Frontiers in Bioscience Landmark

*Front. Biosci. (Landmark Ed)* **2024**; 29(1): 24
https://doi.org/10.31083/j.fbl2901024

*Review*

# Polycystic Ovary Syndrome Pathophysiology: Integrating Systemic, CNS and Circadian Processes

George Anderson[1,*]

[1]CRC Scotland & London, SW1V 1PG London, UK
*Correspondence: anderson.george@rocketmail.com (George Anderson)

Academic Editor: Xudong Huang
Submitted: 4 September 2023 Revised: 28 November 2023 Accepted: 11 December 2023 Published: 18 January 2024

**Abstract**

The conceptualization of polycystic ovary syndrome (PCOS) has primarily focused on hormonal alterations driven by changes within the hypothalamus and ovarian granulosa cells, with treatment by the contraceptive pill and weight loss. However, a growing body of data implicates wider systemic and central nervous system (CNS) changes in the pathoetiology and pathophysiology of PCOS, with consequent implications for targeted treatments. It is proposed that there is a significant role for night-time interactions of factors acting to regulate whether the rising level of cortisol over the night and during the morning cortisol awakening response (CAR) is able to induce the nuclear translocation of the glucocorticoid receptor (GR), thereby influencing how the immune and glial systems regulate cellular function in preparation for the coming day. Factors affording protection in PCOS also inhibit GR nuclear translocation including gut microbiome-derived butyrate, and pineal/local melatonin as well as melatonin regulated bcl2-associated athanogene (BAG)-1. A significant pathophysiological role in PCOS is attributed to the aryl hydrocarbon receptor (AhR), which shows heightened levels and activity in PCOS. The AhR is activated by ligands of many systemic processes, including white adipocyte-derived kynurenine, implicating obesity in the pathophysiological changes occurring in the hypothalamus and ovaries. AhR activation has consequences for the physiological function in the hypothalamic paraventricular nucleus, granulosa cells and adipocytes, partly mediated by AhR upregulation of the mitochondrial N-acetylserotonin/melatonin ratio, thereby decreasing melatonin availability whilst increasing local stress plasticity in the paraventricular nucleus. This article reviews in detail the wider systemic and CNS changes in PCOS highlighting interactions of local and pineal melatonergic pathway, gut microbiome-derived butyrate, white adipocyte-derived kynurenine, the hypothalamic paraventricular nucleus tanycytes/astrocytes, and the hypothalamus-pituitary-adrenal (HPA) axis driven glucocorticoid receptor activation in PCOS pathophysiology. This integrates a wide array of previously disparate data on the biological underpinnings of PCOS, including how PCOS associates with many other currently classified medical conditions, such as depression, bipolar disorder, type 1 diabetes mellitus and the autism spectrum. Numerous future research and treatment implications are detailed.

**Keywords:** polycystic ovary syndrome; depression; gut microbiome; melatonin; N-acetylserotonin; aryl hydrocarbon receptor; kynurenine; tanycytes; paraventricular nucleus; adipocytes; treatment; tyrosine kinase receptor B

## 1. Introduction

Polycystic ovary syndrome (PCOS) affects between 5%–15% of women, approximately 80% of whom are obese and 20% lean. PCOS symptomatology is currently defined by hormonal imbalance, predominantly hyperandrogenism leading to ovarian cysts, irregular menstrual periods, hirsutism, and reproductive difficulties driven by pathophysiological changes in the ovaries and hypothalamus [1]. Treatment is hormonal birth control medication and type 2 diabetes mellitus (T2DM) medications/lifestyle modifications. There is a growing resentment that such classification and treatment is reflective of implicit sexism in medicine [2], which is proposed to restrict the investigation of more fundamental, gender-neutral processes underlying hormonal dysregulation, thereby limiting the discovery of more gender-neutral treatment targets. The implication of 'implicit sexism' seems derived from the frustration arising from the complexity of processes underpinning PCOS pathophysiology, which involves changes in, and interactions among, the brain, stress system, adipocytes, circadian system and ovary. This article provides one possible framework for integrating such diverse data.

Numerous systemic processes show alterations in PCOS, many of which may be intimately linked to alterations in the circadian rhythm and how the circadian rhythm regulates stress responses across adipocytes, the hypothalamus, gut and ovaries. Circadian rhythm dysregulation and suppressed pineal and local melatonin levels are significant aspects of PCOS pathophysiology [3,4], with melatonin (2 mg for 6 months) significantly decreasing PCOS symptoms [5]. Heightened stress and hypothalamic-pituitary-adrenal (HPA) axis activation are evident in PCOS [6], with effects mediated by cortisol activation of the glucocorticoid receptor (GR), leading to the GR being translocated to the nucleus, where it can induce thousands of genes in almost all body and CNS cells. Gut microbiome derived butyrate, melatonin and melatonin regulated bcl-2 associated athanogene (BAG)-1 prevent GR nuclear translocation, whilst

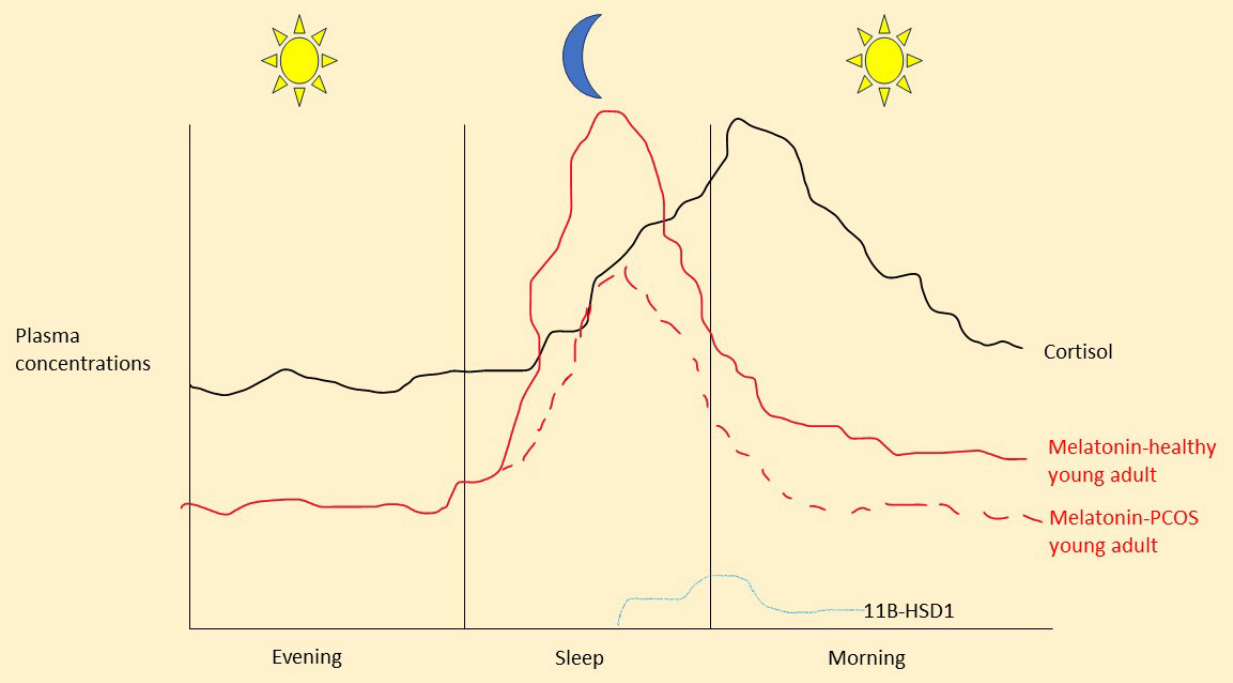


**Fig. 1. Circadian variations in cortisol and melatonin.** Shows the rising levels of cortisol at night and variations in pineal melatonin in healthy young adults compared to young adults with medical conditions, such as type 2 diabetes and PCOS. As melatonin suppresses the nuclear translocation of the activated glucocorticoid receptor, the suppression of pineal melatonin in PCOS and related conditions will increase glucocorticoid receptor effects at night and during the accelerated rise of cortisol upon awakening (cortisol awakening response). Heightened stress and glucocorticoid receptor activation and nuclear translocation increases local cortisol production via the induction of 11$\beta$-hydroxysteroid dehydrogenase type 1 (11$\beta$-HSD1), leading to local cortisol production and a heightened 'local stress' response. As a decrease in pineal melatonin and heightened stress are linked to increased gut dysbiosis, thereby decreasing butyrate and butyrate's inhibition of glucocorticoid receptor activation, suppressed butyrate production will also increase glucocorticoid receptor effects across body cells (not shown for clarity).

BAG-1 can also chaperone the GR to mitochondria [7–9]. Melatonin maintains the GR in a cytoplasmic complex with heat shock protein (hsp)90 [10], whilst gut microbiome-derived butyrate also inhibits GR nuclear translocation via the acetylation of the GR and hsp90, thereby implicating the gut microbiome in stress-linked PCOS pathophysiology [11,12]. The GR modulates key PCOS sites, namely granulosa cell/oocyte interactions [13,14] and hypothalamic function [15], implicating variations in circadian melatonin, gut butyrate, and BAG-1 in modulating GR-linked PCOS pathophysiology. The changes in cortisol and melatonin production over a typical circadian rhythm [9] are shown in Fig. 1. The consequences of decreased pineal melatonin in PCOS at night and early morning on glucocorticoid receptor nuclear translocation and therefore on glucocorticoid receptor effects can be clearly seen. Glucocorticoid receptor nuclear translocation will also be enhanced by a decrease in gut microbiome butyrate (not shown in Fig. 1 for clarity).

Importantly, pineal melatonin is directly released into the cerebrospinal fluid (CSF) of the third ventricle, where it is maintained at a prolonged heightened level, versus circulatory melatonin levels [16]. The third ventricle is lined by tanycytes, which interact with hypothalamic astrocytes to regulate hypothalamic neuronal function and fluxes, thereby allowing pineal melatonin to modulate hypothalamic function [1]. Pineal melatonin is dramatically decreased over aging [17], as well as in PCOS-associated conditions [18], implicating suppressed pineal melatonin in hypothalamic dysregulation in PCOS. Pineal melatonin also has indirect effects on the hypothalamus and ovary (oocytes, granulosa cells) via the gut microbiome [19], with gut dysbiosis/permeability associated with PCOS, thereby increasing circulating lipopolysaccharide (LPS) [20]. LPS activates toll-like receptor (TLR)4 on pineal microglia, thereby increasing tumor necrosis factor (TNF)-$\alpha$ to suppress pineal melatonin [21]. Gut dysbiosis/permeability is therefore intimately linked to circadian processes regulating hypothalamic and ovarian function. This includes melatonin's upregulation of aromatase, and therefore the conversion of testosterone to estrogen [22]. Such data highlights how systemic and circadian interactions may be aspects of 'core' processes that underpin hormonal changes and form a novel conceptualization of the biological underpinnings of PCOS.

Melatonin is produced in all mitochondria-containing cells, allowing the tryptophan-melatonin pathway to be an important determinant of cell function. Importantly, pineal and local melatonin can be 'backward' converted to N-acetylserotonin (NAS) by aryl hydrocarbon receptor (AhR) activation. This has significant consequences, as NAS and melatonin have differential consequences for cell survival, proliferation and stress-linked plasticity [9]. Notably, raised AhR levels, activation and susceptibility alleles are evident in PCOS, driving an increase in NAS linked glucocorticoid receptor (GR)/stress plasticity in the hypothalamus [23–25] and ovarian oocyte-granulosa cell interactions [26,27]. The AhR may therefore be intimately linked to the heightened local stress/GR activation, including from locally induced 11$\beta$-hydroxysteroid dehydrogenase type 1 (11$\beta$-HSD1) in the hypothalamus and ovary (see Fig. 1). As many of the beneficial effects of gut microbiome derived butyrate are mediated via melatonin upregulation [9], the AhR induced increase in the NAS/melatonin ratio will also change the consequences of butyrate effects. Altered regulation of the melatonergic pathway and gut butyrate are also intimately linked to wider PCOS symptomatology, including obesity and type 2 diabetes, which are associated with a decrease in brown adipocytes (BATs) and increase in white adipocytes (WATs) [28]. Such data highlights some of the systemic and circadian changes that underpin the complexity of PCOS pathophysiology, which are integrated in this article to provide a novel conceptualization of PCOS.

This article reviews wide bodies of data on PCOS, proposing that PCOS is significantly determined by the effects of the NAS/melatonin ratio, BAG-1, butyrate and white adipocyte (WAT)-derived kynurenine in the activation of the AhR, which shape whether the cortisol rise at night and during the course of the morning cortisol awakening response (CAR) results in GR nuclear translocation across body and CNS cells. This leads to the proposition that the wider changes in PCOS are not comorbidities of a hormonal conceptualization of PCOS, but rather underpin the hormonal changes in PCOS. As the tryptophan-melatonin pathway is an important aspect of physiological changes in PCOS, it is briefly reviewed first.

## 2. Tryptophan-Melatonin Pathway

The tryptophan-melatonin pathway seems evident in all human cells [9]. Mitochondria are the major sites of melatonin production, with relevance to a wide array of diverse medical conditions [16,29,30]. Tryptophan is an essential amino acid that is primarily diet-derived but may also be provided by the gut microbiome's shikimate pathway, which is significantly regulated by *Akkermansia muciniphila* [31]. Tryptophan availability can also be limited by pro-inflammatory cytokine and glucocorticoid receptor (GR) induced indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), respectively, which convert tryptophan to kynurenine, thereby increasing kynurenine activation of the aryl hydrocarbon receptor (AhR), as well as neuroregulatory kynurenine pathway products, such as the excitotoxic quinolinic acid and excitatory picolinic acid [32]. AhR induced cytochrome P450 (CYP)1A2 and CYP1B1 O-demethylate melatonin to N-acetylserotonin (NAS) [33,34]. As NAS is a brain-derived neurotrophic factor (BDNF) mimic via the activation of the BDNF receptor, tyrosine receptor kinase (Trk)B [35], NAS has distinct effects to melatonin, giving physiological relevance to variations in the NAS/melatonin ratio. Within the hypothalamus, the AhR induced increase in the NAS/melatonin ratio contributes to the hypothalamic TrkB activation that drives stress-linked hypothalamic plasticity [36,37]. TrkB activation in the paraventricular nucleus (PVN) enhances corticotrophin-releasing hormone (CRH) release and therefore increases hypothalamic-pituitary-adrenal (HPA) axis activation via cortisol effects at the glucocorticoid receptor (GR) [38]. As well as initiating the HPA axis, CRH also has independent effects that are relevant to PCOS pathophysiology, including increasing gut permeability [39]. By decreasing melatonin availability and increasing NAS, the AhR can have dramatic consequences for hypothalamic function and fluxes as well as glucocorticoid receptor (GR) nuclear translocation and transcriptional effects. The suppression of melatonin and gut microbiome derived butyrate increases GR nuclear translocation, thereby enhancing stress linked HPA axis activation, and GR effects at night and in the course of the morning cortisol awakening response (CAR). This has significant systemic consequences driven by variations in how GR activation drives changes in the transcription of thousands of genes across all body and CNS cells.

The tryptophan-melatonin pathway is initiated by the large amino acid transporters, which take up circulating tryptophan. Cellular tryptophan is rapidly converted to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH), with 5-HTP quickly converted by aromatic-L-amino acid decarboxylase (AADC) to serotonin (5-HT). TPH1 (body) and TPH2 (brain) require stabilization by 14-3-3 [40]. Platelets and serotonergic neurons are also sources of serotonin for the mitochondrial melatonergic pathway. Serotonin is converted to N-acetylserotonin (NAS) by 14-3-3 stabilized aralkylamine N-acetyltransferase (AANAT), in the presence of acetyl-coenzyme A (acetyl-CoA) as a necessary cosubstrate. As acetyl-CoA availability is strongly dependent on the mitochondrial pyruvate dehydrogenase complex (PDC) conversion of pyruvate to acetyl-CoA, the melatonergic pathway is intimately linked to mitochondrial function and metabolism. NAS is converted to melatonin by acetylserotonin methyltransferase (ASMT). See Fig. 2.

As evident in Fig. 2, the tryptophan-melatonin pathway can be regulated by the numerous cellular processes and factors that modulate tryptophan availability, tryptophan uptake, 14-3-3 isoforms, tryptophan hydroxylase (TPH), AADC, ASMT, and acetyl-coenzyme A (acetyl-

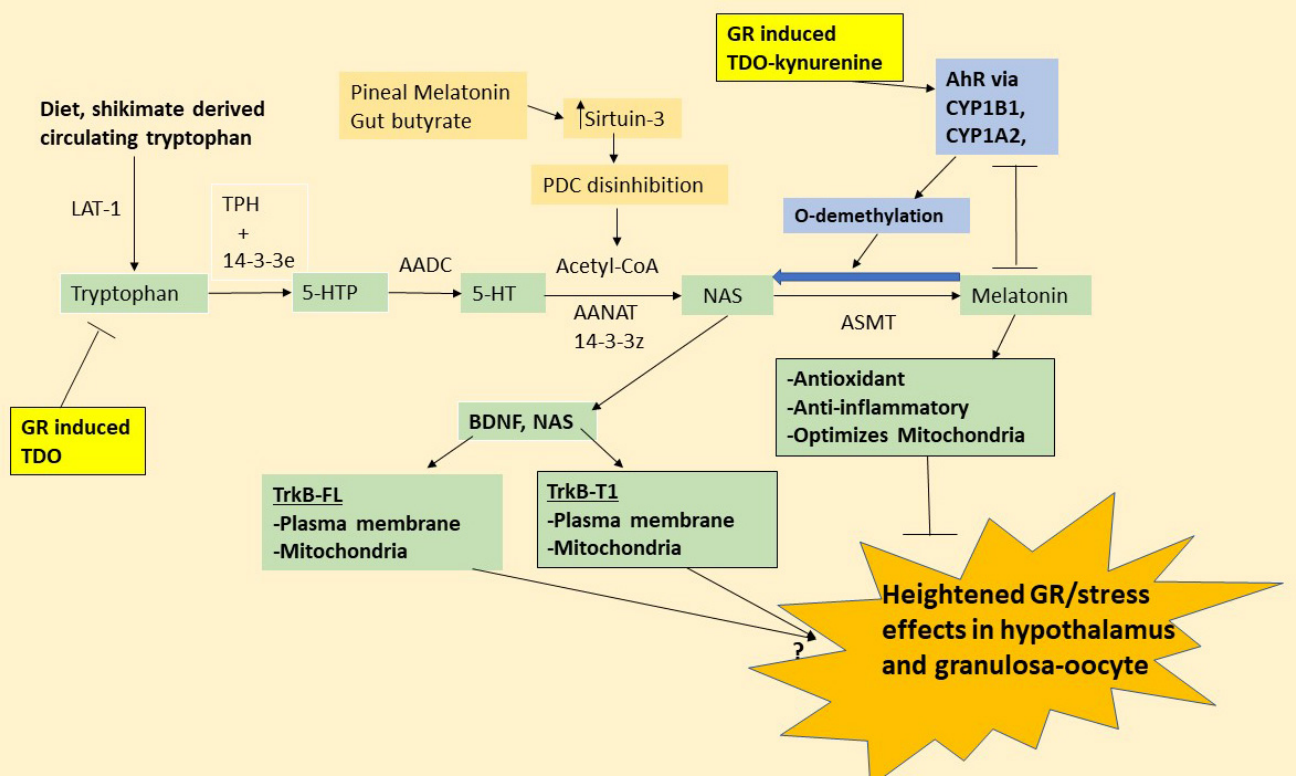


**Fig. 2. Tryptophan-melatonin pathway regulation.** Shows how the tryptophan-melatonin pathway (green shade) interacts with the aryl hydrocarbon receptor (AhR)-driven O-demethylation of melatonin to NAS (blue shade) to determine the NAS/melatonin ratio and the differential consequences of NAS and melatonin in the hypothalamus and on oocyte-granulosa cell functional interactions. The AhR and melatonin generally have reciprocal negative interactions. Tryptophan is primarily derived from the diet, but also from the gut microbiome shikimate pathway, being another route whereby variations in the gut microbiome can regulate systemic processes and mitochondrial function. Circulating tryptophan is taken up by the large amino acid transporter (LAT)-1. 14-3-3, including the 14-3-3$\varepsilon$ isoform, is required to stabilize tryptophan hydroxylase (TPH)1- and TPH2-, thereby allowing the conversion of tryptophan to 5-HTP. AADC converts 5-HTP to serotonin (5-HT), which is metabolized by 14-3-3$\zeta$ stabilized AANAT, in the presence of acetyl-CoA, to N-acetylserotonin (NAS). ASMT then converts NAS to melatonin. Acetyl-CoA levels are significantly linked to optimized mitochondrial function via pyruvate dehydrogenase complex (PDC) disinhibition, including from pineal melatonin and gut microbiome-derived butyrate that increase sirtuin-3, which deacetylates and disinhibits the PDC. Disinhibited PDC enhances the conversion of pyruvate to acetyl-CoA. The AhR, via CYP1B1 and CYP1A2, 'backward' converts melatonin to NAS via O-demethylation. The AhR induction of CYP1B1/CYP1A2/CYP1A1 can also hydroxylate melatonin to 6-hydroxymelatonin, further suppressing melatonin levels and enhancing the NAS/melatonin ratio. NAS and melatonin have some common effects but also important differential effects. NAS activates the BDNF receptor, TrkB, as well as inducing BDNF, thereby enhancing TrkB activation. The consequences of TrkB activation are variable depending whether full-length (TrkB-FL) and/or truncated (TrkB-T1) isoforms are present, as well as whether TrkB-FL and TrkB-T1 are present on the mitochondrial and/or plasma membranes. Melatonin is generally beneficial in PCOS, with benefits in both the hypothalamus and ovary, including via the suppression on glucocorticoid receptor (GR) effects, whilst TrkB activation in the hypothalamus can drive stress-linked plasticity, including enhancing corticotrophin-releasing hormone (CRH) to potentiate the hypothalamic-pituitary-adrenal (HPA) axis and cortisol activation of the glucocorticoid receptor (GR). GR-induced TDO suppresses tryptophan availability, whilst GR-TDO-derived kynurenine activates the AhR (yellow shading). The association of raised AhR levels and alleles with PCOS may be mediated via such AhR effects on the tryptophan-melatonin pathway that suppresses melatonin and increases NAS linked stress plasticity. Abbreviations: 5-HT, serotonin; 5-HTTP, 5-hydroxytryptophan; AADC, aromatic-L-amino acid decarboxylase; AANAT, acetyl-CoA, acetyl-coenzyme A; aralkylamine N-acetyltransferase; AhR, aryl hydrocarbon receptor; ASMT, N-acetylserotonin O-methyltransferase; BAG-1, bcl2-associated athanogene 1; BDNF, brain-derived neurotrophic factor; CRH, corticotrophin releasing hormone; GR, glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; LAT-1, large amino acid transporter 1; NAS, N-acetylserotonin; PCOS, polycystic ovary syndrome; TrkB-FL, tyrosine receptor kinase B-full length; TrkB-T1, tyrosine receptor kinase B-truncated.

