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PCOS AND GUT MICROBIOTA: NEW APPROACH, NEW TREATMENT

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Abstract:

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder with a worldwide prevalence of 5-15% of women of reproductive age. This syndrome is characterised by a chronic state of inflammation and insulin resistance (IR). The ethology of PCOS remains unclear, although studies indicate that both genetic and environmental factors contribute to it. The importance of PCOS in women's health lies in its relationship with not only endocrine disorders (hormonal imbalances), but also metabolic disorders (IR, glucose intolerance, type II diabetes, dyslipidaemia, non-alcoholic fatty liver disease); as well as a chronic state of inflammation and even, psychiatric disorders. The pathogenesis of PCOS remains unclear, however, the latest studies relate the genesis of these mechanisms to the gut microbiota, as it seems that gut microbiota composition in patients with PCOS is significantly altered compared with healthy controls. We recognize specific bacteria associated with the microbiome of patients with PCOS vs control. Gut microbiota can change under PCOS condition, but it has also been proposed that dysbiosis of gut microbiota may be a potential pathogenic factor in the development of PCOS. In this background, it may be engaging to research on new therapies based on the modulation of the gut microbiota, focusing especially on an anti-inflammatory diet and probiotics. Regarding to the diagnosis, recent research shows that the study of metabolites could be crucial, since diagnosis by exclusion may no longer be warranted.

Resum:

La síndrome de l'ovari poliquístic (SOP) és un trastorn endocrí-metabòlic comú amb una prevalença mundial del 5-15% de les dones en edat reproductiva. Aquesta síndrome es caracteritza per un estat crònic d'inflamació i resistència a la insulina (RI). L'etiologia del SOP encara no està clara, tot i que els estudis indiquen que tant els factors genètics com els ambientals hi contribueixen. La importància del SOP en la salut de les dones resideix en la seva relació no només amb trastorns endocrins (desequilibris hormonal), sinó també amb trastorns metabòlics (RI, intolerància a la glucosa, diabetis tipus II, dislipèmia, fetge gras no alcohòlic); així com un estat crònic d'inflamació i, fins i tot, trastorns psiquiàtrics. La patogènesi del SOP encara no està clara, però, els darrers estudis relacionen la gènesi d'aquests mecanismes amb la microbiota intestinal, ja que sembla que la composició de la microbiota intestinal en pacients amb SOP es troba significativament alterada en comparació amb els controls sans. Reconeixem bacteris específics associats al microbioma de pacients amb SOP vs. control. La microbiota intestinal pot canviar en condicions de SOP, però també s'ha proposat que la disbiosis d'aquesta microbiota pot ser un factor patogènic potencial en el desenvolupament del SOP. En aquest context, pot ser interessant investigar sobre noves teràpies basades en la modulació de la microbiota intestinal, centrant-se especialment en una dieta antiinflamatòria i probiòtics. Pel que fa al diagnòstic, investigacions recents demostren que l'estudi dels metabòlits podria ser crucial, ja que així podria deixar d'estar justificat el diagnòstic per exclusió.

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REVIEW

PCOS and Gut Microbiota: New Approach, New Treatment

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder with a worldwide prevalence of 5-15% of women of reproductive age. This syndrome is characterised by a chronic state of inflammation and insulin resistance (IR). The ethology of PCOS remains unclear, although studies indicate that both genetic and environmental factors contribute to it. The importance of PCOS in women's health lies in its relationship with not only endocrine disorders (hormonal imbalances), but also metabolic disorders (IR, glucose intolerance, type II diabetes, dyslipidaemia, non-alcoholic fatty liver disease); as well as a chronic state of inflammation and even, psychiatric disorders. The pathogenesis of PCOS remains unclear, however, the latest studies relate the genesis of these mechanisms to the gut microbiota, as it seems that gut microbiota composition in patients with PCOS is significantly altered compared with healthy controls. We recognize specific bacteria associated with microbiomes of patients with PCOS vs control. Gut microbiota can change under PCOS condition, but it has also been proposed that dysbiosis of gut microbiota may be a potential pathogenic factor in the development of PCOS. In this background, it may be engaging to research on new therapies based in the modulation of gut microbiota, focusing especially on an anti-inflammatory diet and probiotics. Regarding to the diagnosis, recent research shows that the study of metabolites could be crucial, since diagnosis by exclusion may no longer be warranted.

