary nutrients induces the already hypertrophic adipocytes to increase the expression of proinflammatory pathways and a distinct chemokine, monocyte chemoattractant protein-1 (MCP-1).[89] There is a shifting paradigm that renders immune cells as pivotal mediators in adipose tissue inflammation. Such notion is supported by the upregulated transcription levels of pro-inflammatory genes seen with increased adipocyte mass.[120] Interestingly, patients undergoing bariatric surgery followed by a substantial weight loss were able to retain an elevated number of adipose macrophages, $CD4^+$, $CD8^+$, and dendritic cells.[121] This evidence illustrates that inflammation of the adipose tissue and immune cell accumulation are not specific to obesity. Banaszewska et al[122] observed that markers of endotoxemia (LPS, LPS/high-density lipoprotein ratio, and LPS binding protein [LBP]) were significantly elevated in women with PCOS with no significant metabolic abnormalities (eg, obesity). Additionally, all measures of endotoxemia were in positive correlation with hs-CRP and ovarian volume. The study discussed earlier with experimental endotoxemia from intravenous LPS administration prompts the consideration of a link between the LPS-induced enzymes of the KP, serum CRP levels, and PCOS. It is therefore plausible to consider that CRP levels in PCOS patients may reflect the IDO-1/TDO2 enzymatic activity, although more research is warranted. Similarly, the previously discussed KYN/TRP ratio reflects not only the enzymatic activity of IDO, but also that of TDO, KMO, KUNY, and KAT. This notion would imply that in the absence of direct proof of IDO activity, caution should be exercised to determine the contributions of other enzymes with respect to the KYN/TRP ratio.[15] A good example is a study with cirrhotic patients with acute-on-chronic liver failure with or without kidney failure.[123] Contrary to expectations, in those

patients with active infection there was no increase in IDO activity or KYN/TRP ratio. The explanation behind this observation was the inhibited TDO enzyme in cirrhotic patients, which would counteract an IDO-induced Trp depletion and explain the normal KYN/TRP ratio. Likewise, patients undergoing hemodialysis did not have an elevated KYN/TRP ratio, although this finding was independent of upregulated IDO activity.[124] The most likely explanation was the enhanced activity of hepatic TDO, which has been demonstrated in renal failure.

There is no definite proof of the origin of IR in patients with PCOS, although data suggests that excessive serine phosphorylation of the insulin receptor and descending signaling proteins could be the primary reason.[125] IR and/or hyperinsulinemia are common endocrine imbalances found in women with PCOS notwithstanding the presence of obesity. IR essentially pertains to the impaired glucose uptake and tissue utilization from endogenous or exogenously administered insulin. A search for the endogenous, universal metabolite to screen for the presence of HI in women with PCOS led to reduced urine levels of pyruvate and 3-phenylpropionate.[126] However, a recent study by Hou et al[10] analyzing follicular fluid with gas chromatography-mass spectrometry in combination with correlation network suggested that low pyruvate levels were unrelated to HI in women with this condition. The same study further provided evidence of significantly increased levels of L-tyrosine and L-tryptophan as compensatory energy sources secondary to insufficient TCA cycle. Moreover, phenylalanine, Tyr, and Trp were also in positive correlation with the serum levels of testosterone and androstenedione, suggesting a positive association between these amino acids and hyperandrogenism, the hallmark feature of PCOS. Owing to its heterogenous phenotypic and pathophysiological nature, the discovery of a single biomarker to predict PCOS to date has not been established.

Whipps et al[127] in 1988 first proposed and defined the term *microbiome* as "characteristic microbial community occupying a reasonably well defined habitat, which has distinct physiochemical properties." A more contemporary literature delineates the terms *microbiota* as an assembly of microorganisms in a defined environment, and *microbiome* as a collection of genes and genomes of microbiota members.[128] The compositional dysbiosis of gastrointestinal microbiota specific to patients with PCOS was found to correlate with that seen in obese individuals. Obese women with PCOS generally harbored increased number of gram-negative bacteria of the genera Escherichia/Shigella and Bacteroides.[129] LPS produced by the gram-negative bacteria has been implicated in IR, obesity, and chronic inflammation.[130] A diet rich in sugar and fat, yet poor in fiber, has been linked to gut microbiota imbalance, decreased expression of zone occludens (ZO)-1 and subsequent destruction of intestinal epithelial cells.[131] This, in turn, increases gut permeability ("leaky gut") and facilitates the introduction of LPS into the systemic circulation[132]; LPS binds to TLR4 on immune cells and through LBP, CD14 and MD-2 activates downstream signaling pathways to promote the expression of IL-6 and TNF-α, which in turn leads to IR.[133-135] The TLR4, CD14 mRNA, and MD-2 were also found expressed on granulosa cells, and LPS treatment of these cells caused suppressed estradiol secretion.[136] Overall, poor nutrition in women with PCOS leads to a state of endotoxemia that was discussed earlier in the text.