CoA). Pineal melatonin and gut butyrate induce sirtuin-3, which deacetylates and disinhibits the pyruvate dehydrogenase complex (PDC) [41,42], thereby increasing the conversion of pyruvate to acetyl-CoA. This provides acetyl-CoA for the mitochondrial melatonergic pathway as well as for enhanced ATP production by the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). This optimization of mitochondrial energy production is accompanied by suppressed mitochondrial oxidant production, and therefore reactive oxygen species (ROS)-driven microRNAs [43]. The regulation of mitochondrial metabolism and the mitochondrial melatonergic pathway induction are therefore intimately linked to patterned gene expression and cellular fluxes. Consequently, the mitochondrial melatonergic pathway in a given cell is intimately intertwined with cellular mitochondrial function and intercellular fluxes, as well as with wider systemic and circadian processes.

Importantly, N-acetylserotonin (NAS) effects as a BDNF mimic are complicated by the presence of TrkB-full length (TrkB-FL) and TrkB-truncated (mostly TrkB-T1) receptors, with both TrkB-FL and TrkB-T1 being present in some cells on the plasma membrane and/or mitochondrial membrane [32]. NAS may also be metabolized to N-(2-(5-hydroxy-1H-indol-3-yl) ethyl)-2-oxopiperidine-3-carboxamide (HIOC) [44], which may not activate TrkB, allowing the metabolism of NAS to HIOC to have significant consequences for proliferative conditions [45,46], including PCOS linked stress via TrkB activation-driven hypothalamic stress plasticity. The conversion of melatonin to NAS will also attenuate melatonin's induction of other genes, including the immune-regulatory effect of the alpha 7 nicotinic acetylcholine receptor [47]. The aryl hydrocarbon receptor (AhR)-driven O-demethylation of melatonin to NAS, metabolism of NAS to HIOC and the TrkB isoforms sites of expression allows the tryptophan-melatonin pathway to have considerable plasticity in the regulation of intracellular and intercellular function [32,33]. Overall, changes in pineal melatonin, gut butyrate, and AhR activation significantly regulate the tryptophan-melatonin pathway, in association with changes in stress-linked HPA axis activation and glucocorticoid receptor (GR) nuclear translocation.

Melatonin is also intimately linked to glucocorticoid receptor (GR) regulation, and therefore the consequences of morning cortisol awakening response (CAR) and HPA axis activation, which are reviewed next.

## 3. HPA Axis and Cortisol Awakening Response

The hypothalamic-pituitary-adrenal (HPA) axis is widely investigated as a consequence of glucocorticoid receptor (GR) activation during stress. Stress and GR activation are also important aspects of PCOS pathophysiology [48]. The HPA axis is initiated by the hypothalamic paraventricular nucleus (PVN) release of corticotrophin-releasing hormone (CRH), which induces pituitary adrenocorticotropic hormone (ACTH) release that activates the $G_s$-coupled melanocortin-2 receptor on the zona fasciculata cells of the adrenal cortex to drive cortisol release and GR activation across body and brain cells. Cortisol levels gradually increase over the course of sleep at night, culminating in an accelerated rise at the point of awakening and for the next 30 minutes, known as the morning cortisol awakening response (CAR) [49]. CAR and the night-time cortisol rise are considerably less investigated than stress induced HPA axis activation. Although heightened cortisol activation of the GR can have dramatic impacts on immune and glia cells as well as most other body cells across diverse medical conditions [50–53], the general purpose of the night-time cortisol rise, and morning CAR requires clarification beyond 'preparing the body for the coming day' by upregulating respiration and blood pressure.

Although occasionally expressed on the plasma membrane, where it can drive intracellular signaling pathways [54], the glucocorticoid receptor (GR) is primarily located in a cytoplasmic complex with heat shock protein (hsp)90 and p23 [55]. Cortisol activation of the GR leads to GR translocation to the nucleus where it activates genes expressing the glucocorticoid response element (GRE) in gene promotors, thereby impacting patterned gene expression. However, the GR can be regulated by a number of factors, including melatonin, gut butyrate and BAG-1, and can mediate its effects via a number of processes [56], as indicated in Fig. 3.

### *3.1 Glucocorticoid Receptor, Tryptophan 2,3-Dioxygenase and the Aryl Hydrocarbon Receptor*

Glucocorticoid receptor (GR) activation has effects that are intimately intertwined with the tryptophan-melatonin pathway. The cortisol activated GR, via GRE on the TDO promotor, induces tryptophan 2,3-dioxygenase (TDO), thereby taking tryptophan away from the tryptophan-melatonin pathway to produce kynurenine, and kynurenine pathway products such as kynurenic acid (KYNA) and quinolinic acid. As kynurenic acid and quinolinic acid have opposing effects on the glutamatergic n-methyl-d-aspartate receptor (NMDAr), the upregulation of the kynurenine pathway following GR activation can have dramatic and diverse effects on neuronal activity [57]. The glucocorticoid receptor (GR) induced tryptophan 2,3-dioxygenase (TDO) can therefore upregulate kynurenine and KYNA, both of which activate the AhR, thereby increasing the N-acetylserotonin (NAS)/melatonin ratio as well as regulating a wide array of diverse processes [32]. This is one route whereby heightened stress levels, via GR activation of TDO (as well as pro-inflammatory cytokine induction of IDO), can influence patterned neuronal activity across the brain. See Fig. 2.

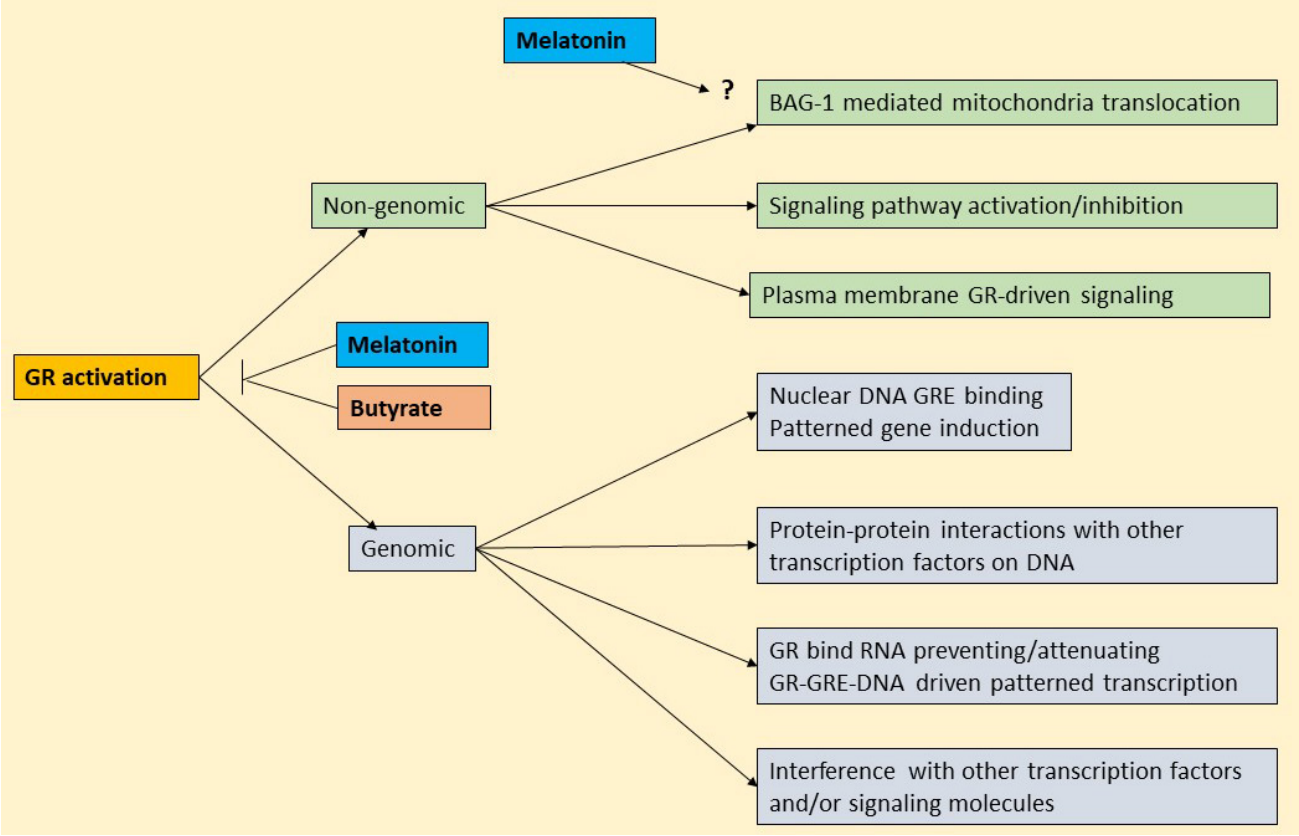


**Fig. 3. The glucocorticoid receptor (GR) has diverse effects and is regulated by melatonin, butyrate and BAG-1.** Shows the array of consequences arising from GR activation, which can impact on both genomic and non-genomic processes. Melatonin suppresses GR genomic and non-genomic effects, partly by maintaining the GR in a cytoplasmic complex with hsp90 as well as by the epigenetic, and perhaps direct, BAG-1 upregulation. Butyrate prevents GR nuclear translocation by acetylating the GR and/or heat shock protein (hsp) 90 to keep the GR in a cytoplasmic complex with hsp90 and p23. Abbreviations: BAG-1, bcl2-associated athanogene 1; GR, glucocorticoid receptor; GRE, glucocorticoid response element.

The aryl hydrocarbon receptor (AhR) is a major target for a host of diverse ligands, both endogenous and environmental. Endogenous ligands include the gut microbiome-derived indole-3-acetate and its derivatives, whilst exogenous ligands include air pollutants and cigarette smoke derived 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). AhR activation has diverse effects on different cells, as well as differential effects in the same cell when the AhR is activated by different ligands [58]. The AhR is importantly involved in the metabolism of many factors, including estrogen [59], via CYP1A2, CYP1B1 and CYP1A1 induction. AhR effects are further complicated by its expression on the mitochondrial membrane where it acts to regulate mitochondrial function [60], including via interaction with translocator protein 18 (TSPO) [61]. The GR-tryptophan 2,3-dioxygenase (TDO)-kynurenine/kynurenic acid (KYNA)/AhR pathway therefore has a number of diverse consequences [51]. As AhR upregulation and activation, as well as AhR alleles, are susceptibility factors for PCOS [23–25], heightened AhR activation may be intimately linked to PCOS alterations in mitochondrial function, including via the AhR increasing the NAS/melatonin ratio to enhance stress-related signaling and plasticity in the hypothalamus of PCOS patients. Interestingly, the suppression of tryptophan availability is strongly associated with AhR upregulation and heightened AhR activation by kynurenine [62], highlighting how the attenuation of the tryptophan-melatonin pathway from a decrease in the tryptophan/kynurenine ratio may be intimately coordinated with heightened AhR levels and activity, as evident in PCOS [23–25]. GR-TDO-kynurenine/KYNA-AhR pathway driven increase in NAS will have diverse effects that are dependent upon the expression of TrkB-FL, vs TrkB-T1, as well as by the presence of these TrkB isoforms on the plasma, vs mitochondrial, membrane [63]. The specificity of such potential diverse effects in PCOS pathophysiology requires clarification in future investigations. Overall, stress-linked GR activation via the induction of tryptophan 2,3-dioxygenase (TDO) not only decreases tryptophan availability for the tryptophan-melatonin pathway, but also increases kynurenine to activate the AhR, thereby altering patterned neuronal activity across the brain.

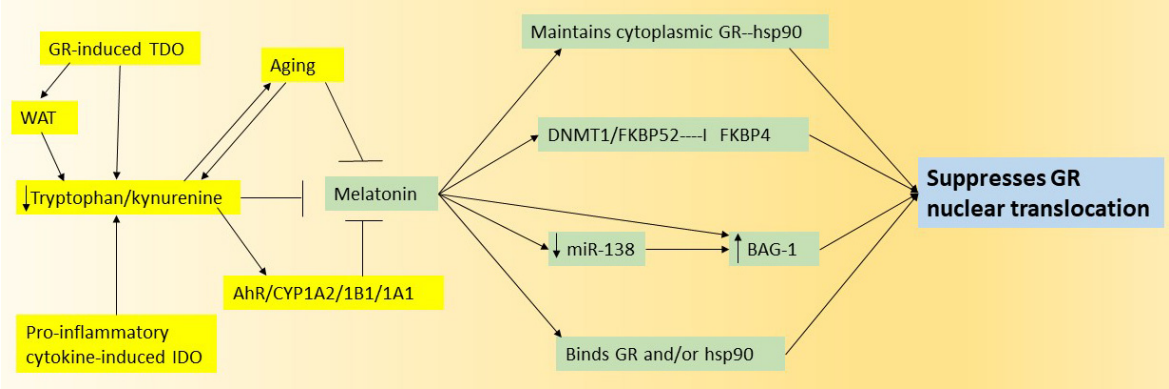


**Fig. 4. Melatonin can suppress the glucocorticoid receptor (GR) by a number of processes.** Shows how factors suppressing pineal and/or local melatonin (yellow shade) can contribute to heightened glucocorticoid receptor (GR) nuclear translocation, with melatonin proposed to suppress GR nuclear translocation via a variety of mechanisms (green shade). Abbreviations: AhR, aryl hydrocarbon receptor; BAG-1, bcl2-associated athanogene-1; CYP, cytochrome P450; DNMT1, DNA methyltransferase 1; FKBP, FK506 binding protein; GR, glucocorticoid receptor; hsp, heat shock protein; IDO, indoleamine 2,3-dioxygenase; miR, microRNA; TDO, tryptophan 2,3-dioxygenase; WAT, white adipocyte.

### *3.2 Melatonin Interactions with the HPA Axis and Cortisol Awakening Response*

Melatonin suppresses glucocorticoid receptor (GR) nuclear translocation and effects across diverse cell types and pathophysiological processes [64–68], as well as the hyperactivated HPA axis evident in PCOS linked conditions, such as type 2 diabetes mellitus (T2DM) [69]. Melatonin can therefore suppress GR effects at both cellular and systemic levels, including at night and during the morning CAR, leading to a differential priming in how night-time cortisol and the morning CAR 'prepare the body for the coming day'. Melatonin has been proposed to suppress GR nuclear translocation [8], by at least four processes, namely: (1) by the maintenance of the GR-hsp90 cytoplasmic complex [10]; (2) by enhancing DNA methyltransferase 1 (DNMT1)-mediated FK506 binding protein (FKBP)52 promoter hypermethylation, which suppresses the GR co-chaperone, FKBP prolyl isomerase 4 (FKBP4), thereby attenuating GR nuclear translocation and mitophagy [70,71]; (3) by derepressing, and perhaps directly upregulating, BAG-1 [72,73], including by melatonin's suppression of miR-138, which, like the AhR, is linked a host of aging-linked medical conditions [74]; and (4) by melatonin's receptor promiscuity [75], allowing melatonin to inhibit GR nuclear translocation via direct GR and/or hsp90 binding. See Fig. 4.

The suppressed pineal (and perhaps local) melatonin levels in PCOS enhance glucocorticoid receptor (GR) nuclear translocation thereby heightening stress-linked changes in the hypothalamus, ovary and more widely in PCOS. The effects of heightened cortisol (corticosteroid) activation of the GR are mediated via 11$\beta$-hydroxysteroid dehydrogenase type 1 (11$\beta$-HSD1) upregulation, as indicated in Fig. 1. 11$\beta$-HSD1 is increased in the granulosa cells by raised cortisol activation of the GR as well as by pro-inflammatory cytokines, leading to dysregulated granulosa cell modulation of oocyte development [76]. 11$\beta$-HSD1 upregulation in PCOS is therefore a consequence of the decrease in night-time melatonin and butyrate, which suppress both the GR and pro-inflammatory cytokines [9]. As melatonin also upregulates aromatase to increase the conversion of testosterone to estrogen in granulosa cells [22], the suppression of pineal, and especially ovarian, melatonin contributes to the 'gender-neutral' core processes underpinning the current conceptualization of PCOS as a hormonal disorder. In contrast to melatonin, a glucocorticoid receptor (GR)/tryptophan 2,3-dioxygenase (TDO)/kynurenine/aryl hydrocarbon receptor (AhR)-driven increase in the N-acetylserotonin (NAS)/melatonin ratio will allow NAS to activate hypothalamic paraventricular nucleus (PVN) TrkB to potentiate HPA axis activation and corticotrophin-releasing hormone (CRH) production and effects, which suppress aromatase [65], as well as modulating estrogen metabolism and driving anovulation via the suppression of the granulosa cell luteinizing hormone receptor, as is common in stress conditions [77], such as PCOS. Importantly, the suppression of pineal melatonin in PCOS will enhance the levels of GR nuclear translocation following the morning cortisol awakening response (CAR), thereby differentially priming body cells, including immune and glial cells, with effects partly mediated via local 11$\beta$-HSD1 upregulation.

## 4. Melatonin, Glucocorticoid Receptor and Wider PCOS Symptomatology

An aryl hydrocarbon receptor (AhR)-driven increase in the N-acetylserotonin (NAS)/melatonin ratio, coupled to a heightened hypothalamic-pituitary-adrenal (HPA) axis, heightened night-time cortisol at the GR increasing11$\beta$-

HSD1 and elevated morning CAR/11$\beta$-HSD1 driving glucocorticoid receptor (GR) activation may therefore be core aspects of PCOS pathophysiology, including in driving the increased levels of anxiety, depression and gut dysbiosis/permeability that contribute to obesity and T2DM [31,78,79]. As white adipocytes (WAT) are the major sources of kynurenine in obesity and heightened pro-inflammatory cytokines induce indoleamine 2,3-dioxygenase (IDO) and kynurenine in depression [57], the emergence of depression and obesity will contribute to the maintenance of PCOS pathophysiology. As the hypothalamus regulates basic survival functions, such as eating, drinking, aggression, sex drive and reproductive hormones, the heightened hypothalamic stress response driven by enhanced glucocorticoid receptor (GR) signaling and an increased aryl hydrocarbon receptor (AhR)-driven NAS/melatonin ratio will have wider systemic consequences via alterations in hypothalamic function. The increased levels of circulating cortisol in PCOS, as indicated by the 2-fold increase in hair cortisol [80], will have significant consequences for the array of currently conceptualized 'comorbidities' in PCOS, such as depression, anxiety and obesity.

Dysregulated hypothalamic and ovarian function may therefore arise from a heightened 'local stress' response driven by elevated GR activation as cortisol levels rise over the course of sleep (see Fig. 1), with heightened night-time GR activation increasing 11$\beta$-HSD1 levels [76]. This does not necessitate a heightened morning CAR level, as the suppression of night-time melatonin and butyrate will allow elevated GR activation in the absence of raised cortisol levels per se. However, raised GR activation will induce 11$\beta$-HSD1, thereby increasing local stress responses [76]. As noted, this does not necessarily indicate a heightened CAR level of activity but would indicate enhanced and dysregulated GR nuclear translocation in the course of daily morning CAR, thereby differentially preparing body cells/microenvironment/systems, for the coming day [51]. It is important to note that all cells exist within microenvironments with other cells, allowing the differential regulation of GR effects at night in the different cells of a given microenvironment to alter the microenvironment homeostatic interactions. This may be of some importance as melatonin and butyrate can have dramatically distinct effects on cellular function, versus cortisol activation of the GR, including distinct effects in the different cells of a given microenvironment, thereby changing the homeostatic interactions in a given microenvironment [51,70]. It is the impact on microenvironment interaction from variations in GR nuclear translocation and levels its GR nuclear translocation inhibitors (melatonin, butyrate and BAG-1) that is proposed to initiate the pathoetiology of diverse medical conditions, such as cancer, neurodegenerative conditions and autoimmune disorders [51,70]. This also has implications for PCOS and wider PCOS symptomatology, including adipocyte alterations in PCOS.