Keywords: PCOS; gut microbiota; microbiome; dysbiosis

Key teaching points:

- Mechanisms involving the gut microbiota may be a potential pathogenic factor in the development of PCOS.
- Microbiota composition in PCOS patients differs from healthy controls.
- Studies suggest that obesity, IR and hormonal imbalance could lead to dysbiosis.
- Modulation of the gut microbiome through diet as a potential PCOS treatment.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder among women of reproductive age, with an estimated worldwide prevalence of 5-15% when the Rotterdam Consensus criteria are used (1). The syndrome, defined by these criteria, is characterised by the presence of at least two of the three classical features of PCOS: menstrual irregularity (oligomenorrhoea or amenorrhoea), hyperandrogenism (acne, hirsutism), and enlarged "polycystic" ovaries on pelvic ultrasound. It is also characterised by a chronic state of inflammation and insulin resistance (IR). It is believed that IR and inflammation are responsible for the increased risk of diabetes, metabolic syndrome and cardiovascular disease observed in long term PCOS patients (2).

The ethology of PCOS remains unclear, although studies indicate that both genetic and environmental factors contribute to the syndrome (3). In anterior years, reviews had described adolescent hyperandrogenism or precocious pubarche such as signs that may indicate the early stages of PCOS (4).

It is known that a big diversity exists in the clinical presentation of PCOS, because not all women diagnosed with it have the same pathological phenotypes (3). PCOS phenotypes vary with ethnicity, weight, age and region (5). Even though, the most common phenotype is the metabolic one, related to insulin resistance.

PCOS is a diagnosis of exclusion, meaning that secondary causes need to be excluded before treating it. Nowadays, due to the prevalence of this syndrome, alternative treatments are gaining much interest among population. It is for this reason that scientific

evidence is focusing a lot on the relationship between PCOS and a possible dysbiosis as one of the causes or worsening of the clinical picture.

Materials and Methods

In order to write this bibliographic review a systemic search of different concepts has been performed in PubMed and Scopus databases. Keywords used to filter through the databases were: PCOS AND ("gut microbiota" OR microbiome OR dysbiosis). For this review, the articles included were those that reported the alterations of the intestinal microbiota under PCOS condition. Additionally, specific terms were used in the search of information regarding to hepatic steatosis (PCOS AND NAFLD) and the urinary metabolites (PCOS AND urinary AND metabolites). Regarding the information related to dietary treatment, different articles were searched directly from "Nutrients" website. Selection criteria for all articles was restricted to a 10-year window, even though most of the selected ones have been published in recent 4 years. Furthermore, studies without humans as subjects were excluded. Finally, in order to obtain a higher level of scientific evidence, the primary selected sources were meta-analyses, reviews and systematic reviews; even so, there are as well some clinical research articles.

Results

Overall, a summary of 24 articles were included, as specifying into the different topics, a total of 37 articles and an online website were added later. Based on the first information obtained from the abstracts of the articles, a script was made. The following includes the topics that will be discussed:

1. The human microbiota

The microbiota is the set of microorganisms that colonize the epidermal surface and the mucous membranes. Throughout the body, humans have a colonizable total of 400m.

The microbiota is changing with life, as we grow, we create our microbiota. In the elderly, the gut microbiota become compositionally unstable and less diverse, events that are associated with coexisting conditions and age-related declines in immunocompetence (6). The microbiota will change over a lifetime and there are many external factors that will influence it. Endogenous and exogenous factors influence the gut microbiota:

- Endogenous: host genetic features, sex and age.
- Exogenous: diet, birth, xenobiotics and other drugs, tobacco, ambiental contamination, infections, diurnal rhythm… These factors are modifiable.

It exists a symbiotic relationship between the bacteria that inhabit our intestines and ourselves, since we provide them with a place to live and, in return, they help us in multitudes of functions and can even produce essential substances.

The main functions are (7):

- Antimicrobial protection: avert colonization by other pathogens microorganisms, thanks to the "barrier" effect that prevents the entry of bacteria, parasites and foreign viruses.
- Immunomodulation: stimulate the immune system, allowing the individual's own substances to be recognized from those that are not.
- Nutrient metabolism: help digest food and absorb nutrients, providing extremely important substances that contribute to the proper functioning of the body. Moreover, produce vitamins, such as B and K, which the human body is not capable of synthesize.