An interplay between immune and cancer cells illustrates the inhibitory effects of effector (CD8+) T cells on cancer development, while the reverse is true for T regulatory cells (Tregs). The buildup of KYN from increased IDO-1 activity has been found to inhibit natural killer (NK) cells/CD8+ T cells and stimulate the activation of regulatory T cells (Treg cells).[137] A growing number of clinical trials are being conducted with the purpose of investigating the treatment efficacy and safety profile of INCB024360 (Epacadostat; an IDO-1 inhibitor) in different cancer types. Therefore, the ongoing research is especially valuable in malignancies of the colon, ovaries, lungs, and melanoma, which are known to overexpress and utilize IDO-1 to promote local tolerance to the cancer.[138] One such study (NCT01961115) with 11 participants was performed with a primary objective to determine the extent to which a regimen of INCB024360 and MELITAC 12.1 multipeptide vaccine alters the tumor-infiltrating CD8+ lymphocytes in melanoma through serial biopsies with immunohistochemistry. Serum Kyn/Trp ratio was used as a marker of biological effect of INCB024360 on IDO-1 function. The results showed that in only 2 patients the combination therapy was considered safe with transient dose-limiting toxicities, including grade 3 syncope and grade 3 transaminase elevation, both of which resolved. Another phase 2, randomized, double-blind study (NCT03322540) with 154 participants investigated the efficacy and safety of pembrolizumab (MK-3475) with INCB024360 versus pembrolizumab with placebo in patients with metastatic non-small cell lung cancer (mNSCLC) with high expression levels of programed cell death ligand (PD-L1). The results showed a tumor objective response rate (ORR; the tumor burden following a treatment) of 32.5% versus 39.0% in combination group versus pembrolizumab alone, respectively. There were similar rates of adverse events reported in all groups. A more recent, randomized phase I/IIb trial (NCT02166905) investigated the efficacy of DEC-205/NY-ESO-1 fusion protein CDX-1401 with adjuvant poly ICLC (polyinosinic-polycytidylic acid) given as a vaccine in combination with INCB024360 in treating patients with primary peritoneal, fallopian tube, or ovarian cancer who no longer have evidence of disease. The idea in this study was to provide stronger and more long-lasting anti-cancer immune responses in patients with primary peritoneal, fallopian tube, or ovarian cancer through administration of DEC-205/NY-ESO-1 fusion protein CDX-1401 with poly ICLC and

IDO-1 inhibitor INCB024360. At the time of writing of this manuscript, the results of this study had not yet been published. Even though clinical trials with Epacadostat are either actively recruiting participants or pending results, still the paucity of these trials with IDO1 inhibitors in ovarian pathology prompts more research in order to provide definite recommendations about the efficacy and safety of this drug in PCOS.

Conclusion

Ongoing research related to the pathophysiological aspects of PCOS continues to provide novel information about this complex condition. The KP has been recognized as a useful intermediate facilitating our understanding of numerous diseases and in this review, we attempted to further elaborate on the relationship between the KP and PCOS. We have summarized the influence of sex steroid hormones on different enzymes of the KP; inflammatory nature of PCOS and CRP as a marker of IDO/TDO activity; and how altered gut flora in women with PCOS introduces LPS into the bloodstream and produces endotoxemia with subsequent KP enzyme activation. With a plethora of emerging and evolving technologies, we are optimistic that new advancements will allow for a more comprehensive analyses of metabolites that would deepen our understanding of different pathogenic mechanism interactions, especially those of KP in PCOS.

Author Contributions

FJ—conceptualization, design, drafting, revising the article, and designing the figure. AS and NNK revised the manuscript. All authors read the final version and approved it.

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