### *4.1 Obesity: Brown and White Adipocytes*

Melatonin increases brown adipocyte (BAT) function and beiges white adipocytes (WAT) as well as generating beige adipocytes from mesenchymal stem cells [81,82] via sirtuin-3/pyruvate dehydrogenase complex (PDC) optimization of mitochondrial function [83]. Suppressed pineal melatonin levels and effects will lower the BAT/WAT ratio in PCOS, via direct effects in adipocytes, as well as indirectly [84]. The indirect effects of melatonin on adipocyte phenotypes involve the loss of melatonin's induction of aromatase in granulosa cells, thereby increasing testosterone [84]. The enhanced androgen levels in PCOS suppress BAT levels and thermogenesis, at least partly via the reduction of BAT mitochondrial respiration [84]. This provides a pathway whereby decreased pineal and local melatonin increases testosterone, leading to lower BAT levels and suppressed thermogenesis, contributing to the alterations in adipocyte regulation in PCOS.

The heightened WAT levels, GR induced TDO and heightened pro-inflammatory cytokine induced IDO in PCOS raise kynurenine levels to activate the aryl hydrocarbon receptor (AhR). AhR activation is a major suppressor of BAT function and contributes to obesity [85]. Clearly, the glucocorticoid receptor (GR)/tryptophan 2,3-dioxygenase (TDO)/kynurenine/AhR pathway suppression of the tryptophan-melatonin pathway couples heightened AhR activation to suppressed BAT mitochondrial function, thereby allowing this pathway and associated suppressed melatonin to attenuate BAT induction and function in PCOS. As the benefits of exercise and fat loss in PCOS indicate [86], alterations in adipocyte phenotypes and fluxes are intimately linked to wider PCOS pathophysiology. Although adipocytes may release many factors relevant to brain, immune, ovary and circadian function, including adiponectin, hormone-sensitive lipase, IL-6, leptin, and microRNA containing exosomes [46,84–86], for clarity the role of released kynurenine is highlighted in the current article.

Prolonged glucocorticoid exposure induces metabolic syndrome and increases white adipocytes (WAT), which can be attenuated by a glucocorticoid receptor (GR) antagonist [87]. GR activation accelerates both BAT and WAT development [88]. However, concurrently suppressed melatonin and heightened aryl hydrocarbon receptor (AhR) activation dramatically increases the WAT/BAT ratio, highlighting the importance of interactions of the circadian melatonin and cortisol levels with wider systemic processes. Preclinical data indicate that raised testosterone levels and androgen receptor activation have cell specific effects in BATs that enhance GR effects [89]. However, the expression of BAG-1 and the consequences of GR mitochondrial translocation in BAT and WAT have still to be determined. Clearly, GR effects in BATs and WATs require further investigation, including whether the GR induces tryptophan 2,3-dioxygenase (TDO) in WATs and whether

local melatonin production in adipocytes as well as BAG-1 would prevent the deleterious effects of GR activation in adipocytes.

Adipocytes, primarily WAT, are a major source of the raised circulating kynurenine levels in obesity [90]. This is proposed to be driven by heightened pro-inflammatory cytokine-induced indoleamine 2,3-dioxygenase (IDO) and an IDO/kynurenine/AhR/signal transducer and activator of transcription (STAT)3/IL-6 signaling pathway [90]. Interestingly, STAT3 suppresses pineal AANAT and the melatonergic pathway via miR-7 upregulation and the suppression of 14-3-3z, which is necessary for AANAT stabilization [91,92]. The extent to which the melatonergic pathway is suppressed in WAT, in the course of increasing kynurenine production will be important to determine, including as to the role of endogenous WAT melatonin in the course of WAT beiging.

Notably, over 60% of brain kynurenine is derived from the periphery [93], indicating that adipocyte-derived kynurenine may be a significant contributor to aryl hydrocarbon receptor (AhR)-driven changes in the brain, including the hypothalamic paraventricular nucleus (PVN), thereby allowing potentially direct links to hypothalamic stress-linked plasticity via kynurenine/KYNA/AhR/CYP1A1/1B1 increasing the N-acetylserotonin (NAS) activation of TrkB (see Fig. 2). Raised levels of circulating kynurenine and KYNA are evident in PCOS, with kynurenine and KYNA levels positively correlating with levels of anti-Mullerian hormone (AMH), luteinizing hormone, and fasting insulin, as well as Homeostatic model assessment for insulin resistance (HOMA-IR) [94]. Granulosa cell derived anti-Mullerian hormone (AMH) is frequently mooted as a PCOS biomarker [1]. However, the above would suggest that it may be downstream from decreased pineal and local melatonin coupled to heightened AhR activation by WAT derived kynurenine. WAT derived kynurenine may therefore be a relevant contributor to wider PCOS pathophysiology in granulosa cells as well as in the hypothalamus.

As testosterone can increase indoleamine 2,3-dioxygenase (IDO) [95], the suppression of melatonin's induction of aromatase in granulosa cells may therefore allow raised testosterone to also enhance WAT indoleamine 2,3-dioxygenase (IDO)-derived kynurenine to activate the AhR/N-acetylserotonin(NAS)/TrkB and therefore hypothalamic and oocyte/granulosa cell local stress reactivity. In the presence of suppressed pineal and local melatonin, this would be coupled to heightened glucocorticoid receptor (GR) transcriptional effects and thereby increased local 11$\beta$-hydroxysteroid dehydrogenase type 1 (11$\beta$-HSD1) [76]. This indicates significant interactions across adipocytes, hypothalamus and the granulosa cell-oocyte 'complex' arising from suppressed melatonin and associated increase in GR nuclear translocation. Tanycytes have a powerful role in determining hypothalamic function [26], including in the regulation of paraventricular nucleus (PVN) gonadotrophin releasing hormone (GnRH) and systemic metabolism [26,27]. Tanycytes may therefore be an important hub for hypothalamic alterations and subsequent hypothalamic interactions with adipocytes and the ovary. Alterations in the 'homeostatic' interactions of the hypothalamus, adipocytes, pineal/local melatonin, gut butyrate and the ovary may be parsimonious with the partial efficacy of single-focus treatments, such as melatonin supplementation or exercise/weight loss, given the diverse impacts of such single-focus treatments on wider PCOS pathophysiology [6]. See Fig. 5.

### *4.2 Depression and PCOS*

As well as obesity, people with PCOS often meet current criteria for comorbid classification of depression and anxiety [77]. Depression criteria are often met for conditions where an increase in pro-inflammatory cytokines/cortisol/IDO/TDO suppresses the tryptophan-melatonin pathway, coupled to raised kynurenine and aryl hydrocarbon receptor (AhR) activation that dysregulates the mitochondrial melatonergic pathway and enhances glucocorticoid receptor (GR) activation. The cytokine/IDO-GR/TDO-kynurenine pathway coupled to a suppressed tryptophan-melatonin pathway and 'comorbid' depression are often evident in diverse medical conditions, such as cancer [51], T2DM [96], rheumatoid arthritis [97], autoimmune disorders more widely [98] and an array of neuropsychiatric disorders [99]. Depression in such circumstances may be seen as a corollary of the above processes in the pathophysiology of these diverse conditions, and therefore less a comorbidity and more of a consequence of core pathophysiological processes having multiple systemic and CNS effects [100].

Depression, when presenting as the main symptom, is typically associated with raised levels of pro-inflammatory cytokines, circadian dysregulation, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, with weight gain or loss, coupled to an amotivational state and some cognitive impairment [101]. These dysregulated processes in depression are also relevant aspects of PCOS pathophysiology, as highlighted throughout, and are also evident in Bipolar Disorder, which shows an increased comorbidity with PCOS [102]. Brain areas classically researched as major drivers and treatment targets of depression include the hippocampus, amygdala, frontal cortex and the brain reward system (ventral tegmental area and nucleus accumbens) [103,104]. More recent work highlights the role of astrocytes and microglia and how such CNS glial cells may be regulated by alterations in the gut microbiome, opioidergic system and the HPA axis. This has consequences for glia and immune cell mitochondrial function with impacts on how these reactive cells regulate neuronal survival and activity, including patterned inter-area activity [105,106]. This provides

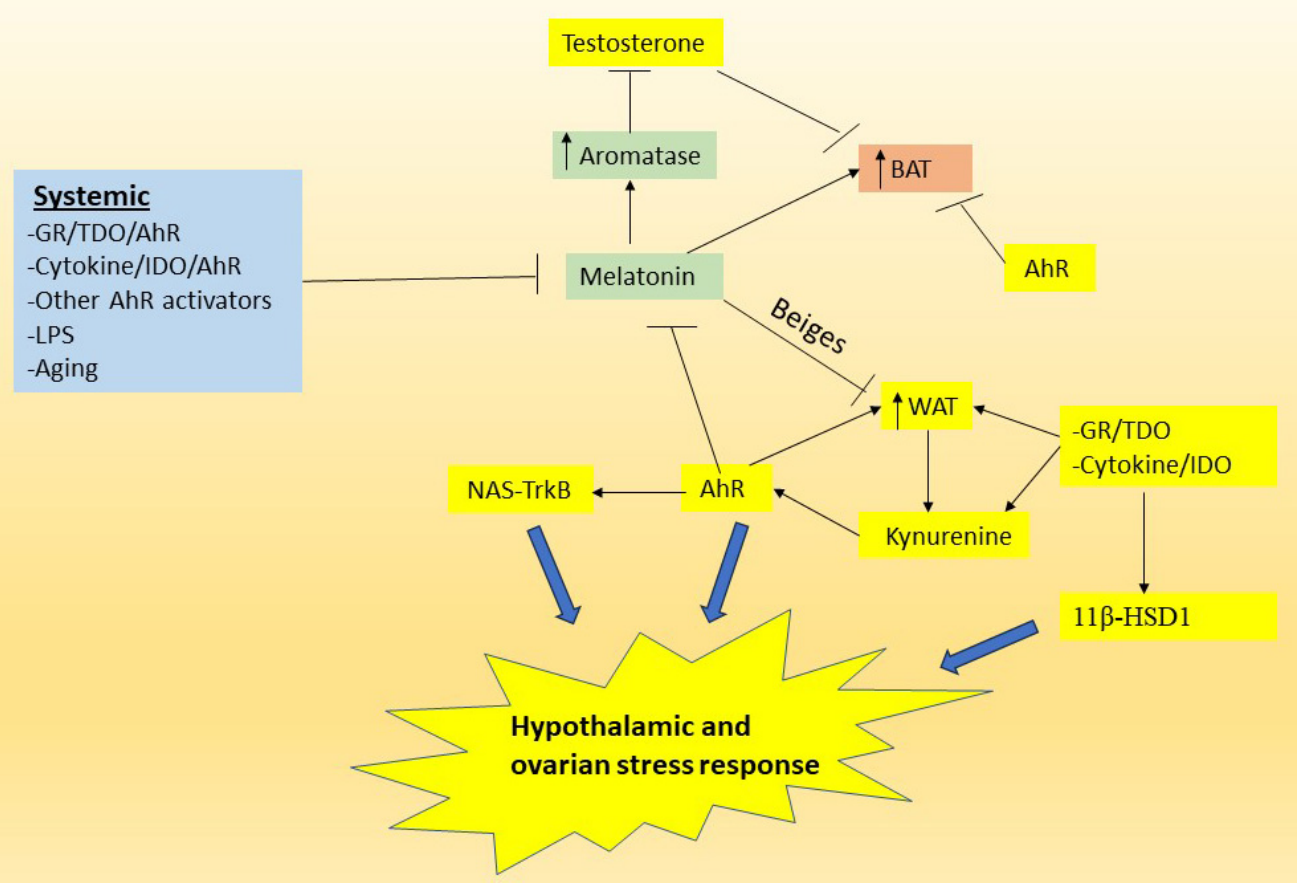


**Fig. 5. Systemic inhibitors of melatonin modulate hypothalamic and ovarian stress.** Shows how systemic inhibitors (blue shade) of pineal and/or local melatonin will regulate white adipocytes (WAT) and brown adipocytes (BAT), with consequences for a heightened hypothalamic and ovarian stress response. Melatonin increases BATs and beiges WATs. Suppressed melatonin contributes to decreased aromatase thereby increasing testosterone, which suppresses BATs. Raised GR/TDO and pro-inflammatory cytokines in PCOS increase WAT, which are major contributors to circulating kynurenine in obesity. Kynurenine activation of the brain and systemic aryl hydrocarbon receptor (AhR), along with GR/TDO and proinflammatory cytokine/IDO, will contribute to an enhanced hypothalamic and ovarian stress response in PCOS. The GR and pro-inflammatory cytokine induction of 11$\beta$-HSD1, under conditions of suppressed melatonin, butyrate and BAG-1 will also significantly contribute to heightened hypothalamic and ovarian stress responses. Abbreviations: 11$\beta$-HSD1, 11$\beta$-hydroxysteroid dehydrogenase 1; AhR, aryl hydrocarbon receptor; GR, glucocorticoid receptor; IDO, indoleamine 2,3-dioxygenase; LPS, lipopolysaccharide; NAS, N-acetylserotonin; TDO, tryptophan 2,3-dioxygenase; TrkB, tyrosine receptor kinase B; WAT, white adipocyte.

a distinct, and less neurocentric, conceptualization of CNS function in which to integrate the emergence of depression in PCOS.

Stress/glucocorticoid receptor (GR) mediated upregulation of corticotrophin-releasing hormone (CRH) in the paraventricular nucleus (PVN), amygdala and hippocampus are inhibited by oxytocin acting on astrocyte oxytocin receptors [107,108]. PVN oxytocin neurons project to the central amygdala where oxytocin inhibits CRH, thereby inhibiting the CRH induction of dynorphin and the dynorphin activation of the $\kappa$-opioid receptor [109,110]. Dynorphin activation of the amygdala $\kappa$-opioid receptor induces dysphoria that is proposed to underpin the affective dysregulation evident in Borderline personality disorder [111], and unipolar depression [106]. Dynorphin at the $\kappa$-opioid receptor also significantly suppresses motivation driven by ventral tegmental area (VTA) dopamine inputs to the Nucleus Accumbens (N.Acc) [112], suggesting direct effects of oxytocin [113] and/or oxytocin regulated amygdala modulation of the VTA-N.Acc junction. Overall, suppressed PVN oxytocin production may be a significant regulator of how PCOS associates with mood dysregulation. As PVN oxytocin also suppresses the HPA axis, PVN oxytocin (from oxytocin neuronal dense core vesicles acting on PVN astrocyte oxytocin receptors) allows oxytocin to modulate the HPA axis, night-time cortisol rise and morning CAR and therefore the consequences arising from stress, night-time cortisol and CAR driven GR activation. Notably, oxytocin receptor alleles are susceptibility factors for PCOS [114]. Preclinical data also shows PVN oxytocin projections to the

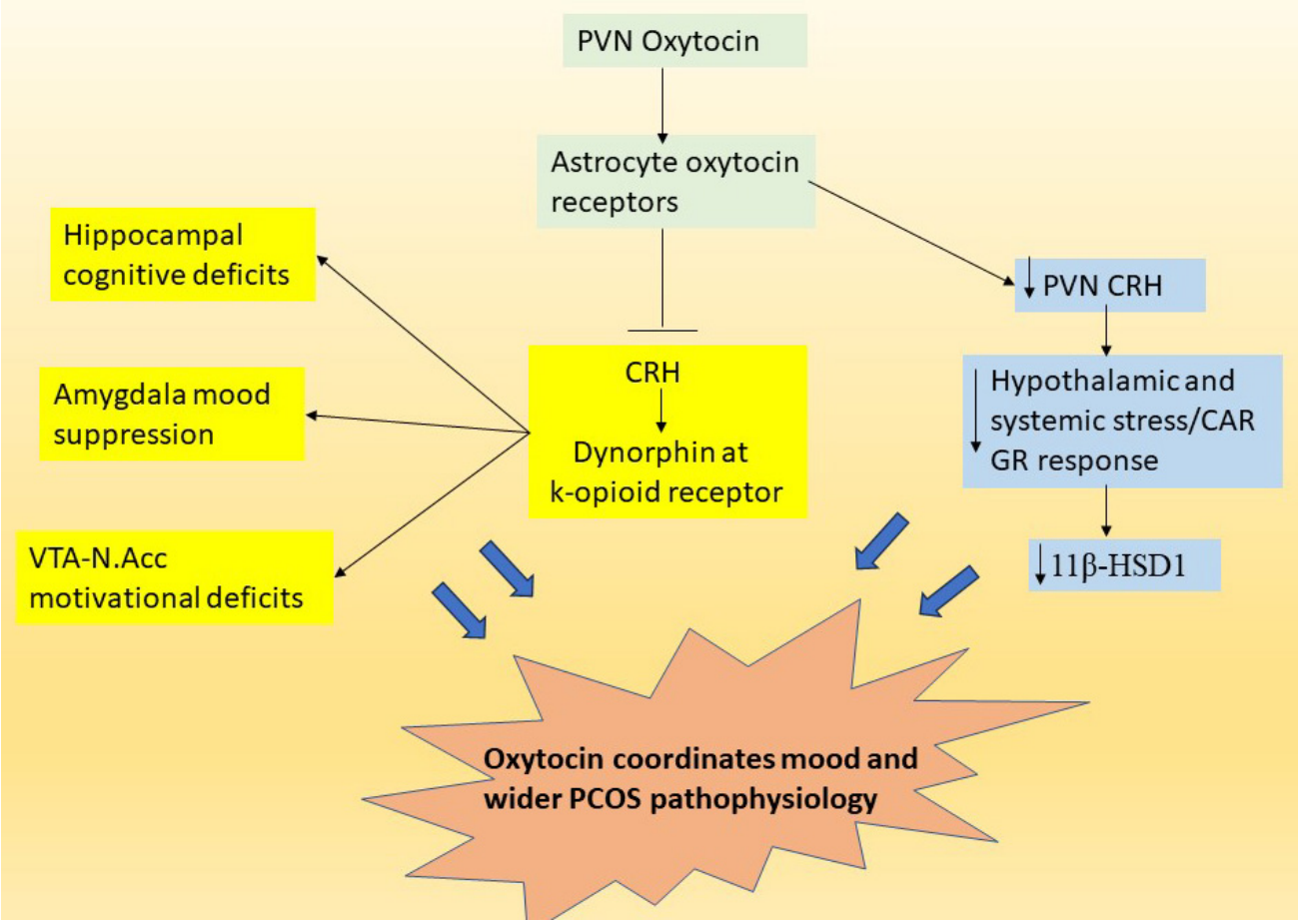


**Fig. 6. Oxytocin modulation of mood and wider symptomatology in PCOS.** Shows how hypothalamic oxytocin, via astrocyte oxytocin receptors suppresses the corticotrophin-releasing hormone (CRH)/dynorphin-$\kappa$-opioid receptor pathway by coordinating mood linked changes in the hippocampus, amygdala and VTA-N.Acc junction. This is coordinated with wider systemic regulation of GR activation during hypothalamic-pituitary-adrenal (HPA) axis driven stress and the cortisol awakening response (CAR). The oxytocin suppression of the HPA axis and GR activation will contribute to suppressed local stress in the hypothalamus and ovary via decreased 11$\beta$-HSD1 induction. Abbreviations: 11$\beta$-HSD1, 11$\beta$-hydroxysteroid dehydrogenase 1; CRH, corticotrophin releasing hormone; N.Acc, nucleus accumbens; PVN, hypothalamic paraventricular nucleus; VTA, ventral tegmental area.

hippocampus [115], indicating a role for suppressed oxytocin in PCOS [116] in the regulation of stress/CRH effects on the cognitive dysregulation that can occur in depression and PCOS, including when associated with early life stressors [117]. PVN oxytocin may therefore modulate the cognitive, affective and motivational aspects of depression in PCOS, with effects, at least partly mediated by the suppression of dynorphin activation of the $\kappa$-opioid receptor in these brain regions. See Fig. 6.

As indicated above (section 3.2), the effects of raised night-time GR activation, night-time cortisol rise and morning CAR may be importantly primed by alterations in factors during sleep that regulate how CAR primes body cells, including immune and glial cells. Suppressed pineal melatonin, butyrate and BAG-1 levels, as well as raised adipocyte-derived kynurenine, aryl hydrocarbon receptor (AhR) levels and activation will all impact on how CAR prepares systemic and brain cells for the coming day [51], including the hypothalamus, which has a long-standing association with mood dysregulation [118,119]. Alterations in the cortex, hippocampus and amygdala are more classically seen as reflecting inputs into consciousness related processes [120] that generate the phenomenological state of depression. However, mood dysregulation may also be powerfully driven by implicit processes, including circadian priming of the hypothalamus [121,122]. Such 'implicit' processing will also be regulated by systemic factors, such as white adipocyte (WAT)-derived kynurenine and alterations in how gut butyrate regulates glucocorticoid receptor (GR) translocation and effects [11,12]. The emergence of depression in PCOS may therefore be a downstream corollary of implicit and systemic processes in the regulation of explicit processes underpinning the phenomenological state. The above would indicate that the impact of implicit processes may be occurring at night during sleep. The classical conceptualization of depression as de-

fined by explicit processes has parallels to conceptualizations of PCOS as a 'hormonal' condition when it may be more accurately determined by more fundamental implicit, circadian and systemic processes.