2. Gut microbiota in PCOS

During the past decade, science has heavily focussed on the microbiota and its broad functions throughout the body. The link between the dysbiosis of gut microbiota and the development of PCOS was initially studied in 2012 by Tremellen and Pearce. Afterwards, many clinical studies confirmed that gut microbiome composition differs when comparing PCOS patients with healthy controls.

Gut dysbiosis, brought about a poor diet, creates an increase in gut mucosal permeability, with a resultant decrease of protective bacteria and increase in the passage

of lipopolysaccharide from Gram negative colonic bacteria into the system circulation (2).

Intestinal microorganisms produce short-chain fatty acids (SCFAs), these metabolites play a crucial role in immunomodulation (8). One of the metabolites produced for these gut microbes (bile acids) may contribute to the pathogenesis and development of diabetes and obesity (9), two typical features in PCOS women.

The intestinal microbiota is also able to regulate circulating estrogen levels (we refer to the stroboloma), therefore, is associated with gynecopathy such as endometrial hyperplasia, endometriosis, PCOS and infertility (10).

We actually know that not only women with PCOS have alterations of enteric microorganisms, but also particular species are related to PCOS phenotypes (11).

There are different possible mechanisms through which gut microbes are associated with PCOS. Referring to that, intestinal microbes influence the progression of PCOS by upregulating or downregulating hormone secretion, gut-brain mediators, cytokines and metabolite production.

For instance, alterations of the gut microbiota and less microbial diversity led to (11):

- Decreased quantity of interleukin-22 (IL-22). One of the mechanisms refers to IL-22 upregulating the expression of brown fat-related genes, they can't be regulated with poor levels of IL-22, consequently, brown adipose tissue couldn't help

improving PCOS symptoms. On the other hand, IL-22 might target ovarian granulosa cells to ameliorate inflammation.

- Decreased quantity of short-chain fatty acids (SCFAs) in their faecal samples. This low production of SCFAs increases the inflammation and insulin resistance problem by worsening the translocation of endotoxins across the gut wall.

- Lower levels of gut-brain mediators. The hypothalamus-pituitary gland-gonad axis does not work the way it is supposed to due to the lack of YY peptide (PYY) and ghrelin.

- Higher concentrations of androgen hormones. Thus, PCOS condition seems to have higher levels of *Provotella* (related with androgen levels such as testosterone and androstenedione) and decreased abundance of *Lactobacilli* (correlated with estrone and estradiol levels). This specific microbiota contributes to an androgen excess.

Even the PCOS factors remains unclear, we already know that the pathogenesis of PCOS not only depends on the genetic factors, but also on the epigenetics. Within the environmental factors, our everyday higher exposure to the environmental endocrine disruptors have been proposed as an important factor in the develop of the syndrome, as they have the ability to interfere with hormone sensitivity systems (12). Knowing that gut microbiota is affected by sex hormones, it is worth bearing in mind that, even more research is required, it could exist a forthright alteration in gut bacteria due to these disruptors (13).

One of the most studied endocrine disruptors in relation to PCOS is tributyltin (TBT) (14). TBT has a negative role in PCOS, as inhibits aromatase, an enzyme whose function is to convert testosterone into estradiol. As a result, reducing the function of this enzyme could increase testosterone, worsening the symptoms of PCOS. Other disruptors are bisphenol A (BPA) or certain herbicides.

2.1 Specific bacteria and obesity

Body weight could be influenced by the gut microbiota due to its complex effects on appetite and metabolism (15).

As said before, it is currently known that women with PCOS have an altered microbiota, different from those without the disease. We can say that women with PCOS have decreased alfa-diversity, which represents within-subject microbial diversity, and significantly altered microbial composition (beta-diversity) compared with controls without PCOS. Several bacterial taxa associated with PCOS status was also correlated with metabolic biomarkers such as waist-to-hip ratio and triglycerides (TG) (16).

Comparing bacteria of the microbiomes of patients with PCOS and healthy (control) patients, a relationship between PCOS and specific gut microbiota has been found (1). An increase in several taxa at the genus levels, specifically the genus *Lactobacillus*, *Escherichia/Shigella* (especially in the gut of PCOS women with obesity (17)) and *Bacteroides* was showed compared with controls.