Importantly, alterations in the gut microbiome/permeability are intimately linked to these more fundamental processes in depression as well as PCOS pathophysiology more widely [123].

## 5. Gut Microbiome and PCOS

A recent meta-analysis of ten studies comparing PCOS, vs controls, on microbiome composition shows PCOS to be associated with decreased $\alpha$-diversity, indicative of gut dysbiosis [124], with gut dysbiosis/permeability also evident in the PCOS 'comorbidities', obesity and type 2 diabetes mellitus (T2DM) [125]. Both obesity and T2DM are intimately linked to driving PCOS symptomatology [126], at least partly via white adipocyte (WAT)-derived kynurenine/ aryl hydrocarbon receptor (AhR) activation [62] and suppressed gut butyrate [127]. A preliminary study shows butyrate to be decreased in PCOS, in correlation with raised testosterone levels [128].

Butyrate acts via different processes, including G-protein coupled receptor (GPR)41, GPR43 and GPR109 activation as well as epigenetic regulation via its capacity as a pan-histone deacetylase (HDAC) inhibitor. Butyrate also increases sirtuin-3, thereby enhancing oxidative phosphorylation (OXPHOS) and acetyl-CoA via pyruvate dehydrogenase complex (PDC) deacetylation and disinhibition [129], thereby upregulating the mitochondrial melatonergic pathway [130,131], (see Fig. 2). Butyrate maintains the gut barrier and crosses into the general circulation. As with the LPS suppression of pineal melatonin, decreased butyrate not only attenuates butyrate's induction of the mitochondrial melatonergic pathway but has consequences for metabolism, resilience, and stress responses [130].

Circulating butyrate regulates systemic processes directly as well as indirectly via mucosal immune cells, including by upregulating IL-22 in mucosal CD4+ t cells and Innate lymphoid cells (ILCs) via GPR41 and histone deacetylase inhibition (HDACi) [132]. Gut dysbiosis suppresses IL-22 levels, which not only attenuates optimal gut immunity but also contributes to suboptimal granulosa cell mitochondrial function in preclinical PCOS models [133]. Butyrate therefore has an expected treatment efficacy in PCOS given that some of the benefits of oral contraceptive medication in PCOS patients is mediated via IL-22 upregulation [134]. Gut mucosal immune cell derived IL-22 may also afford benefits in pancreatic $\beta$-cells where it attenuates insulin resistance via the IL-22r/Janus kinase 1 (JAK1)/signal transducer and activator of transcription-3 (STAT3) pathway in a PCOS preclinical model [135], with effects in the pancreas being another aspect of how the gut microbiome regulates the obesity/T2DM pathophysiological consequences in PCOS. Stress attenuates gut IL-22 production, which is reversed by oral melatonin [136], indicating that the benefits of oral melatonin in PCOS patients [5] may be mediated by diverse processes, including via the gut microbiome and its interaction with the mucosal immune system. STAT3-miR-7 is also an important regulator of the melatonergic pathway as well as oocyte-granulosa cell interactions over the menstrual cycle [137,138], indicating possible wider systemic consequences of butyrate induced IL-22 in mucosal immune cells.

Importantly, butyrate suppresses inflammation in PCOS granulosa cells [139]. These authors showed that serum butyrate levels are decreased in clinical PCOS where obesity is evident [139], which the authors attribute to enhanced mRNA modification by $N^6$-methyladenosine (m6A), especially the m6A modification of FOSL2 (FOS Like 2, AP-1 Transcription Factor Subunit). FOSL2 encodes the protein, Fos-related antigen 2 (FRA2). Granulosa cell data shows butyrate to suppress METTL3, possibly via the induction of Yes-Associated Protein (YAP) [140], and thereby suppressing the m6A modification of FOSL2, concurrent to a decrease in the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, IL-6 and TNF-$\alpha$. See Fig. 7. As LPS potentiates FOSL2 [139], an increase in gut permeability and circulating LPS, (as evident in PCOS with concurrent obesity [20]), may be another means by which the gut modulates ovarian function in PCOS. As butyrate increases sirtuin-3 and the mitochondrial melatonergic pathway [131], butyrate optimizes granulosa cell mitochondrial function and therefore the intercellular interactions of granulosa cells with oocytes as well as with other granulosa cells. The capacity of butyrate to regulate PCOS pathophysiology may be attenuated under conditions of tryptophan-melatonin pathway suppression, including in granulosa cells where the suppression of melatonin and the melatonin induction of aromatase increases the testosterone that contributes to wider PCOS symptomatology. Notably, gut permeability (or uterine infection) induced LPS suppresses aromatase in granulosa cells, and increases NF-kB driven inflammatory processes, which histone deacetylase inhibition (HDACi) prevents [141]. Such data would indicate significant roles for gut dysbiosis and gut permeability/uterine infection in PCOS granulosa cells, which may be particularly pertinent when PCOS is accompanied by obesity in association with suppressed butyrate and melatonin levels. See Fig. 7.

As well as regulating PCOS granulosa cell aromatase and inflammatory processes [141], butyrate also modulates adipocytes. The consequences of decreased butyrate in obesity, T2DM and PCOS are intimately linked to butyrate's pan-HDACi capacity, which increases mitochondrial oxidative phosphorylation to drive a brown adipocyte (BAT)/browning phenotype during adipogenesis [142,143]. Other histone deacetylase (HDAC) inhibitors also show the capacity to brown/beige white adipocytes (WATs) [144]. Whether this is coordinated with butyrate's

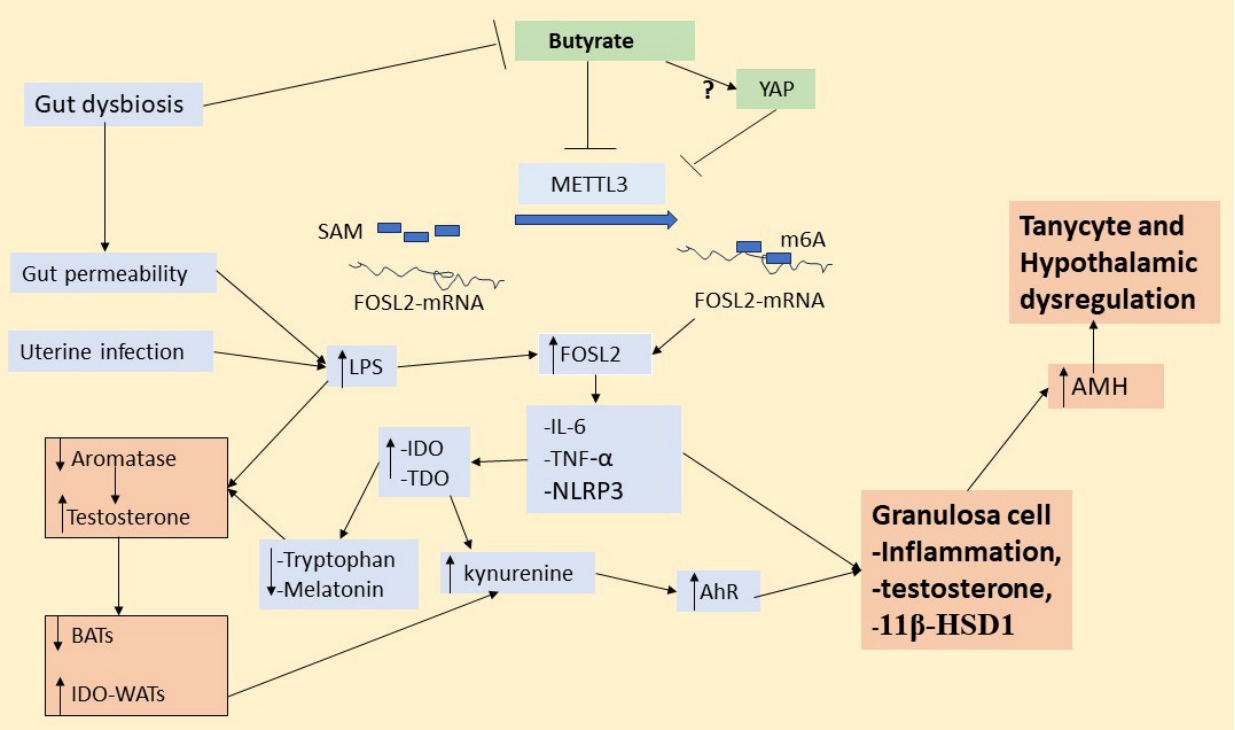


**Fig. 7. Gut dysbiosis/permeability regulates granulosa cells.** Shows how gut dysbiosis and associated increased gut permeability (light blue shade) can regulate granulosa cell inflammation (gold shade), as well as suppress aromatase to increase testosterone, including via decreased melatonin in granulosa cells. Gut microbiome-derived butyrate suppresses the METTL3 conversion of SAM to m6A of FOSL2 mRNA (dark blue shade), possibly via butyrate induction of YAP. Increased FOSL2, including from LPS arising from gut permeability and/or uterine infection, enhances IL-6, TNF-$\alpha$ and the NLRP3 inflammasome, thereby increasing granulosa cell inflammation. Pro-inflammatory cytokines also increase IDO and TDO to convert tryptophan to kynurenine, whilst decreasing local melatonin's induction of aromatase, thereby increasing testosterone. Raised testosterone levels decrease brown adipocytes (BATs) and increase IDO, including possibly in white adipocytes (WATs), thereby increasing adipocyte-derived kynurenine to activate the aryl hydrocarbon receptor (AhR). Kynurenine activation of the AhR dysregulates granulosa cells and other ovarian cells. Gut dysbiosis, including via decreased butyrate and increased gut permeability is therefore intimately linked to adipocyte and ovarian alterations in PCOS and the impact this has for wider systemic processes, including increased AMH, which drives tanycyte and hypothalamic dysregulation. Pro-inflammatory cytokines, like raised GR activation, increase local stress responses via 11$\beta$-HSD1 upregulation. Abbreviations: 11$\beta$-HSD1, 11$\beta$-hydroxysteroid dehydrogenase 1; AhR, aryl hydrocarbon receptor; AMH, anti-Mullerian hormone; FOSL2, FOS Like 2, AP-1 Transcription Factor Subunit; IDO, indoleamine 2,3-dioxygenase; IL-, interleukin; LPS, lipopolysaccharide; m6A, $N^6$-methyladenosine; METTL3, m6A methyltransferase; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; SAM, S-adenosylmethionine; TDO, tryptophan 2,3-dioxygenase; TNF, tumor necrosis factor; YAP, Yes-Associated Protein.

suppression of the glucocorticoid receptor (GR)/tryptophan 2,3-dioxygenase (TDO)/kynurenine/aryl hydrocarbon receptor (AhR) [62] in adipocytes via the acetylation of the adipocyte GR and hsp90 [11,12] will be important to determine, including the role of butyrate's induction of the mitochondrial melatonergic pathway in adipocytes. Gut microbiome-derived butyrate can therefore regulate systemic processes by a multitude of means. These include the suppression of GR nuclear translocation during stress-linked hypothalamic-pituitary-adrenal (HPA) axis activation and the rising levels of cortisol over sleep culminating in the cortisol awakening response (CAR). Butyrate can therefore impact on how night-time cortisol and morning CAR 'prepare the body for the coming day', including in adipocytes, granulosa cells and hypothalamic cells, thereby regulating the nature of the homeostatic interactions of any cell in its given microenvironment. As GR activation also increases gut permeability, butyrate will act in the gut to limit the effects of cortisol/stress/corticotrophin-releasing hormone (CRH) on gut permeability and gut dysbiosis, as well as regulating the circadian entrainment of intestinal epithelial cells [145]. Consequently, butyrate's regulation of gut permeability/dysbiosis will determine butyrate circulatory levels and therefore butyrate impacts on adipocytes and other cells.

Importantly, gut microbiome production of short-chain fatty acids varies over the circadian rhythm, with butyrate being mostly produced during fasting when on a reg-

ular, but not a high-fat, diet, as shown in preclinical studies [146]. Subsequent preclinical data shows the application of melatonin to establish a gut circadian rhythm of butyrate production even under a high fat diet, supporting a role for pineal melatonin in the circadian regulation of gut microbiome-derived butyrate [147]. The implications for this in humans have still to be investigated, including the role of varying butyrate levels over the circadian rhythm in the regulation of stress linked HPA axis activation and rising cortisol effects at night and during the morning CAR. Existing preclinical data indicate that butyrate levels vary over the circadian rhythm, being increased during fasting and sleep, thereby allowing butyrate to suppress the glucocorticoid receptor (GR) nuclear translocation in the course of morning CAR as well as in stress linked HPA axis activation, especially if stress arises at a time of 'fasting' [146,147]. Exogenous butyrate significantly increases circulating butyrate levels for over 6 hours [148], suggesting fasting-linked enhanced butyrate over sleep will modulate not only the rising cortisol effects at night but also the morning CAR activation and nuclear translocation of the GR. As evident in the use of corticosteroid medications, GR activation can dramatically alter immune and glial cell activation, indicating that variations in night-time melatonin, butyrate and BAG-1 will impact on how these reactive cells are primed, including ovarian granulosa cells, by GR activation during the night and in the course of the morning CAR. Given the powerful role of the GR in PCOS pathophysiology, such gut and pineal interactions with CAR and HPA axis driven GR nuclear translocation are likely to be of some importance in the pathophysiology of PCOS, as with many other medical conditions [51].

### *5.1 Hypothalamic Stress/Plasticity and Gut Microbiome*

As well as heightened 'hypothalamic stress/plasticity' being modulated by interactions with adipocytes and the oocyte/granulosa cell complex, the gut microbiome is also an important regulator of hypothalamic function directly, as well as via adipocytes and the ovaries. Decreased gut butyrate heightens the glucocorticoid receptor (GR)/tryptophan 2,3-dioxygenase (TDO)/kynurenine/aryl hydrocarbon receptor (AhR)/N-acetylserotonin (NAS)/TrkB pathway [36,37], thereby impacting on many aspects of hypothalamic function and fluxes. For example, butyrate suppresses the raised corticotrophin-releasing hormone (CRH) levels, including via the upregulation of oxytocin receptors in hypothalamic astrocytes (see Section 5.2 below). Butyrate therefore attenuates the CRH initiation of the HPA axis that determines stress, night-time cortisol and morning CAR cortisol levels [38] as well as CRH independent effects, such as increasing gut permeability [39]. Butyrate and LPS effects in the hypothalamus are significantly modulated by the heightened and prolonged melatonin (and NAS and NAS induced BDNF [149]) levels in the third ventricle over the duration of sleep [16], which modulate tanycyte and astrocyte regulation of hypothalamic neurons and neuronal fluxes [1] (see Section 6).

Butyrate effects will be partly determined by the NAS/melatonin ratio, given that butyrate effects involve the upregulation of acetyl-CoA and therefore the mitochondrial melatonergic pathway [131]. However, a butyrate upregulation of NAS, versus melatonin, will have dramatic consequences in the hypothalamus, given NAS directly, as well as indirectly via BDNF upregulation, activates the stress plasticity linked hypothalamic TrkB. Butyrate, via histone deacetylase inhibition (HDACi), can enhance AhR activation by endogenous AhR ligands [150,151], suggesting that the effects of butyrate on hypothalamic plasticity may be importantly determined by the levels of AhR ligands, including WAT-derived kynurenine. How decreases in gut microbiome-derived butyrate may interact with core and wider PCOS pathophysiology is shown in Fig. 8.

### *5.2 Gut Microbiome and Oxytocin*

The complexity of gut microbiome influence in PCOS is further highlighted by data indicating that the gut bacteria, *Limosilactobacillus reuteri*, upregulates oxytocin, possibly involving vagal nerve activation and alterations in the adaptive immune response [152]. *L. reuteri* effects are proposed to be mediated via the adaptive immune system during the acceleration of wound healing, although the immune response does not modulate the *L. reuteri* effects on social behavior in autism preclinical models [153]. *L. reuteri* upregulates the gut hormone, secretin, which is produced in enteroendocrine cells, and which increases oxytocin production in human gut enterocytes [154]. Enterocyte-derived oxytocin maintains the gut barrier and dampens mucosal immunity [155]. *L. reuteri* also upregulates gut butyrate production as well as increasing other butyrate-producing bacteria, as shown consistently in 6 human volunteers [156]. By increasing gut butyrate, *L. reuteri* will also increase brown adipocytes (BATs), the mitochondrial melatonergic pathway and optimize mitochondrial metabolism, as well as suppressing glucocorticoid receptor (GR) nuclear translocation via GR and hsp90 acetylation [11,12], thereby impacting on hypothalamic-pituitary-adrenal (HPA) axis, night-time rising cortisol and morning CAR effects. Butyrate, and other histone deacetylase inhibitors (HDACi), upregulate oxytocin receptor levels and activation [157], suggesting that *L. reuteri* effects may be partly mediated via butyrate upregulating oxytocin receptors and levels, including in gut enterocytes, as well as via secretin upregulation [154]. Interestingly, preclinical data shows sodium butyrate to increase pancreatic secretin [158], possibly indicative of a role for butyrate in the upregulation of secretin from gut enteroendocrine cells and consequent gut enterocyte oxytocin upregulation. Importantly, gut oxytocin also suppresses enteric glial cell activation to stress [159], thereby impacting on how these 'gut as-

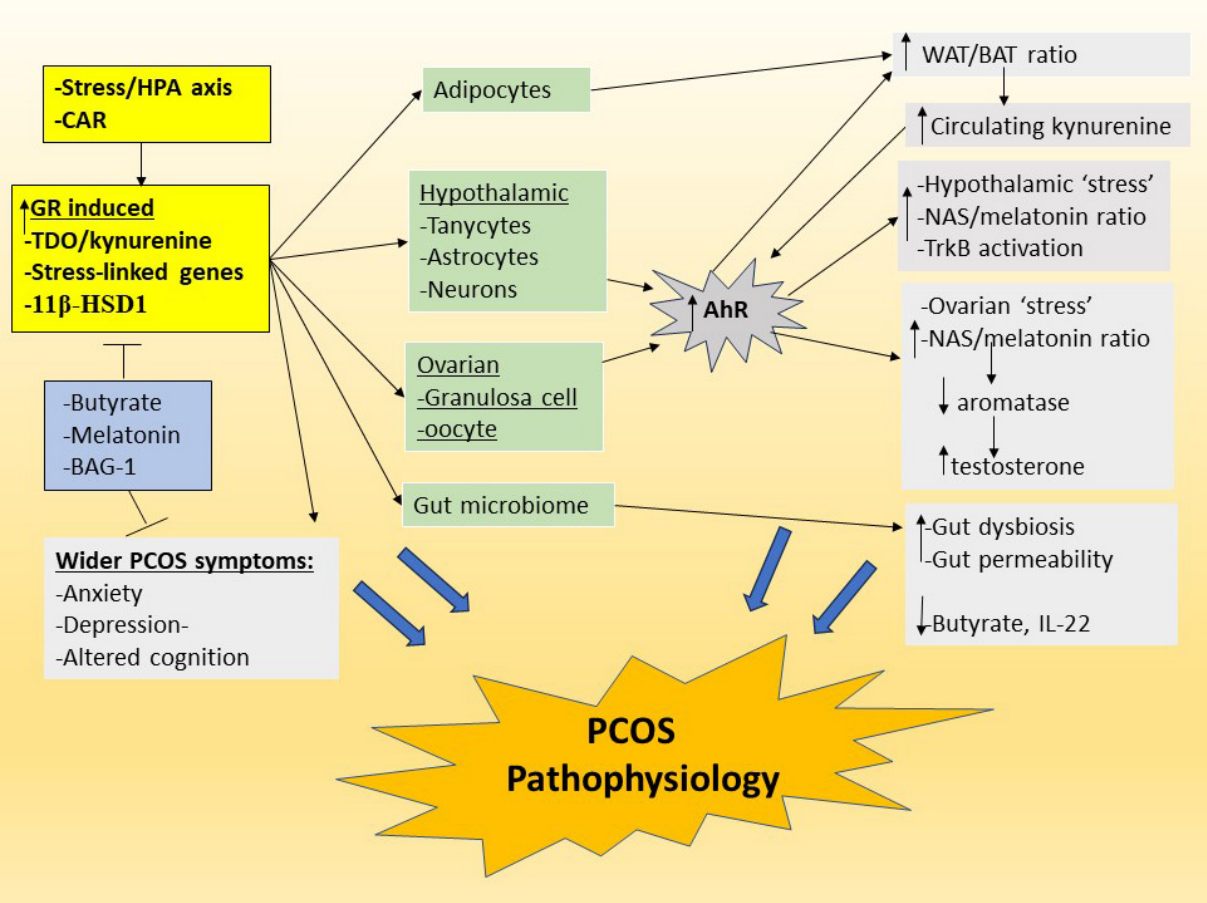


**Fig. 8. Systemic PCOS pathophysiology.** Shows PCOS pathophysiology to be driven by heightened stress responses in the hypothalamus and oocyte-granulosa cell complex, as well as in the gut microbiome. Stress/HPA axis, night-time rising cortisol levels and morning CAR drive glucocorticoid receptor (GR) activation, which when transported to the nucleus increases TDO and kynurenine, including in adipocytes when obesity is present. GR also induces other stress-linked and immune/glial cell regulatory genes. The suppression of pineal and local melatonin as well as butyrate and BAG-1 enhance GR nuclear translocation, with effects in adipocytes, hypothalamus, ovary and gut. The GR/TDO/kynurenine pathway suppresses tryptophan levels for the tryptophan-melatonin pathway as well as increasing kynurenine and kynurenic acid, which activate the AhR to suppress melatonin and increase NAS to drive ovarian and hypothalamic stress plasticity. Aryl hydrocarbon receptor (AhR) activation, like testosterone, also increases the white adipocyte (WAT)/brown adipocyte (BAT) ratio, thereby increasing kynurenine, which may further induce and activate the AhR. Enhanced GR activation, combined with decreased butyrate, melatonin and BAG-1, coupled to raised pro-inflammatory cytokines underpin wider PCOS symptomatology, including depression, anxiety and cognitive changes. Heightened local stress effects will be significantly determined by 11$\beta$-HSD1 upregulation by the GR and pro-inflammatory cytokines. Abbreviations: 11$\beta$-HSD1, 11$\beta$-hydroxysteroid dehydrogenase 1; AhR, aryl hydrocarbon receptor; BAG-1, bcl2-associated anthanogene-1; BAT, brown adipocyte; CAR, cortisol awakening response; GR, glucocorticoid receptor; HPA, hypothalamus-pituitary-adrenal; IL, interleukin; NAS, N-acetylserotonin; PCOS, polycystic ovary syndrome; TrkB, tyrosine receptor kinase B; WAT, white adipocyte.

trocytes' determine the interactions of the gut/microbiome, enteric nervous system, vagal nerve and mucosal immune system. Whether the suppression of enteric glial cell activation by butyrate [160], includes oxytocin receptor upregulation in enteric glial cells in association with gut oxytocin production will be important to determine. Notably, *L. reuteri* prevents PCOS in a circadian rhythm disruption preclinical PCOS model [161].

Whether butyrate upregulates oxytocin receptors on tanycytes and astrocytes in the preclinical paraventricular nucleus (PVN), amygdala, hippocampus, and VTA-N.Acc junction [108,115], thereby regulating the consequences of PVN-derived oxytocin will be important to determine. The gut microbiome may therefore have a more significant role in the regulation of hypothalamic function in the course of PCOS pathophysiology than previously appreciated, with consequences for wider patterned interarea connectivity across the brain [162], as well as the regulation of diverse systemic processes.

## 6. Hypothalamic Tanycytes and Astrocytes: Core Hub in PCOS Symptomatology

Hypothalamic symptomatology in PCOS is classically linked to enhanced hypothalamic gonadotropin-releasing hormone (GnRH) pulsatile levels, arising from a 40% increase in pulsatile frequency, thereby raising pituitary luteinizing hormone (LH) secretion, which is a major contributor to enhanced ovarian androgen production [163]. Raised serum anti-Mullerian hormone (AMH) levels are frequently used as a PCOS biomarker, with raised AMH from granulosa cells associating with heightened hypothalamic glia-neuronal activation in PCOS patients [1]. These authors proposed that AMH induces tanycyte retraction, thereby allowing greater entry of GnRH neurons to the median eminence blood capillaries, implicating a core role for tanycyte/glia-neuronal dysregulation in PCOS hormonal pathophysiology [1]. Structural changes in tanycytes may therefore be an important aspect of hypothalamic 'stress plasticity', including as induced by paraventricular nucleus (PVN) TrkB activation. Alterations in tanycyte function are also an important aspect of obesity, as indicated by: (1) tanycyte loss in the arcuate nucleus and median eminence in obesity [164]; (2) tanycyte retraction driven by changes in tanycyte mitochondrial function, including alterations in the mitochondrial import inner membrane translocase TIM50, heat shock protein (hsp)40, hsp60, hsp70, hsp90a and hsp110, coupled to raised levels of Agouti-related protein (AgRP), thereby increasing food intake [165,166]. Knockout of the glucose transporter (GLUT)2 in tanycytes disrupts the hypothalamic glucose-sensing mechanism, increasing food consumption and weight gain, indicating the powerful role that tanycytes have on systemic metabolism and the hypothalamic regulation of 'core' behaviors [167]. Such data would indicate a significant role of tanycytes in PCOS, perhaps especially when obesity is present, as well as indicating how changes in granulosa cells, such as increased AMH, modulates tanycyte and hypothalamic function. The ablation of tanycytes in mice increases weight gain, feeding and insulin insensitivity as well as visceral adiposity, further highlighting the importance of tanycytes to systemic metabolism, which is partly mediated by their capacity to sense and transport glucose and leptin [168].

Tanycytes are also important regulators of hypothalamic neurogenesis and gliogenesis being conceptualized as 'stem-like' cells, which may also be an aspect of tanycyte regulation of metabolism and reproduction. Tanycytes are classically subdivided into $\alpha$1, $\alpha$2, $\beta$1 and $\beta$2 tanycytes, although their plasticity would indicate that tanycyte phenotyping may simply reflect a gradation of responses to dynamic environmental changes [169]. $\alpha$-tanycytes seem to have true 'stem-cell' properties, whereas $\beta$-tanycytes seem to be more limited as 'neuronal progenitor cells', with tanycytes providing neurons and astrocytes for the median eminence and other hypothalamic nuclei, as well as for renewal of tanycytes lost at the barrier with the cerebrospinal fluid (CSF) [170]. Tanycyte morphology and putative subtype biomarkers show significant changes over aging, with heightened glial fibrillary acidic protein (GFAP) expression, most typical of astrocytes, being upregulated over age, whilst the brain-CSF barrier may also show gaps over aging where tanycytes have not been replaced [169]. Like astrocytes, tanycytes form networks that can transmit $Ca^{2+}$ waves triggered by ATP activation of purinergic P2Y1 receptors, indicating a coordinated regulation of hypothalamic processes. Tanycytes are proposed to have systemic anti-aging properties, possibly via their release of exosomal miRNAs into the CSF and/or their stem cell capacity [171]. These complex and interesting cells also regulate the blood-hypothalamic barrier and interact with the pituitary and thyroid gland in the regulation of core hypothalamic functions, including reproduction in males and females, metabolism, food/water intake and aggression [172–175].

The structural plasticity of tanycytes has been most extensively investigated, although it is highly likely that the plasticity in their morphology is accompanied by a complexity of alterations in cellular fluxes, metabolism and intercellular interactions. At diestrus, gonadotropin output is low due to GnRH secretory nerve terminals being completely ensheathed by tanycytes, thereby blocking GnRH release into the pituitary portal blood vessels. Tanycytes are structurally remodeled on the proestrus day allowing the preovulatory GnRH/luteinizing hormone (LH) surge. Tanycyte structural plasticity is therefore a crucial aspect of reproduction. Progesterone attenuates GnRH release via semaphoring (Sema)7A/B1-integrin-mediated ensheathment of GnRH nerve terminals by tanycytes, thereby suppressing the pulsatile increase in LH release [176]. In contrast, estradiol induces the release of endothelial nitric oxide (NO), leading to tanycyte retraction, coupled to an increase in cyclooxygenase (COX)1 and COX2-induced prostaglandin (PG)E2, which increases GnRH release [169]. Tanycytes are therefore major determinants of hormonal alterations and their treatment in PCOS.

The expression/activation/ablation of the tanycyte outer mitochondrial membrane protein, translocator protein-18 (TSPO), induces an AMPK-dependent lipophagy and increases ATP [177], indicating a significant role for tanycytic TSPO via macroautophagy/autophagy-regulated lipid metabolism. Interestingly, data in other cell types shows TSPO to 'crosstalk' with the mitochondrial aryl hydrocarbon receptor (AhR), with both showing heightened expression/activity when the other is knocked out, whilst both are required for optimal mitochondrial function [61]. Notably, TSPO effects at mitochondria may require activation of the BDNF-mammalian target of rapamycin (mTOR) pathway [178], suggesting that the AhR induction of N-acetylserotonin (NAS), a BDNF mimic at plasma membrane and mitochondria located TrkB, may play a significant role in the interactions of the AhR and TSPO in tanycytes. This would indicate a

possible role for the tanycyte mitochondria melatonergic pathway in the regulation of AhR and TSPO 'crosstalk' in tanycytes and therefore in the regulation of systemic metabolism. Overall, wider data on tanycytes highlights the role of alterations in tanycyte mitochondrial proteins in the regulation of systemic metabolism and implicates a possible significant role for the putative tanycyte mitochondrial melatonergic pathway. Tanycyte regulation is therefore a crucial aspect of hormone-linked reproduction and metabolism/obesity, which are core classical features of PCOS symptomatology.

Interestingly, the pineal gland, via the pineal recess in the posterodorsal aspect of the third ventricle releases melatonin (and presumably NAS) directly into the cerebrospinal fluid (CSF), with prolonged and heightened (fourfold) melatonin levels evident at night in the third ventricle compared to the general circulation [16]. This is proposed to allow melatonin to have a stronger influence on the circadian rhythm via heightened effects at the hypothalamic suprachiasmatic nucleus (SCN) [16]. However, melatonin released into the third ventricle will have direct effects on the cells that predominantly line this ventricle, namely tanycytes. This suggests that decreased pineal melatonin in PCOS [2] will have significant impacts on hypothalamic function, including via suppressed melatonin effects on tanycytes and tanycyte mitochondrial function. Given the importance of tanycyte mitochondrial function to hypothalamic regulation, tanycyte retraction and systemic metabolism, suppressed pineal melatonin in the third ventricle will deprive tanycytes of melatonin's mitochondria-optimizing effects. It is unknown, although highly likely, whether tanycytes express the melatonergic pathway, nor is it known whether variations in pineal N-acetylserotonin (NAS)/melatonin ratio effluxed into the third ventricle would differentially modulate tanycyte and wider hypothalamic function. BDNF, TrkB-FL and TrkB-T1 are expressed in tanycytes and adjacent hypothalamic astrocytes [179], suggesting that pineal NAS, as well as the O-demethylation of melatonin to NAS by aryl hydrocarbon receptor (AhR)-induced CYP1A2 and CYP1B1, will activate tanycyte TrkB-FL and/or TrkB-T1. Both TrkB-FL and TrkB-T1 can be expressed in the plasma membrane and mitochondrial membrane, indicating diverse effects on mitochondrial function and patterned gene transcription that may be dependent upon the chaperoning of TrkB to mitochondria and therefore the state of cell and intercellular processes that would regulate the site of TrkB isoforms. This study also shows stress to be associated with BDNF upregulation in tanycytes and adjacent astrocytes [179], indicating that BDNF and TrkB activation in tanycytes and adjacent astrocytes are an integral aspect of the hypothalamic stress plasticity response [36,37,165].

Melatonin, both pineal and local, may act to modulate BDNF/TrkB driven stress plasticity during metabolic regulation and possibly wider hypothalamic regulatory functions [51]. Whether an increased pineal NAS/melatonin ratio would prime tanycytes and adjacent astrocytes to a different stress response via NAS activation of TrkB will be important to determine [36,37,165]. AhR activation is a significant driver of a heightened NAS/melatonin ratio, with the AhR an important modulator of tanycyte function, including tanycyte capacity as stem cells following insult, as shown in preclinical models [180]. AhR activation is also strongly associated with dysregulated GnRH neuronal function and GnRH release coupled to suppression of the circadian gene, Period (Per)1 [181]. Much of this data has been collected following TCDD-induced AhR activation and requires investigation as to the effects of kynurenine activation of the AhR and the consequences this has for the NAS/melatonin ratio, including in hypothalamic GnRH neurons [181]. The role of enhanced white adipocyte (WAT)-derived kynurenine in activating the tanycyte AhR, thereby altering tanycyte hormonal regulation requires investigation.

The aryl hydrocarbon receptor (AhR) induced CYP1A1/2 significantly regulate sex hormones, including estrogen metabolism to quinol, as well as the hydroxylation of estrogen, progesterone and testosterone [182]. Estrogen, via the estrogen receptor (ER)$\alpha$, downregulates AhR induced CYP1A1 via DNMT1 mediated epigenetic processes [183], whilst AhR activation downregulates ER$\alpha$ [184]. The AhR, via testosterone metabolism, also decreases androgen receptor levels [185], as well as suppressing porcine luteal cell progesterone [186]. Such AhR effects on the main sex hormones and their receptors associated with classical PCOS pathophysiology has wide systemic consequences, including for tanycyte regulation where estrogen and progesterone modulate tanycyte function, hormonal regulation and systemic metabolism [187]. Such data highlights the direct and indirect effects of AhR activation on tanycyte function in PCOS pathophysiology, and how the AhR levels, ligands and activation can be significantly determined by diverse systemic and CNS processes.

Unlike the endogenous AhR ligand, 6-formylindolo[3,2-b]carbazole (FICZ), the induced tryptophan-derived AhR ligands, kynurenine and kynurenic acid as well as the gut microbiome derived ligands, indole-3-propionate, indole-3-acetate, indole-3-lactate and indole-3-carboxaldehyde, are not metabolized by AhR-induced CYP1/2, suggesting that these induced ligands show a prolonged duration of action at the human AhR [188]. This would indicate that the prolonged AhR activation of these induced ligands would be dependent upon the AhR induction of its own repressor, the AhR repressor (AhRR) in order to prevent the heightened stress-linked plasticity in the hypothalamic paraventricular nucleus (PVN) by AhR-induced N-acetylserotonin (NAS) and BDNF. As noted, the AhRR is a PCOS susceptibility gene [23], indicating that the heightened AhR activation

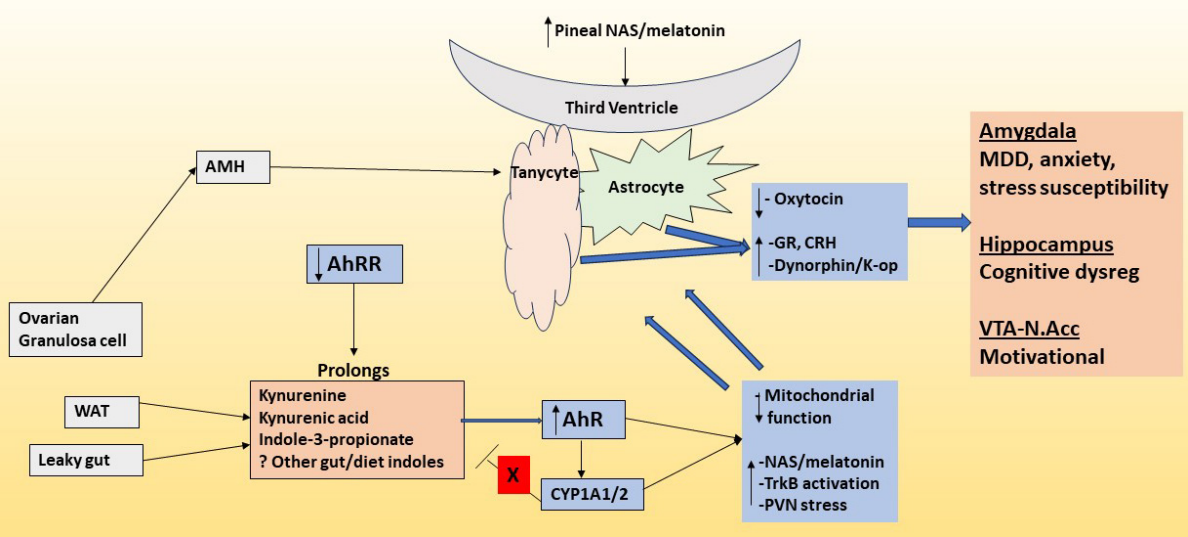


**Fig. 9. Hypothalamic tanycytes and astrocytes interact with systemic processes.** Shows how hypothalamic tanycytes and astrocytes not only interact with systemic processes to regulate gonadal hormones but are also important hubs for how adipocyte, gut, ovarian, and genetic factors drive PCOS mood, cognitive and motivational dysregulation. Aryl hydrocarbon receptor (AhR) ligands derived from white adipocyte (WAT) and the gut microbiome (and dietary) have prolonged effects on AhR activation due to the inability of AhR induced CYP1A1, CYP1B1, CYP1A2 to metabolize these AhR ligands. AhR normally induces its own repressor, AhRR, which is susceptibility gene for PCOS, with the susceptibility allelel leading to prolonged AhR activation. Consequent increases in the NAS/melatonin ratio and TrkB activation will drive paraventricular nucleus (PVN) stress-like plasticity, likely involving alterations in the mitochondrial function and mitochondrial melatonergic pathway in both tanycytes and astrocytes. The latter will be contributed to by the suppression of pineal melatonin, and possibly an increase in the pineal NAS/melatonin ratio, in the third ventricle. The ensuing alterations in PVN neuronal regulation include the suppression of oxytocin, thereby decreasing the oxytocin inhibition of glucocorticoid receptor (GR)/corticotrophin-releasing hormone (CRH)/dynorphin/$\kappa$-opioid receptor. This will drive mood and stress dysregulation in the amygdala, associated cognitive dysregulation in the hippocampus and motivational deficits in the VTA/N.Acc. Alterations in ovarian hormones, including AMH, will also alter tanycyte and astrocyte function. Abbreviations: AhR, aryl hydrocarbon receptor; AhRR, aryl hydrocarbon receptor repressor; AMH, anti-mullerian hormone; CRH, corticotrophin releasing hormone; GR, glucocorticoid receptor; k-op, $\kappa$-opioid receptor; MDD, major depressive disorder; N.Acc, nucleus accumbens; NAS, N-acetylserotonin; PVN, paraventricular nucleus; TrkB, tyrosine kinase receptor B; VTA, ventral tegmental area; WAT, white adipocyte.

and induced AhR ligands in PCOS will have prolonged effects. These prolonged effects include driving changes in tanycyte and PVN astrocyte mitochondrial melatonergic pathway, allowing prolonged AhR activation to increase the NAS/melatonin ratio and drive stress linked PVN plasticity. Such enhanced PVN plasticity will have concurrent consequences for mitochondrial function in tanycytes and astrocytes, with impacts on patterned hypothalamic neuronal interactions and PVN fluxes. Of the induced gut microbiome derived tryptophan metabolites, only indole-3-proptionate seems to achieve measurable circulatory levels, suggesting the circulating kynurenine and not gut-derived AhR ligands will be clinically relevant [188]. See Fig. 9.

Overall, the presence of TrkB-FL and TrkB-T1 in tanycytes and adjacent astrocytes allow variations in pineal (and local) NAS/melatonin ratio to modulate core aspects of hypothalamic function [179]. Data on the role of the AhR in PCOS pathophysiology would indicate that AhR suppression of melatonin, coupled to increased N-acetylserotonin (NAS) and BDNF activation of TrkB in hypothalamic tanycytes and astrocytes will contribute to a heightened 'stress' plasticity response in the hypothalamus, as well as in granulosa cells (see Fig. 2). This will alter the nature of the fluxes between the hypothalamus and granulosa cell/oocyte complex, such as increased granulosa cell anti-Mullerian hormone (AMH) to tanycytes thereby leading to tanycyte retraction and heightened GnRH levels to enhance pituitary luteinizing hormone release [1]. See Fig. 7. Systemic processes in adipocytes, gut microbiome and the cortisol awakening response (CAR) will also be influenced by alterations in the dynamic interactions of hypothalamic tanycytes and astrocytes with the granulosa cell/oocyte complex. As indicated in Fig. 6, the interactions of tanycytes with astrocytes in the regulation of neuronal function in the paraventricular nucleus (PVN), especially as influenced by oxytocin on PVN astrocytes, will be a significant determinant of the common mood dysregulation evident in many PCOS patients. Such data would strongly suggest that depression, anxiety and systemic metabolic dysregulation are not PCOS

'comorbidities' but are aspects of tanycyte and astrocyte regulation of hypothalamic neuronal function, which may ultimately be determined by systemic and circadian factors influencing mitochondrial function in hypothalamic astrocytes and tanycytes, perhaps especially the mitochondrial melatonergic pathway.

## 7. Future Research Implications

A number of future research directions have been indicated throughout the article. A couple are worth highlighting:

- Is the aryl hydrocarbon receptor (AhR) evident in tanycytes?

- Is BAG-1 present in brown adipocyte (BAT) and white adipocyte (WAT), with differential consequences for glucocorticoid receptor (GR) translocation to the nucleus versus mitochondria?

- Does the availability of night-time butyrate, like melatonin, modulate the rising cortisol levels at night and morning cortisol awakening response (CAR) as well as stress-linked hypothalamic-pituitary-adrenal (HPA) axis activation?