It is important to point out that the taxa *Parabacteroides distasonis* was not only elevated in PCOS obese women but also in lean women with PCOS. This shows us an altered gut microbiota not only in overweight but also in lean women with this syndrome (18).

2.2 Dysbiosis and Insulin Resistance (IR)

One of the commonest conditions in women's suffering of PCOS is insulin resistance (IR). IR occurs when cells in the muscle, fat, and liver don't respond well to insulin and can't easily take up glucose from the blood (extra glucose stays in the bloodstream rather than entering the cells). As a result, pancreas makes more insulin to help glucose enter to the cells. Over time, this plight could develop type 2 diabetes (T2D) (19).

Gut microbiota dysbiosis is one of the factors that mediate the appearance of IR (and even the subsequent obesity). It seems that IR led to metabolic sequelae such as polycystic ovary syndrome or non-alcoholic fatty liver disease (15).

He F and Li Y (20) studied the relation between the gut microbial composition in PCOS with IR. Comparing with PCOS without IR and healthy controls, a difference in the microbial composition of PCOS with IR was found. It does exist a relative abundance *of Rothia* and *Enterococcus* in this PCOS-IR patients, suggesting that the bacteria of the gut microbiota could potentially play a key role in the pathogenesis of PCOS by regulating the glucagon-like peptidin-1 (GLP-1) level.

In this context, major risk factors for T2D such as overnutrition and low dietary fibre involve the gut and have been found to be associated with increased IR, decreased glucose tolerance and local or systemic low-grade inflammation (21).

The leaky gut hypothesis also has to do with alterations of the insulin receptor (16). This hypothesis relates to the permeability of the intestinal barrier, caused for a high sugar/fat and low dietary fibre diet. As previously stated, this permeability will induce a passage into the blood stream of toxins, antigens and bacteria; what finally leads to a systemic inflammatory response. The insulin receptor will be altered for this chronic state of inflammation, that affects multiple organs and follicular development. IR and the following hyperinsulinemia might promote synthesis of testosterone, which, at high concentrations can intervene in follicular development (2) ***[Figure 1 near here]***.

Gut dysbiosis and its intestinal mucosal permeability not only increase the passage of the mentioned inflammatory mediators, but also produces branch-chain amino acid (BCAA) by intestinal microflora. This increase in circulating BCAA activates the immune response of the body and reduce insulin sensitivity (22). A recent study found that imidazole propionate (microbial metabolite produced from amino acid) was shown to have higher concentrations in the portal and peripheral blood of patients with T2D (23).

This excess of insulin in PCOS play an elementary role in the already known hyperinsulinemia (24). Excess circulating insulin promote secretion of androgen by ovary and adrenal gland, therefore, increasing levels of free testosterone by inhibiting the synthesis of sex hormone binding globulin (SHBG) (25). To wit, PCOS with IR is a

metabolic problem that causes an alteration in the ovary too.

Furthermore, IR is also related with dyslipidaemia (lipid metabolism disorders); pathology seen as the first metabolic disorder in the referred ovary syndrome (26).

Fang-fang He and Yu-mei Li (22) write about the implication of the gut microbiota (as an indispensable microbial organ) in the pathogenesis of PCOS. We have seen that insulin is linked to the microbiota. Even though, other studies highlight the interaction, through signal pathways and transduction, between IR and chronic subclinical inflammation (28). Then, gut microbiota might be involved in the pathogenesis of PCOS by mediating systemic low-grade inflammation and insulin resistance, also affecting the change in sex hormones leading to the acquaintance hyperandrogenism (22,27).

2.3. Association between hyperandrogenism and gut microbial dysbiosis

Hyperandrogenism is a medical condition characterized by high levels of androgens in females. This androgen excess results, between other symptoms, in ovulation disorders (22), a very representative clinical parameter in women with PCOS.

A recent study (29) had a deeper investigation in the correlation between gut microbiome and hyperandrogenism. This study enrolled 73 PCOS women, of which 62 had hyperandrogenism. Among the findings, it stands out that the lower alfa-diversity of the microbiome (seen as typical in PCOS) strongly correlated (negative correlation) with hyperandrogenism, specifically with total testosterone level and hirsutism. It seems that

androgens may change the gut microbiome, worsening like this the progression and pathology of PCOS.