- Is the TrkB activation in hypothalamic tanycytes, astrocytes and neurons mediated via TrkB-FL, and/or TrkB-T1 at the plasma and/or mitochondrial membrane? Are TrkB isoforms and site integral aspects of hypothalamic plasticity dependent upon mitochondrial function in a given cell?

- Recent work has indicated a role for glyphosate-based herbicides (GBH) in the pathoetiology of amyotrophic lateral sclerosis (ALS) [189]. GBH suppress aromatase [190,191], and alters the histological function of the ovaries, including granulosa cells and oocytes, in preclinical models [192,193] as well as inducing gut dysbiosis [189], suppressing adipocyte differentiation [194] and dysregulating hypothalamic reproductive hormones [195]. The role of GBH in PCOS pathoetiology and ongoing pathophysiology, perhaps especially in the lean PCOS phenotype, will be important to determine.

## 8. Treatment Implications

- Melatonin upregulates aromatase and the conversion of testosterone to estrogen in granulosa cells [4,22]. Melatonin also stimulates progesterone from human and bovine granulosa cells [196], thereby upregulating the progesterone deficiency that is evident in PCOS patients [197]. The utility of melatonin (2 mg for 6 months) on PCOS symptomatology more widely [5], including in the gut microbiome/permeability, adipocytes, hypothalamus and granulosa cell-oocyte complex as well as its suppression of the glucocorticoid receptor (GR) indicate that it is underutilized in PCOS treatment. Optimization of melatonin dose, timing and mode of application will be important to determine in future clinical trials of PCOS.

- The refinement of currently available technologies/treatment, such as MSC-derived exosomes, that target phenotypes of particular cells would allow for the tryptophan-melatonin pathway to modulated in given cells, such as granulosa cells, tanycytes or white adipocytes (WATs). This would modulate the influence that these cells have on systemic processes, including by action at night in the regulation of night-time cortisol and the morning cortisol awakening response (CAR), thereby optimizing the circadian and systemic processes and suppressing PCOS symptomatology. Melatonin treated MSC shows significant alterations in exosome constituents [198], which may also allow a readily available improved targeting of cellular processes via different miRNAs. However, the targeted upregulation of the mitochondrial melatonergic pathway in particular phenotypes of given cells would induce a level of precision, ultimately driving alterations in the local microenvironment intercellular interactions. This is a feasible target for technological development relevant to the pathophysiological processes underpinning a host of diverse medical conditions, including cancer, neurodegenerative disorders and 'autoimmune'/'immune-mediated' conditions [51,70,199].

- A recent meta-analysis of probiotic use in PCOS management indicates beneficial effects on metabolic aspects of PCOS symptomatology, including body mass index, fasting plasma glucose, and lipid profiles [200]. The gut microbiome is a significant treatment target, including by the utilization of sodium butyrate.

- A number of nutriceuticals that inhibit the aryl hydrocarbon receptor (AhR), including epigallocatechin gallate (EGCG) [201], resveratrol [202], curcumin [203], folate [204], vitamin B12 [205], and propolis [206] show efficacy in suppressing PCOS symptomatology. However, none of the PCOS studies of these AhR antagonists have investigated or implicated a role for the AhR. As well as having ready utility in helping to alleviate PCOS symptomatology, it will be important for future research to clarify the role of the AhR and its modulation of core processes of PCOS pathophysiology by beneficial nutriceuticals.

- Alterations in autonomic nervous system activity are common in PCOS [207], with the hypothalamic paraventricular nucleus (PVN) acting as the 'conductor' of the autonomic nervous system [208]. Recent data indicates an increase in the sympathetic/parasympathetic nervous system ratio in PCOS, with clinical utility of transcutaneous auricular vagal nerve stimulation [209] These authors propose that such vagal nerve stimulation has already shown efficacy for isolated PCOS symptoms, including obesity, insulin resistance, T2DM, depression, and gut microbiome symptoms, as well as cardiovascular disease [209], and therefore is likely to favorably regulate many of the systemic changes occurring in PCOS.

## 9. Conclusions

The conceptualization of PCOS pathophysiology and derived treatment has focused on hormonal alterations and weight loss, leaving many PCOS patients with a feeling that the sexism of the 'wandering womb' may still be present in medical practice [2]. This article has reviewed in some detail the wider systemic and CNS changes in PCOS, providing a conceptualization based on 'gender-neutral' processes that underpin how hormones are dysregulated in PCOS, as well as how the common comorbidities of PCOS such as obesity and mood disorders can be integrated as aspects of PCOS pathophysiology rather than distinct 'comorbidities'. The article highlights the interactions of the local and pineal melatonergic pathway, gut microbiome-derived butyrate, white adipocyte-derived kynurenine, paraventricular nucleus (PVN) tanycytes and astrocytes, the hypothalamus-pituitary-adrenal (HPA) axis and night-time glucocorticoid receptor (GR) regulation in PCOS pathophysiology. A significant role is indicated for the night-time interactions of factors, such as melatonin, butyrate and BAG-1, which can suppress GR nuclear translocation in the course of the morning cortisol awakening response (CAR). The dynamic interactions of the above processes at different sites and in different cell types may ultimately be having their major impacts by regulating how the night-time cortisol rise and morning CAR modulate systemic and CNS cells, as the body is 'prepared for the coming day'. Given the powerful role of reactive cells, such as immune and glial cells, in determining the function of other systemic and CNS cells, the actions of night-time cortisol and morning CAR on these cells will be important to clarify, especially how CAR is modulated by variations in night-time melatonin, butyrate, and BAG-1 as well as the white adipocyte (WAT)-derived kynurenine activation of the aryl hydrocarbon receptor (AhR). All of these factors not only regulate the GR but also mitochondrial function, thereby significantly impacting on the determining role of mitochondrial metabolism in the regulation of glia, immune and tanycyte function. The mitochondrial regulation by melatonin, butyrate, BAG-1 and kynurenine/AhR are intimately linked to the mitochondrial melatonergic pathway. This provides a conceptualization that integrates a wide array of previously disparate data on the biological underpinnings of PCOS, including how PCOS associates with many 'comorbidities'. The investigation of the numerous future research implications indicated should provide a less 'sexist' conceptualization of PCOS [2], with treatments targeted to core processes that underpin hormonal and weight dysregulation.

## Abbreviations

11$\beta$-HSD1, 11$\beta$-hydroxysteroid dehydrogenase type 1; 5-HT, serotonin; 5-HTTP, 5-hydroxytryptophan; $\alpha$7nAChR, alpha 7nicotinic acetylcholine receptor; AADC, aromatic-L-amino acid decarboxylase; AANAT, aralkylamine N-acetyltransferase; acetyl-CoA, acetyl-coenzyme A; ACTH, adrenocorticotropic hormone; AhR, aryl hydrocarbon receptor; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; ASMT, N-acetylserotonin O-methyltransferase; BAG-1, bcl-2 associated athanogene 1; BAT, brown adipocyte; BDNF, brain-derived neurotrophic factor; CAR, cortisol awakening response; CRH, corticotrophin releasing hormone; CSF, cerebrospinal fluid; CYP, cytochrome P450; DNMT1, DNA methyltransferase 1; FKBP4, FKBP prolyl isomerase 4; GR, glucocorticoid receptor; GRE, glucocorticoid receptor element; HDAC, histone deacetylase; HPA, hypothalamic-pituitary-adrenal; hsp, heat shock protein; IDO, indoleamine 2,3-dioxygenase; lnc, long non-coding; LAT-1, large amino acid transporter 1; mGluR, metabotropic glutamate receptor; MERTK, MER Proto-Oncogene, Tyrosine Kinase; MHC, major histocompatibility complex; mTOR, mammalian target of rapamycin; N.Acc, nucleus accumbens; NAS, N-acetylserotonin; NK, natural killer; OXPHOS, oxidative phosphorylation; P2Y1r, purinergic P2Y1 receptor; PCOS, polycystic ovary syndrome; PDC, pyruvate dehydrogenase complex; PINK1, PTEN-associated kinase 1; PVN, paraventricular nucleus; T1DM, type 1 diabetes mellitus; TCA, tricarboxylic acid; TDO, tryptophan 2,3-dioxygenase; TIM, mitochondrial import inner membrane translocase subunit; TOM, mitochondrial import outer receptor subunit; TPH, tryptophan hydroxylase; TrkB-FL, tyrosine receptor kinase B-full length; TrkB-T1, tyrosine receptor kinase B-truncated; VTA, ventral tegmental area; WAT, white adipocyte.

## Author Contributions

GA confirms sole responsibility for the following: study conception and design and manuscript writing. GA read and approved the final manuscript.

## Ethics Approval and Consent to Participate

Not applicable.


## Acknowledgment

Not applicable.

## Funding

This research received no external funding.


## Conflict of Interest

The author declares no conflict of interest.

## References


[1] Barbotin AL, Mimouni NEH, Kuchcinski G, Lopes R, Viard R, Rasika S, *et al*. Hypothalamic neuroglial plasticity is regulated by anti-Müllerian hormone and disrupted in polycystic ovary syndrome. EBioMedicine. 2023; 90: 104535.

[2] Dubrosa F, Sangiuolo K, Terala A, Cheng E, Wang X, O'Connor

M, *et al*. Hashtag Hormones: Characterizing PCOS-Related Content on TikTok. IMPE Abstracts. 2023; 96: P134.
[3] Li H, Liu M, Zhang C. Women with polycystic ovary syndrome (PCOS) have reduced melatonin concentrations in their follicles and have mild sleep disturbances. BMC Women's Health. 2022; 22: 79.
[4] Chu W, Li S, Geng X, Wang D, Zhai J, Lu G, *et al*. Long-term environmental exposure of darkness induces hyperandrogenism in PCOS *via* melatonin receptor 1A and aromatase reduction. Frontiers in Cell and Developmental Biology. 2022; 10: 954186.
[5] Tagliaferri V, Romualdi D, Scarinci E, Cicco SD, Florio CD, Immediata V, *et al*. Melatonin Treatment May Be Able to Restore Menstrual Cyclicity in Women With PCOS: A Pilot Study. Reproductive Sciences (Thousand Oaks, Calif.). 2018; 25: 269–275.
[6] Wang F, Zhang ZH, Xiao KZ, Wang ZC. Roles of Hypothalamic-Pituitary-Adrenal Axis and Hypothalamus-Pituitary-Ovary Axis in the Abnormal Endocrine Functions in Patients with Polycystic Ovary Syndrome. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. Acta Academiae Medicinae Sinicae. 2017; 39: 699–704.
[7] Luo S, Hou Y, Zhang Y, Feng L, Hunter RG, Yuan P, *et al*. Bag-1 mediates glucocorticoid receptor trafficking to mitochondria after corticosterone stimulation: Potential role in regulating affective resilience. Journal of Neurochemistry. 2021; 158: 358–372.
[8] Quiros I, Mayo JC, Garcia-Suarez O, Hevia D, Martin V, Rodríguez C, *et al*. Melatonin prevents glucocorticoid inhibition of cell proliferation and toxicity in hippocampal cells by reducing glucocorticoid receptor nuclear translocation. The Journal of Steroid Biochemistry and Molecular Biology. 2008; 110: 116–124.
[9] Anderson G. Why are aging and stress associated with dementia, cancer, and other diverse medical conditions? Role of pineal melatonin interactions with the HPA axis in mitochondrial regulation via BAG-1. Melatonin Research. 2023; 6: 345–371.
[10] Singh AK, Haldar C. Melatonin modulates glucocorticoid receptor mediated inhibition of antioxidant response and apoptosis in peripheral blood mononuclear cells. Molecular and Cellular Endocrinology. 2016; 436: 59–67.
[11] Kim M, Lee HA, Cho HM, Kang SH, Lee E, Kim IK. Histone deacetylase inhibition attenuates hepatic steatosis in rats with experimental Cushing's syndrome. The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2018; 22: 23–33.
[12] Zhu K, Zhang Y, Zhang J, Zhou F, Zhang L, Wang S, *et al*. Acetylation of Hsp90 reverses dexamethasone-mediated inhibition of insulin secretion. Toxicology Letters. 2020; 320: 19–27.
[13] Jeon H, Choi Y, Brännström M, Akin JW, Curry TE, Jo M. Cortisol/glucocorticoid receptor: a critical mediator of the ovulatory process and luteinization in human periovulatory follicles. Human Reproduction (Oxford, England). 2023; 38: 671–685.
[14] Čikoš Š, Babeľová J, Špirková A, Burkuš J, Kovaříková V, Šefčíková Z, *et al*. Glucocorticoid receptor isoforms and effects of glucocorticoids in ovulated mouse oocytes and preimplantation embryos†. Biology of Reproduction. 2019; 100: 351–364.
[15] Boutagouga Boudjadja M, Culotta I, De Paula GC, Harno E, Hunter J, Cavalcanti-de-Albuquerque JP, *et al*. Hypothalamic AgRP neurons exert top-down control on systemic TNF-$\alpha$ release during endotoxemia. Current Biology: CB. 2022; 32: 4699–4706.e4.
[16] Reiter RJ, Sharma R, Cucielo MS, Tan DX, Rosales-Corral S, Gancitano G, *et al*. Brain washing and neural health: role of age, sleep, and the cerebrospinal fluid melatonin rhythm. Cellular and Molecular Life Sciences: CMLS. 2023; 80: 88.
[17] Karasek M, Reiter RJ. Melatonin and aging. Neuro Endocrinology Letters. 2002; 23: 14–16.
[18] Patel R, Parmar N, Pramanik Palit S, Rathwa N, Ramachandran AV, Begum R. Diabetes mellitus and melatonin: Where are we? Biochimie. 2022; 202: 2–14.
[19] Iesanu MI, Zahiu CDM, Dogaru IA, Chitimus DM, Pircalabioru GG, Voiculescu SE, *et al*. Melatonin-Microbiome Two-Sided Interaction in Dysbiosis-Associated Conditions. Antioxidants (Basel, Switzerland). 2022; 11: 2244.
[20] González F, Considine RV, Abdelhadi OA, Acton AJ. Saturated Fat Ingestion Promotes Lipopolysaccharide-Mediated Inflammation and Insulin Resistance in Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology and Metabolism. 2019; 104: 934–946.
[21] da Silveira Cruz-Machado S, Pinato L, Tamura EK, Carvalho-Sousa CE, Markus RP. Glia-pinealocyte network: the paracrine modulation of melatonin synthesis by tumor necrosis factor (TNF). PloS One. 2012; 7: e40142.
[22] Cheng JC, Fang L, Li Y, Wang S, Li Y, Yan Y, *et al*. Melatonin stimulates aromatase expression and estradiol production in human granulosa-lutein cells: relevance for high serum estradiol levels in patients with ovarian hyperstimulation syndrome. Experimental & Molecular Medicine. 2020; 52: 1341–1350.
[23] Chung YK, Kim JJ, Hong MA, Hwang KR, Chae SJ, Yoon SH, *et al*. Association Between Polycystic Ovary Syndrome and the Polymorphisms of Aryl Hydrocarbon Receptor Repressor, Glutathione-S-transferase T1, and Glutathione-S-transferase M1 Genes. Gynecological Endocrinology: the Official Journal of the International Society of Gynecological Endocrinology. 2021; 37: 558–561.
[24] Kunitomi C, Harada M, Kusamoto A, Azhary JM, Nose E, Koike H, *et al*. Induction of aryl hydrocarbon receptor in granulosa cells by endoplasmic reticulum stress contributes to pathology of polycystic ovary syndrome. Molecular Human Reproduction. 2021; 27: gaab003.
[25] Eini F, Kutenaei MA, Foroutan T, Salehi E. High levels of follicular fluid testosterone could impair oocyte developmental competency via affecting aryl hydrocarbon receptor pathway in PCOS patients. BMC Molecular and Cell Biology. 2022; 23: 47.
[26] Imbernon M, Saponaro C, Helms HCC, Duquenne M, Fernandois D, Deligia E, *et al*. Tanycytes control hypothalamic liraglutide uptake and its anti-obesity actions. Cell Metabolism. 2022; 34: 1054–1063.e7.
[27] Desroziers E. Unusual suspects: Glial cells in fertility regulation and their suspected role in polycystic ovary syndrome. Journal of Neuroendocrinology. 2022; 34: e13136.
[28] Bril F, Ezeh U, Amiri M, Hatoum S, Pace L, Chen YH, *et al*. Adipose Tissue Dysfunction in Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology and Metabolism. 2023; dgad356.
[29] Davoodvandi A, Nikfar B, Reiter RJ, Asemi Z. Melatonin and cancer suppression: insights into its effects on DNA methylation. Cellular & Molecular Biology Letters. 2022; 27: 73.
[30] Tobeiha M, Jafari A, Fadaei S, Mirazimi SMA, Dashti F, Amiri A, *et al*. Evidence for the Benefits of Melatonin in Cardiovascular Disease. Frontiers in Cardiovascular Medicine. 2022; 9: 888319.
[31] Anderson G. Type I Diabetes Pathoetiology and Pathophysiology: Roles of the Gut Microbiome, Pancreatic Cellular Interactions, and the 'Bystander' Activation of Memory CD8$^+$ T Cells. International Journal of Molecular Sciences. 2023; 24: 3300.
[32] Anderson G, Maes M. Interactions of Tryptophan and Its Catabolites With Melatonin and the Alpha 7 Nicotinic Receptor in Central Nervous System and Psychiatric Disorders: Role of the Aryl Hydrocarbon Receptor and Direct Mitochondria Regulation. International Journal of Tryptophan Research: IJTR. 2017; 10: 1178646917691738.

[33] Mokkawes T, de Visser SP. Melatonin Activation by Cytochrome P450 Isozymes: How Does CYP1A2 Compare to CYP1A1? International Journal of Molecular Sciences. 2023; 24: 3651.
[34] Ma X, Idle JR, Krausz KW, Gonzalez FJ. Metabolism of melatonin by human cytochromes p450. Drug Metabolism and Disposition: the Biological Fate of Chemicals. 2005; 33: 489–494.
[35] Jang SW, Liu X, Pradoldej S, Tosini G, Chang Q, Iuvone PM, *et al*. N-acetylserotonin activates TrkB receptor in a circadian rhythm. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107: 3876–3881.
[36] Sosanya NM, Garza TH, Stacey W, Crimmins SL, Christy RJ, Cheppudira BP. Involvement of brain-derived neurotrophic factor (BDNF) in chronic intermittent stress-induced enhanced mechanical allodynia in a rat model of burn pain. BMC Neuroscience. 2019; 20: 17.
[37] Schaich CL, Wellman TL, Einwag Z, Dutko RA, Erdos B. Inhibition of BDNF signaling in the paraventricular nucleus of the hypothalamus lowers acute stress-induced pressor responses. Journal of Neurophysiology. 2018; 120: 633–643.
[38] Jeanneteau FD, Lambert WM, Ismaili N, Bath KG, Lee FS, Garabedian MJ, *et al*. BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 1305–1310.
[39] Vanuytsel T, van Wanrooy S, Vanheel H, Vanormelingen C, Verschueren S, Houben E, *et al*. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut. 2014; 63: 1293–1299.
[40] Winge I, McKinney JA, Ying M, D'Santos CS, Kleppe R, Knappskog PM, *et al*. Activation and stabilization of human tryptophan hydroxylase 2 by phosphorylation and 14-3-3 binding. The Biochemical Journal. 2008; 410: 195–204.
[41] Shafiei G, Moghani-Ghoroghi F, Miyan J, Almasi M, Kashani IR, Nikzad H, *et al*. Melatonin protects against visible light-induced oxidative stress and promotes the implantation potential of mouse blastocyst in vitro. Research in Veterinary Science. 2023; 155: 29–35.
[42] Chen M, Hui S, Lang H, Zhou M, Zhang Y, Kang C, *et al*. SIRT3 Deficiency Promotes High-Fat Diet-Induced Nonalcoholic Fatty Liver Disease in Correlation with Impaired Intestinal Permeability through Gut Microbial Dysbiosis. Molecular Nutrition & Food Research. 2019; 63: e1800612.
[43] Kang LL, Zhang DM, Jiao RQ, Pan SM, Zhao XJ, Zheng YJ, *et al*. Pterostilbene Attenuates Fructose-Induced Myocardial Fibrosis by Inhibiting ROS-Driven Pitx2c/miR-15b Pathway. Oxidative Medicine and Cellular Longevity. 2019; 2019: 1243215.
[44] Kang JH, Guo XD, Wang YD, Kang XW. Neuroprotective Effects of N-acetylserotonin and Its Derivative. Neuroscience. 2023; 517: 18–25.
[45] Anderson G, Reiter RJ. Glioblastoma: Role of Mitochondria N-acetylserotonin/Melatonin Ratio in Mediating Effects of miR-451 and Aryl Hydrocarbon Receptor and in Coordinating Wider Biochemical Changes. International Journal of Tryptophan Research: IJTR. 2019; 12: 1178646919855942.
[46] Anderson G. Endometriosis Pathoetiology and Pathophysiology: Roles of Vitamin A, Estrogen, Immunity, Adipocytes, Gut Microbiome and Melatonergic Pathway on Mitochondria Regulation. Biomolecular Concepts. 2019; 10: 133–149.
[47] Markus RP, Silva CLM, Franco DG, Barbosa EM, Jr, Ferreira ZS. Is modulation of nicotinic acetylcholine receptors by melatonin relevant for therapy with cholinergic drugs? Pharmacology & Therapeutics. 2010; 126: 251–262.
[48] Benson S, Arck PC, Tan S, Hahn S, Mann K, Rifaie N, *et al*. Disturbed stress responses in women with polycystic ovary syndrome. Psychoneuroendocrinology. 2009; 34: 727–735.
[49] Stalder T, Lupien SJ, Kudielka BM, Adam EK, Pruessner JC, Wüst S, *et al*. Evaluation and update of the expert consensus guidelines for the assessment of the cortisol awakening response (CAR). Psychoneuroendocrinology. 2022; 146: 105946.
[50] Zhao X, Peng T, Cao X, Hou Y, Li R, Han T, *et al*. *In vivo* G-CSF treatment activates the GR-SOCS1 axis to suppress IFN-$\gamma$ secretion by natural killer cells. Cell Reports. 2022; 40: 111342.
[51] Anderson G. Melatonin, BAG-1 and cortisol circadian interactions in tumor pathogenesis and patterned immune responses. Exploration of Targeted Anti-tumor Therapy. 2023; 4: 962–993.
[52] Dwyer AR, Perez Kerkvliet C, Truong TH, Hagen KM, Krutilina RI, Parke DN, *et al*. Glucocorticoid Receptors Drive Breast Cancer Cell Migration and Metabolic Reprogramming via PDK4. Endocrinology. 2023; 164: bqad083.
[53] Shimba A, Ikuta K. Control of immunity by glucocorticoids in health and disease. Seminars in Immunopathology. 2020; 42: 669–680.
[54] Stojadinovic O, Sawaya A, Pastar I, Tomic-Canic M. Glucocorticoid receptor localizes to adherens junctions at the plasma membrane of keratinocytes. PloS One. 2013; 8: e63453.
[55] Noddings CM, Wang RYR, Johnson JL, Agard DA. Structure of Hsp90-p23-GR reveals the Hsp90 client-remodelling mechanism. Nature. 2022; 601: 465–469.
[56] Lammer NC, Ashraf HM, Ugay DA, Spencer SL, Allen MA, Batey RT, *et al*. RNA binding by the glucocorticoid receptor attenuates dexamethasone-induced gene activation. Scientific Reports. 2023; 13: 9385.
[57] Anderson G, Seo M, Berk M, Carvalho AF, Maes M. Gut Permeability and Microbiota in Parkinson's Disease: Role of Depression, Tryptophan Catabolites, Oxidative and Nitrosative Stress and Melatonergic Pathways. Current Pharmaceutical Design. 2016; 22: 6142–6151.
[58] Sládeková L, Mani S, Dvořák Z. Ligands and agonists of the aryl hydrocarbon receptor AhR: Facts and myths. Biochemical Pharmacology. 2023; 213: 115626.
[59] van den Brand AD, Rubinstein E, de Jong PC, van den Berg M, van Duursen M. Assessing anti-estrogenic effects of AHR ligands in primary human and rat endometrial epithelial cells. Reproductive Toxicology (Elmsford, N.Y.). 2020; 96: 202–208.
[60] Hwang HJ, Dornbos P, Steidemann M, Dunivin TK, Rizzo M, LaPres JJ. Mitochondrial-targeted aryl hydrocarbon receptor and the impact of 2,3,7,8-tetrachlorodibenzo-p-dioxin on cellular respiration and the mitochondrial proteome. Toxicology and Applied Pharmacology. 2016; 304: 121–132.
[61] Steidemann MM, Liu J, Bayes K, Castro LP, Ferguson-Miller S, LaPres JJ. Evidence for crosstalk between the aryl hydrocarbon receptor and the translocator protein in mouse lung epithelial cells. Experimental Cell Research. 2023; 429: 113617.
[62] Solvay M, Holfelder P, Klaessens S, Pilotte L, Stroobant V, Lamy J, *et al*. Tryptophan depletion sensitizes the AHR pathway by increasing AHR expression and GCN2/LAT1-mediated kynurenine uptake, and potentiates induction of regulatory T lymphocytes. Journal for Immunotherapy of Cancer. 2023; 11: e006728.
[63] Anderson G, Carbone A, Mazzoccoli G. Tryptophan Metabolites and Aryl Hydrocarbon Receptor in Severe Acute Respiratory Syndrome, Coronavirus-2 (SARS-CoV-2) Pathophysiology. International Journal of Molecular Sciences. 2021; 22: 1597.
[64] Vishwas DK, Mukherjee A, Haldar C. Melatonin improves humoral and cell-mediated immune responses of male golden hamster following stress induced by dexamethasone. Journal of Neuroimmunology. 2013; 259: 17–25.
[65] Pal Chowdhury J, Haldar C. Stress associated ovarian dysfunctions in a seasonal breeder Funambulus pennanti: Role of glucocorticoids and possible amelioration by melatonin. General and

Comparative Endocrinology. 2022; 316: 113962.
[66] Qin Y, Bai J, Dai J, Zhou J, Zhang T, Zhang S, *et al*. Alterations of Cortisol and Melatonin Production by the Theca Interna Cells of Porcine Cystic Ovarian Follicles. Animals: an Open Access Journal from MDPI. 2022; 12: 357.
[67] Giudice A, Aliberti SM, Barbieri A, Pentangelo P, Bisogno I, D'Arena G, *et al*. Potential Mechanisms by which Glucocorticoids Induce Breast Carcinogenesis through Nrf2 Inhibition. Frontiers in Bioscience (Landmark Edition). 2022; 27: 223.
[68] Shi XT, Zhu HL, Xu XF, Xiong YW, Dai LM, Zhou GX, *et al*. Gestational cadmium exposure impairs placental angiogenesis via activating GC/GR signaling. Ecotoxicology and Environmental Safety. 2021; 224: 112632.
[69] Zhou J, Zhang J, Luo X, Li M, Yue Y, Laudon M, *et al*. Neu-P11, a novel MT1/MT2 agonist, reverses diabetes by suppressing the hypothalamic-pituitary-adrenal axis in rats. European Journal of Pharmacology. 2017; 812: 225–233.
[70] Anderson G, Almulla AF, Reiter RJ, Maes M. Redefining Autoimmune Disorders' Pathoetiology: Implications for Mood and Psychotic Disorders' Association with Neurodegenerative and Classical Autoimmune Disorders. Cells. 2023; 12: 1237.
[71] Kim MJ, Choi GE, Chae CW, Lim JR, Jung YH, Yoon JH, *et al*. Melatonin-mediated FKBP4 downregulation protects against stress-induced neuronal mitochondria dysfunctions by blocking nuclear translocation of GR. Cell Death & Disease. 2023; 14: 146.
[72] Ma F, Zhang M, Gong W, Weng M, Quan Z. MiR-138 Suppresses Cell Proliferation by Targeting Bag-1 in Gallbladder Carcinoma. PloS One. 2015; 10: e0126499.
[73] Hou G, Chen H, Yin Y, Pan Y, Zhang X, Jia F. MEL Ameliorates Post-SAH Cerebral Vasospasm by Affecting the Expression of eNOS and HIF1$\alpha$ via H19/miR-138/eNOS/NO and H19/miR-675/HIF1$\alpha$. Molecular Therapy. Nucleic Acids. 2020; 19: 523–532.
[74] Chen Z, Huai Y, Chen G, Liu S, Zhang Y, Li D, *et al*. MiR-138-5p Targets MACF1 to Aggravate Aging-related Bone Loss. International Journal of Biological Sciences. 2022; 18: 4837–4852.
[75] Williams WR. Dampening of neurotransmitter action: molecular similarity within the melatonin structure. Endocrine Regulations. 2018; 52: 199–207.
[76] Wu H, Zhu Q, Li X, He Y, Wang Y, Lu Y, *et al*. The upregulation of 11$\beta$-HSD1 in ovarian granulosa cells by cortisol and interleukin-1$\beta$ in polycystic ovary syndrome. Gynecological Endocrinology: the Official Journal of the International Society of Gynecological Endocrinology. 2020; 36: 668–672.
[77] Zhang X, Wei Y, Li X, Li C, Zhang L, Liu Z, *et al*. The Corticosterone-Glucocorticoid Receptor-AP1/CREB Axis Inhibits the Luteinizing Hormone Receptor Expression in Mouse Granulosa Cells. International Journal of Molecular Sciences. 2022; 23: 12454.
[78] Nasiri-Amiri F, Faramarzi M, Omidvar S, Alizadeh-Navaei R. Depression and anxiety in adolescents and young women with polycystic ovary syndrome: a systematic review and meta-analysis. International Journal of Adolescent Medicine and Health. 2023; 35: 233–242.
[79] Sun Y, Gao S, Ye C, Zhao W. Gut microbiota dysbiosis in polycystic ovary syndrome: Mechanisms of progression and clinical applications. Frontiers in Cellular and Infection Microbiology. 2023; 13: 1142041.
[80] Gonzalez D, Maidana P, Ibar C, Jamardo J, Jacobsen D, Fritzler A, *et al*. Hair cortisol in polycystic ovary syndrome. Scientific Reports. 2022; 12: 10309.
[81] Xu Z, You W, Liu J, Wang Y, Shan T. Elucidating the Regulatory Role of Melatonin in Brown, White, and Beige Adipocytes. Advances in Nutrition (Bethesda, Md.). 2020; 11: 447–460.
[82] Salagre D, Chayah M, Molina-Carballo A, Oliveras-López MJ, Munoz-Hoyos A, Navarro-Alarcón M, *et al*. Melatonin induces fat browning by transdifferentiation of white adipocytes and *de novo* differentiation of mesenchymal stem cells. Food & Function. 2022; 13: 3760–3775.
[83] Wei T, Huang G, Liu P, Gao J, Huang C, Sun M, *et al*. Sirtuin 3-mediated pyruvate dehydrogenase activity determines brown adipocytes phenotype under high-salt conditions. Cell Death & Disease. 2019; 10: 614.
[84] Lerner A, Kewada D, Ahmed A, Hardy K, Christian M, Franks S. Androgen Reduces Mitochondrial Respiration in Mouse Brown Adipocytes: A Model for Disordered Energy Balance in Polycystic Ovary Syndrome. International Journal of Molecular Sciences. 2020; 22: 243.
[85] Gourronc FA, Markan KR, Kulhankova K, Zhu Z, Sheehy R, Quelle DE, *et al*. Pdgfr$\alpha$-Cre mediated knockout of the aryl hydrocarbon receptor protects mice from high-fat diet induced obesity and hepatic steatosis. PloS One. 2020; 15: e0236741.
[86] Cowan S, Lim S, Alycia C, Pirotta S, Thomson R, Gibson-Helm M, *et al*. Lifestyle management in polycystic ovary syndrome - beyond diet and physical activity. BMC Endocrine Disorders. 2023; 23: 14.
[87] Kroon J, Koorneef LL, van den Heuvel JK, Verzijl CRC, van de Velde NM, Mol IM, *et al*. Selective Glucocorticoid Receptor Antagonist CORT125281 Activates Brown Adipose Tissue and Alters Lipid Distribution in Male Mice. Endocrinology. 2018; 159: 535–546.
[88] Park YK, Ge K. Glucocorticoid Receptor Accelerates, but Is Dispensable for, Adipogenesis. Molecular and Cellular Biology. 2017; 37: e00260–16.
[89] Spaanderman DCE, Nixon M, Buurstede JC, Sips HC, Schilperoort M, Kuipers EN, *et al*. Androgens modulate glucocorticoid receptor activity in adipose tissue and liver. The Journal of Endocrinology. 2018. (online ahead of print)
[90] Huang T, Song J, Gao J, Cheng J, Xie H, Zhang L, *et al*. Adipocyte-derived kynurenine promotes obesity and insulin resistance by activating the AhR/STAT3/IL-6 signaling. Nature Communications. 2022; 13: 3489.
[91] Wu LN, Wei XW, Fan Y, Miao JN, Wang LL, Zhang Y, *et al*. Altered expression of 14-3-3$\zeta$ protein in spinal cords of rat fetuses with spina bifida aperta. PloS One. 2013; 8: e70457.
[92] Beischlag TV, Anderson G, Mazzoccoli G. Glioma: Tryptophan Catabolite and Melatoninergic Pathways Link microRNA, 14-3-3, Chromosome 4q35, Epigenetic Processes and other Glioma Biochemical Changes. Current Pharmaceutical Design. 2016; 22: 1033–1048.
[93] Németh H, Toldi J, Vécsei L. Role of kynurenines in the central and peripheral nervous systems. Current Neurovascular Research. 2005; 2: 249–260.
[94] Wang S, Mu L, Zhang C, Long X, Zhang Y, Li R, *et al*. Abnormal Activation of Tryptophan-Kynurenine Pathway in Women With Polycystic Ovary Syndrome. Frontiers in Endocrinology. 2022; 13: 877807.
[95] Cattelan Souza L, de Brito MLO, Jesse CR, Boeira SP, de Gomes MG, Goes ATR, *et al*. Involvement of kynurenine pathway in depressive-like behaviour induced by nandrolone decanoate in mice. Steroids. 2020; 164: 108727.
[96] Roh E, Kwak SH, Jung HS, Cho YM, Pak YK, Park KS, *et al*. Serum aryl hydrocarbon receptor ligand activity is associated with insulin resistance and resulting type 2 diabetes. Acta Diabetologica. 2015; 52: 489–495.
[97] Sakthiswary R, Uma Veshaaliini R, Chin KY, Das S, Sirasanagandla SR. Pathomechanisms of bone loss in rheumatoid arthritis. Frontiers in Medicine. 2022; 9: 962969.
[98] Pryce CR, Fontana A. Depression in Autoimmune Diseases. Current Topics in Behavioral Neurosciences. 2017; 31: 139–

154.
[99] Tanaka M, Tóth F, Polyák H, Szabó Á, Mándi Y, Vécsei L. Immune Influencers in Action: Metabolites and Enzymes of the Tryptophan-Kynurenine Metabolic Pathway. Biomedicines. 2021; 9: 734.
[100] de Melo LGP, Nunes SOV, Anderson G, Vargas HO, Barbosa DS, Galecki P, *et al*. Shared metabolic and immune-inflammatory, oxidative and nitrosative stress pathways in the metabolic syndrome and mood disorders. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2017; 78: 34–50.
[101] Anderson G, Maes M. Oxidative/nitrosative stress and immuno-inflammatory pathways in depression: treatment implications. Current Pharmaceutical Design. 2014; 20: 3812–3847.
[102] Dai W, Liu J, Qiu Y, Teng Z, Li S, Huang J, *et al*. Shared postulations between bipolar disorder and polycystic ovary syndrome pathologies. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2022; 115: 110498.
[103] Kim JJ, Sapio MR, Vazquez FA, Maric D, Loydpierson AJ, Ma W, *et al*. Transcriptional Activation, Deactivation and Rebound Patterns in Cortex, Hippocampus and Amygdala in Response to Ketamine Infusion in Rats. Frontiers in Molecular Neuroscience. 2022; 15: 892345.
[104] Nunes EJ, Addy NA. L-type calcium channel regulation of dopamine activity in the ventral tegmental area to nucleus accumbens pathway: Implications for substance use, mood disorders and co-morbidities. Neuropharmacology. 2023; 224: 109336.
[105] Anderson G. Depression Pathophysiology: Astrocyte Mitochondrial Melatonergic Pathway as Crucial Hub. International Journal of Molecular Sciences. 2022; 24: 350.
[106] Anderson G, Maes M. Role of Opioidergic System in Regulating Depression Pathophysiology. Current Pharmaceutical Design. 2020; 26: 5317–5334.
[107] Tsushima H, Zhang Y, Muratsubaki T, Kanazawa M, Fukudo S. Oxytocin antagonist induced visceral pain and corticotropin-releasing hormone neuronal activation in the central nucleus of the amygdala during colorectal distention in mice. Neuroscience Research. 2021; 168: 41–53.
[108] Wahis J, Baudon A, Althammer F, Kerspern D, Goyon S, Hagiwara D, *et al*. Astrocytes mediate the effect of oxytocin in the central amygdala on neuronal activity and affective states in rodents. Nature Neuroscience. 2021; 24: 529–541.
[109] Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS, Muglia LJ. Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 12004–12009.
[110] Bruchas MR, Land BB, Lemos JC, Chavkin C. CRF1-R activation of the dynorphin/kappa opioid system in the mouse basolateral amygdala mediates anxiety-like behavior. PloS One. 2009; 4: e8528.
[111] Anderson G. Pathoetiology and pathophysiology of borderline personality: Role of prenatal factors, gut microbiome, mu- and kappa-opioid receptors in amygdala-PFC interactions. Progress in Neuro-psychopharmacology & Biological Psychiatry. 2020; 98: 109782.
[112] Robble MA, Bozsik ME, Wheeler DS, Wheeler RA. Learned avoidance requires VTA KOR-mediated reductions in dopamine. Neuropharmacology. 2020; 167: 107996.
[113] Musardo S, Contestabile A, Knoop M, Baud O, Bellone C. Oxytocin neurons mediate the effect of social isolation via the VTA circuits. eLife. 2022; 11: e73421.
[114] Amin M, Horst N, Wu R, Gragnoli C. Oxytocin receptor (OXTR) is a risk gene for polycystic ovarian syndrome. European Review for Medical and Pharmacological Sciences. 2023; 27: 2634–2639.
[115] Althammer F, Roy RK, Lefevre A, Najjar RS, Schoenig K, Bartsch D, *et al*. Altered PVN-to-CA2 hippocampal oxytocin pathway and reduced number of oxytocin-receptor expressing astrocytes in heart failure rats. Journal of Neuroendocrinology. 2022; 34: e13166.
[116] Jahromi BN, Dabbaghmanesh MH, Bakhshaie P, Parsanezhad ME, Anvar Z, Alborzi M, *et al*. Assessment of oxytocin level, glucose metabolism components and cutoff values for oxytocin and anti-mullerian hormone in infertile PCOS women. Taiwanese Journal of Obstetrics & Gynecology. 2018; 57: 555–559.
[117] Ivy AS, Rex CS, Chen Y, Dubé C, Maras PM, Grigoriadis DE, *et al*. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2010; 30: 13005–13015.
[118] Jiang J, Yang M, Tian M, Chen Z, Xiao L, Gong Y. Intertwined associations between oxytocin, immune system and major depressive disorder. Biomedicine & Pharmacotherapy. 2023; 163: 114852.
[119] Claes SJ. CRH, stress, and major depression: a psychobiological interplay. Vitamins and Hormones. 2004; 69: 117–150.
[120] Franklin DJ, Grossberg S. A neural model of normal and abnormal learning and memory consolidation: adaptively timed conditioning, hippocampus, amnesia, neurotrophins, and consciousness. Cognitive, Affective & Behavioral Neuroscience. 2017; 17: 24–76.
[121] Zadra JR, Proffitt DR. Implicit associations have a circadian rhythm. PloS One. 2014; 9: e110149.
[122] Hidalgo V, Villada C, Almela M, Espín L, Gómez-Amor J, Salvador A. Enhancing effects of acute psychosocial stress on priming of non-declarative memory in healthy young adults. Stress (Amsterdam, Netherlands). 2012; 15: 329–338.
[123] Mukherjee AG, Wanjari UR, Kannampuzha S, Murali R, Namachivayam A, Ganesan R, *et al*. The Implication of Mechanistic Approaches and the Role of the Microbiome in Polycystic Ovary Syndrome (PCOS): A Review. Metabolites. 2023; 13: 129.
[124] Guo J, Shao J, Yang Y, Niu X, Liao J, Zhao Q, *et al*. Gut Microbiota in Patients with Polycystic Ovary Syndrome: a Systematic Review. Reproductive Sciences (Thousand Oaks, Calif.). 2022; 29: 69–83.
[125] Moser B, Milligan MA, Dao MC. The Microbiota-Gut-Brain Axis: Clinical Applications in Obesity and Type 2 Diabetes. Revista De Investigacion Clinica; Organo Del Hospital De Enfermedades De La Nutricion. 2022; 74: 302–313.
[126] Ibáñez L, de Zegher F. Adolescent PCOS: a postpubertal central obesity syndrome. Trends in Molecular Medicine. 2023; 29: 354–363.
[127] Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. Current Obesity Reports. 2019; 8: 317–332.
[128] Liang Y, Ming Q, Liang J, Zhang Y, Zhang H, Shen T. Gut microbiota dysbiosis in polycystic ovary syndrome: association with obesity - a preliminary report. Canadian Journal of Physiology and Pharmacology. 2020; 98: 803–809.
[129] Aleshin VA, Artiukhov AV, Kaehne T, Graf AV, Bunik VI. Daytime Dependence of the Activity of the Rat Brain Pyruvate Dehydrogenase Corresponds to the Mitochondrial Sirtuin 3 Level and Acetylation of Brain Proteins, All Regulated by Thiamine Administration Decreasing Phosphorylation of PDHA Ser293. International Journal of Molecular Sciences. 2021; 22: 8006.

[130] Anderson G, Maes M. Gut Dysbiosis Dysregulates Central and Systemic Homeostasis via Suboptimal Mitochondrial Function: Assessment, Treatment and Classification Implications. Current Topics in Medicinal Chemistry. 2020; 20: 524–539.
[131] Jin CJ, Engstler AJ, Sellmann C, Ziegenhardt D, Landmann M, Kanuri G, *et al*. Sodium butyrate protects mice from the development of the early signs of non-alcoholic fatty liver disease: role of melatonin and lipid peroxidation. The British Journal of Nutrition. 2016; 116: 1682–1693.
[132] Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, *et al*. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. Nature Communications. 2020; 11: 4457.
[133] Luo M, Chen Y, Pan X, Chen H, Fan L, Wen Y. *E. coli Nissle 1917* ameliorates mitochondrial injury of granulosa cells in polycystic ovary syndrome through promoting gut immune factor IL-22 *via* gut microbiota and microbial metabolism. Frontiers in Immunology. 2023; 14: 1137089.
[134] Aksun S, Ersal E, Portakal O, Yildiz BO. Interleukin-22/Interleukin-22 binding protein axis and oral contraceptive use in polycystic ovary syndrome. Endocrine. 2023; 81: 54–57.
[135] Gao Z, Wang G, Ma X, Tan H, Zhang C, Yin X, *et al*. Troxerutin attenuates insulin resistance via pancreatic IL-22/JAK1/STAT3 signaling activation in dihydrotestosterone-induced polycystic ovary syndrome rats. American Journal of Physiology. Endocrinology and Metabolism. 2022; 323: E405–E417.
[136] Gao T, Wang Z, Cao J, Dong Y, Chen Y. Melatonin attenuates microbiota dysbiosis of jejunum in short-term sleep deprived mice. Journal of Microbiology (Seoul, Korea). 2020; 58: 588–597.
[137] Yu J, Lou Y, He K, Yang S, Yu W, Han L, *et al*. Goose broodiness is involved in granulosa cell autophagy and homeostatic imbalance of follicular hormones. Poultry Science. 2016; 95: 1156–1164.
[138] Isa AM, Sun Y, Li Y, Wang Y, Ni A, Yuan J, *et al*. MicroRNAs with non-additive expression in the ovary of hybrid hens target genes enriched in key reproductive pathways that may influence heterosis for egg laying traits. Frontiers in Genetics. 2022; 13: 974619.
[139] Liu K, He X, Huang J, Yu S, Cui M, Gao M, *et al*. Short-chain fatty acid-butyric acid ameliorates granulosa cells inflammation through regulating METTL3-mediated N6-methyladenosine modification of FOSL2 in polycystic ovarian syndrome. Clinical Epigenetics. 2023; 15: 86.
[140] Dai LN, Yan JK, Xiao YT, Wen J, Zhang T, Zhou KJ, *et al*. Butyrate stimulates the growth of human intestinal smooth muscle cells by activation of yes-associated protein. Journal of Cellular Physiology. 2018; 233: 3119–3128.
[141] Mehta A, Ravinder, Onteru SK, Singh D. HDAC inhibitor prevents LPS mediated inhibition of CYP19A1 expression and 17$\beta$-estradiol production in granulosa cells. Molecular and Cellular Endocrinology. 2015; 414: 73–81.
[142] Cricrí D, Coppi L, Pedretti S, Mitro N, Caruso D, De Fabiani E, *et al*. Histone Deacetylase 3 Regulates Adipocyte Phenotype at Early Stages of Differentiation. International Journal of Molecular Sciences. 2021; 22: 9300.
[143] Ferrari A, Longo R, Peri C, Coppi L, Caruso D, Mai A, *et al*. Inhibition of class I HDACs imprints adipogenesis toward oxidative and brown-like phenotype. Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids. 2020; 1865: 158594.
[144] Ma J, Wang Y, Ding J, Zhang S, Yang Y, Sun C. SAHA induces white fat browning and rectifies metabolic dysfunctions via activation of ZFPs. The Journal of Endocrinology. 2021; 249: 177–193.
[145] Fawad JA, Luzader DH, Hanson GF, Moutinho TJ, Jr, McKinney CA, Mitchell PG, *et al*. Histone Deacetylase Inhibition by Gut Microbe-Generated Short-Chain Fatty Acids Entrains Intestinal Epithelial Circadian Rhythms. Gastroenterology. 2022; 163: 1377–1390.e11.
[146] Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, *et al*. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host & Microbe. 2015; 17: 681–689.
[147] Yin J, Li Y, Han H, Ma J, Liu G, Wu X, *et al*. Administration of Exogenous Melatonin Improves the Diurnal Rhythms of the Gut Microbiota in Mice Fed a High-Fat Diet. MSystems. 2020; 5: e00002–20.
[148] van Deuren T, Smolders L, Hartog A, Bouwman FG, Holst JJ, Venema K, *et al*. Butyrate and hexanoate-enriched triglycerides increase postprandrial systemic butyrate and hexanoate in men with overweight/obesity: A double-blind placebo-controlled randomized crossover trial. Frontiers in Nutrition. 2023; 9: 1066950.
[149] Yoo JM, Lee BD, Sok DE, Ma JY, Kim MR. Neuroprotective action of N-acetyl serotonin in oxidative stress-induced apoptosis through the activation of both TrkB/CREB/BDNF pathway and Akt/Nrf2/Antioxidant enzyme in neuronal cells. Redox Biology. 2017; 11: 592–599.
[150] Modoux M, Rolhion N, Lefevre JH, Oeuvray C, Nádvorník P, Illes P, *et al*. Butyrate acts through HDAC inhibition to enhance aryl hydrocarbon receptor activation by gut microbiota-derived ligands. Gut Microbes. 2022; 14: 2105637.
[151] You D, Wen X, Gorczyca L, Morris A, Richardson JR, Aleksunes LM. Increased MDR1 Transporter Expression in Human Brain Endothelial Cells Through Enhanced Histone Acetylation and Activation of Aryl Hydrocarbon Receptor Signaling. Molecular Neurobiology. 2019; 56: 6986–7002.
[152] Poutahidis T, Kearney SM, Levkovich T, Qi P, Varian BJ, Lakritz JR, *et al*. Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. PloS One. 2013; 8: e78898.
[153] Dooling SW, Sgritta M, Wang IC, Duque ALRF, Costa-Mattioli M. The Effect of *Limosilactobacillus reuteri* on Social Behavior Is Independent of the Adaptive Immune System. MSystems. 2022; 7: e0035822.
[154] Danhof HA, Lee J, Thapa A, Britton RA, Di Rienzi SC. Microbial stimulation of oxytocin release from the intestinal epithelium via secretin signaling. BioRxiv: the Preprint Server for Biology. 2023. (Preprint)
[155] Welch MG, Margolis KG, Li Z, Gershon MD. Oxytocin regulates gastrointestinal motility, inflammation, macromolecular permeability, and mucosal maintenance in mice. American Journal of Physiology. Gastrointestinal and Liver Physiology. 2014; 307: G848–G862.
[156] Van den Abbeele P, Goggans M, Deyaert S, Baudot A, Van de Vliet M, Calatayud Arroyo M, *et al*. *Lacticaseibacillus rhamnosus* ATCC 53103 and *Limosilactobacillus reuteri* ATCC 53608 Synergistically Boost Butyrate Levels upon Tributyrin Administration Ex Vivo. International Journal of Molecular Sciences. 2023; 24: 5859.
[157] Wang H, Duclot F, Liu Y, Wang Z, Kabbaj M. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. Nature Neuroscience. 2013; 16: 919–924.
[158] Guilloteau P, Savary G, Jaguelin-Peyrault Y, Romé V, Le Normand L, Zabielski R. Dietary sodium butyrate supplementation increases digestibility and pancreatic secretion in young milk-fed calves. Journal of Dairy Science. 2010; 93: 5842–5850.
[159] Xu S, Qin B, Shi A, Zhao J, Guo X, Dong L. Oxytocin inhibited stress induced visceral hypersensitivity, enteric glial cells activation, and release of proinflammatory cytokines in maternal separated rats. European Journal of Pharmacology. 2018; 818: 578–584.

[160] Kimono D, Sarkar S, Albadrani M, Seth R, Bose D, Mondal A, *et al*. Dysbiosis-Associated Enteric Glial Cell Immune-Activation and Redox Imbalance Modulate Tight Junction Protein Expression in Gulf War Illness Pathology. Frontiers in Physiology. 2019; 10: 1229.
[161] Li S, Zhai J, Chu W, Geng X, Wang D, Jiao L, *et al*. Alleviation of Limosilactobacillus reuteri in polycystic ovary syndrome protects against circadian dysrhythmia-induced dyslipidemia via capric acid and GALR1 signaling. NPJ Biofilms and Microbiomes. 2023; 9: 47.
[162] Baudon A, Clauss Creusot E, Althammer F, Schaaf CP, Charlet A. Emerging role of astrocytes in oxytocin-mediated control of neural circuits and brain functions. Progress in Neurobiology. 2022; 217: 102328.
[163] Abbara A, Dhillo WS. Targeting Elevated GnRH Pulsatility to Treat Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology and Metabolism. 2021; 106: e4275–e4277.
[164] Yoo S, Cha D, Kim S, Jiang L, Cooke P, Adebesin M, *et al*. Tanycyte ablation in the arcuate nucleus and median eminence increases obesity susceptibility by increasing body fat content in male mice. Glia. 2020; 68: 1987–2000.
[165] Porniece Kumar M, Cremer AL, Klemm P, Steuernagel L, Sundaram S, Jais A, *et al*. Insulin signalling in tanycytes gates hypothalamic insulin uptake and regulation of AgRP neuron activity. Nature Metabolism. 2021; 3: 1662–1679.
[166] Cleland NRW, Bruce KD. Fatty acid sensing in the brain: The role of glial-neuronal metabolic crosstalk and horizontal lipid flux. Biochimie. 2022. (online ahead of print)
[167] Barahona MJ, Llanos P, Recabal A, Escobar-Acuña K, Elizondo-Vega R, Salgado M, *et al*. Glial hypothalamic inhibition of GLUT2 expression alters satiety, impacting eating behavior. Glia. 2018; 66: 592–605.
[168] Clayton RW, Lovell-Badge R, Galichet C. The Properties and Functions of Glial Cell Types of the Hypothalamic Median Eminence. Frontiers in Endocrinology. 2022; 13: 953995.
[169] Prevot V, Dehouck B, Sharif A, Ciofi P, Giacobini P, Clasadonte J. The Versatile Tanycyte: A Hypothalamic Integrator of Reproduction and Energy Metabolism. Endocrine Reviews. 2018; 39: 333–368.
[170] Rizzoti K, Lovell-Badge R. Pivotal role of median eminence tanycytes for hypothalamic function and neurogenesis. Molecular and Cellular Endocrinology. 2017; 445: 7–13.
[171] Zhang Y, Kim MS, Jia B, Yan J, Zuniga-Hertz JP, Han C, *et al*. Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. Nature. 2017; 548: 52–57.
[172] Kannangara H, Cullen L, Miyashita S, Korkmaz F, Macdonald A, Gumerova A, *et al*. Emerging roles of brain tanycytes in regulating blood-hypothalamus barrier plasticity and energy homeostasis. Annals of the New York Academy of Sciences. 2023; 1525: 61–69.
[173] Dali R, Estrada-Meza J, Langlet F. Tanycyte, the neuron whisperer. Physiology & Behavior. 2023; 263: 114108.
[174] Butruille L, Batailler M, Cateau ML, Sharif A, Leysen V, Prévot V, *et al*. Selective Depletion of Adult GFAP-Expressing Tanycytes Leads to Hypogonadotropic Hypogonadism in Males. Frontiers in Endocrinology. 2022; 13: 869019.
[175] Kővári D, Penksza V, Szilvásy-Szabó A, Sinkó R, Gereben B, Mackie K, *et al*. Tanycyte specific ablation of diacylglycerol lipase alpha stimulates the hypothalamic-pituitary-thyroid axis by decreasing the endocannabinoid mediated inhibition of TRH release. Journal of Neuroendocrinology. 2022; 34: e13079.
[176] Parkash J, Messina A, Langlet F, Cimino I, Loyens A, Mazur D, *et al*. Semaphorin7A regulates neuroglial plasticity in the adult hypothalamic median eminence. Nature Communications. 2015; 6: 6385.
[177] Kim S, Kim N, Park S, Jeon Y, Lee J, Yoo SJ, *et al*. Tanycytic TSPO inhibition induces lipophagy to regulate lipid metabolism and improve energy balance. Autophagy. 2020; 16: 1200–1220.
[178] Chen J, Zhu W, Zeng X, Yang K, Peng H, Hu L. Paeoniflorin exhibits antidepressant activity in rats with postpartum depression via the TSPO and BDNF mTOR pathways. Acta Neurobiologiae Experimentalis. 2022; 82: 347–357.
[179] Givalois L, Arancibia S, Alonso G, Tapia-Arancibia L. Expression of brain-derived neurotrophic factor and its receptors in the median eminence cells with sensitivity to stress. Endocrinology. 2004; 145: 4737–4747.
[180] Di Giaimo R, Durovic T, Barquin P, Kociaj A, Lepko T, Aschenbroich S, *et al*. The Aryl Hydrocarbon Receptor Pathway Defines the Time Frame for Restorative Neurogenesis. Cell Reports. 2018; 25: 3241–3251.e5.
[181] Solak KA, Wijnolts FMJ, Pralong FP, Blaauboer BJ, van den Berg M, Westerink RH, *et al*. *In vitro* neuroendocrine effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the AhR-expressing hypothalamic rat GnV-3 cell line. Toxicology. 2013; 311: 124–134.
[182] Lu J, Shang X, Zhong W, Xu Y, Shi R, Wang X. New insights of CYP1A in endogenous metabolism: a focus on single nucleotide polymorphisms and diseases. Acta Pharmaceutica Sinica. B. 2020; 10: 91–104.
[183] Hitzman RT, Dunlap TL, Howell CE, Chen SN, Vollmer G, Pauli GF, *et al*. 6-Prenylnaringenin from Hops Disrupts ER$\alpha$-Mediated Downregulation of *CYP1A1* to Facilitate Estrogen Detoxification. Chemical Research in Toxicology. 2020; 33: 2793–2803.
[184] Tarnow P, Zordick C, Bottke A, Fischer B, Kühne F, Tralau T, *et al*. Characterization of Quinoline Yellow Dyes As Transient Aryl Hydrocarbon Receptor Agonists. Chemical Research in Toxicology. 2020; 33: 742–750.
[185] Montazeri-Najafabady N, Chatrabnous N, Arabnezhad MR, Azarpira N. Anti-androgenic effect of astaxanthin in LNCaP cells is mediated through the aryl hydrocarbon-androgen receptors cross talk. Journal of Food Biochemistry. 2021; 45: e13702.
[186] Gregoraszczuk EL, Zabielny E, Ochwat D. Aryl hydrocarbon receptor (AhR)-linked inhibition of luteal cell progesterone secretion in 2,3,7,8-tetrachlorodibenzo-p-dioxin treated cells. Journal of Physiology and Pharmacology: an Official Journal of the Polish Physiological Society. 2001; 52: 303–311.
[187] Cardona-Gómez GP, Chowen JA, Garcia-Segura LM. Estradiol and progesterone regulate the expression of insulin-like growth factor-I receptor and insulin-like growth factor binding protein-2 in the hypothalamus of adult female rats. Journal of Neurobiology. 2000; 43: 269–281.
[188] Dong F, Annalora AJ, Murray IA, Tian Y, Marcus CB, Patterson AD, *et al*. Endogenous Tryptophan-Derived Ah Receptor Ligands are Dissociated from CYP1A1/1B1-Dependent Negative-Feedback. International Journal of Tryptophan Research: IJTR. 2023; 16: 11786469231182508.
[189] Anderson G. Amyotrophic Lateral Sclerosis Pathoetiology and Pathophysiology: Roles of Astrocytes, Gut Microbiome, and Muscle Interactions via the Mitochondrial Melatonergic Pathway, with Disruption by Glyphosate-Based Herbicides. International Journal of Molecular Sciences. 2022; 24: 587.
[190] Defarge N, Takács E, Lozano VL, Mesnage R, Spiroux de Vendômois J, Séralini GE, *et al*. Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels. International Journal of Environmental Research and Public Health. 2016; 13: 264.
[191] Zhang C, Schilirò T, Gea M, Bianchi S, Spinello A, Magistrato A, *et al*. Molecular Basis for Endocrine Disruption by Pesticides Targeting Aromatase and Estrogen Receptor. International Journal of Environmental Research and Public Health. 2020; 17: 5664.

[192] Alarcón R, Ingaramo PI, Rivera OE, Dioguardi GH, Repetti MR, Demonte LD, *et al*. Neonatal exposure to a glyphosate-based herbicide alters the histofunctional differentiation of the ovaries and uterus in lambs. Molecular and Cellular Endocrinology. 2019; 482: 45–56.

[193] Hamdaoui L, Naifar M, Rahmouni F, Harrabi B, Ayadi F, Sahnoun Z, *et al*. Subchronic exposure to kalach 360 SL-induced endocrine disruption and ovary damage in female rats. Archives of Physiology and Biochemistry. 2018; 124: 27–34.

[194] Martini CN, Gabrielli M, Brandani JN, Vila MDC. Glyphosate Inhibits PPAR Gamma Induction and Differentiation of Preadipocytes and is able to Induce Oxidative Stress. Journal of Biochemical and Molecular Toxicology. 2016; 30: 404–413.

[195] Serra L, Estienne A, Vasseur C, Froment P, Dupont J. Review: Mechanisms of Glyphosate and Glyphosate-Based Herbicides Action in Female and Male Fertility in Humans and Animal Models. Cells. 2021; 10: 3079.

[196] Webley GE, Luck MR. Melatonin directly stimulates the secretion of progesterone by human and bovine granulosa cells *in vitro*. Journal of Reproduction and Fertility. 1986; 78: 711–717.

[197] Baracat MCP, Baracat EC, Simões RS, Simões MJ, Maciel GAR, Azziz R, *et al*. Hormonal and Metabolic Factors Influence the Action of Progesterone on the Endometrium of Women with Polycystic Ovary Syndrome. Diagnostics (Basel, Switzerland). 2023; 13: 382.

[198] Heo JS, Lim JY, Yoon DW, Pyo S, Kim J. Exosome and Melatonin Additively Attenuates Inflammation by Transferring miR-34a, miR-124, and miR-135b. BioMed Research International. 2020; 2020: 1621394.

[199] Anderson G. Tumor Microenvironment and Metabolism: Role of the Mitochondrial Melatonergic Pathway in Determining Intercellular Interactions in a New Dynamic Homeostasis. International Journal of Molecular Sciences. 2022; 24: 311.

[200] Angoorani P, Ejtahed HS, Ettehad Marvasti F, Taghavi M, Mohammadpour Ahranjani B, Hasani-Ranjbar S, *et al*. The effects of probiotics, prebiotics, and synbiotics on polycystic ovarian syndrome: an overview of systematic reviews. Frontiers in Medicine. 2023; 10: 1141355.

[201] Hazimeh D, Massoud G, Parish M, Singh B, Segars J, Islam MS. Green Tea and Benign Gynecologic Disorders: A New Trick for An Old Beverage? Nutrients. 2023; 15: 1439.

[202] Ali Fadlalmola H, Elhusein AM, Al-Sayaghi KM, Albadrani MS, Swamy DV, Mamanao DM, *et al*. Efficacy of resveratrol in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomized clinical trials. The Pan African Medical Journal. 2023; 44: 134.

[203] Shen W, Qu Y, Jiang H, Wang H, Pan Y, Zhang Y, *et al*. Therapeutic effect and safety of curcumin in women with PCOS: A systematic review and meta-analysis. Frontiers in Endocrinology. 2022; 13: 1051111.

[204] Haghighi M, Mehdizadeh M, Amjadi F, Zandieh Z, Najafi M, Artimani T, *et al*. Effects of myo-inositol plus folic acid on ovarian morphology and oocyte quality in PCOS mouse model. Zygote (Cambridge, England). 2023; 31: 111–122.

[205] Thornburgh S, Gaskins AJ. B vitamins, polycystic ovary syndrome, and fertility. Current Opinion in Endocrinology, Diabetes, and Obesity. 2022; 29: 554–559.

[206] Abbasi E, Bagherniya M, Soleimani D, Ghasemi-Tehrani H, Abbaspour M, Clark CCT, *et al*. The effects of propolis supplementation on high-sensitivity C-reactive protein, testosterone hormone, and metabolic profile in women with polycystic ovary syndrome: A randomized, triple-blinded, placebo-controlled clinical trial. Phytotherapy Research: PTR. 2023; 37: 5366–5377.

[207] Lansdown AJ, Warnert EAH, Sverrisdóttir Y, Wise RG, Rees DA. Regional Cerebral Activation Accompanies Sympathoexcitation in Women With Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology and Metabolism. 2019; 104: 3614–3623.

[208] Grzęda E, Ziarniak K, Sliwowska JH. The paraventricular nucleus of the hypothalamus - the concertmaster of autonomic control. Focus on blood pressure regulation. Acta Neurobiologiae Experimentalis. 2023; 83: 34–44.

[209] Zhang S, He H, Wang Y, Wang X, Liu X. Transcutaneous auricular vagus nerve stimulation as a potential novel treatment for polycystic ovary syndrome. Scientific Reports. 2023; 13: 7721.