2.4. Psychiatric disorders and gut-brain axis changes

We actually know that there is a permanent relationship between our gut and our brain. The central nervous system has a constant bidirectional connection with the digestive tract. It is for this reason that some people refer to our gut as our "second brain".

An actual study (18) analysed the relationship between alterations in our intestinal microbiota and the prevalence of gut–brain axis changes in PCOS. The study was designed to test the possible involvement of the gut microbiota in metabolic disorders and neurotransmitter production.

The acquired reports of the above-mentioned study identified a difference in the species present in the intestinal microbiota of women with PCOS when compared with women without said diagnosis. The study reflected a possible microbial dysbiosis in women with PCOS that could be associated with neuroendocrine changes, revealing a possible gut-brain axis behind the syndrome.

This dysbiosis can produce an imbalance between gamma aminobutyric acid (GABA) producing and consuming bacteria, leading to a significant increase in GABA-producing bacteria (*Parabacteroides distanosis, Bacteroides fragilis* and *Esterichia coli* were increased in PCOS against controls (18,30)). These bacteria might increase the level

of GABA, acting on the receptors of gonadotrophin-releasing hormone (GnRH) neurons in hypothalamus to stimulate the secretion of luteinizing hormone (LH), causing the neuroendocrine disorders (18) and the typical increased ratio of LH to follicle-stimulating hormone (FSH) in PCOS patients.

Thus far, the point of view has been lately focused on neuroendocrine impairments associated with PCOS. This is because psychiatric disorders are more often diagnosed in this syndrome (31).

Eating disorders are one of the most common disorders among women with PCOS (32). One of the links between PCOS and eating disorders refers to the elevated testosterone concentrations, as these levels may promote food cravings, perhaps via a poor impulse control (33).

Even so, eating disorders disturb neuroendocrine pathways leading to altered biochemical processes. In this way, binge eating, or stress (continuous secretion of cortisol) can increase insulin levels, leading to routes that can increase free circulating testosterone (31).

Seems that the relationship between psychiatric disorders and PCOS will work in both directions, the psychiatric disorders appear to function as cause and consequence.

If we take a deeper look, we can conclude that eating disorders can also modify the gut microbiota, generally, leading to a reduction in diversity associated with poor clinical outcomes (34). Régine P. M. Steegers-Theunissen et al. (31) postulate that the exposure to a variety of psychological stressors during puberty and adolescence induces chronic psychiatric disorders, which, in some individuals, result in repeated episodes of overeating and dieting, collectively contributing to the development of PCOS. This psychological distress and its associated eating disorders during these periods lead to the epigenetic dysregulation of neurohormones (androgens, oestradiol, insulin, ghrelin…) involved in the pubertal upregulation of the hypothalamic–pituitary–gonadal axis (HPG axis), which contributes to the development of PCOS and related comorbidities in these subjects ***[Figure 2 near here]***.

2.5. Hepatic steatosis

We already know that women with PCOS have a specific microbiota, but it appears that the microbiome is also important in the pathogenesis of the so-called non-alcoholic fatty liver disease (NAFLD) (35). It is interesting to study the relationship between PCOS and NAFLD, since its known that women with PCOS have a higher risk of suffering from NAFLD.

At first, a higher prevalence of NAFLD has been observed in women with PCOS with a body mass index (BMI) similar to women without PCOS (36). In the case of adolescent girls with obesity, the prevalence rate ranged from 50% compared to 13% in women without said endocrine disorder (37).

To delve into this topic, we will use the study carried out by Jobira B. et al (38), in which 34 girls with similar parameters were equally divided into two groups: with and without hepatic steatosis (HS). The results of said study were that those adolescents with PCOS, obesity and HS had a dysbiosis of the microbiota compared to those with PCOS and obesity but without HS. Then, a conclusion comes to light, since it seems that not only PCOS and HS affect the microbiota separately, but also, if we add liver dysfunction to the sum of PCOS and obesity, we can see other specific changes in the bacterial taxa of the gut microbiota. Even so, the question remains about what the jumping-off point is and if this relationship between PCOS and HS are causative or just associations.

The study by Del Chierico et al. (39) in pediatric population and the above-mentioned study (38) agree about their findings in lower amounts of *Bacteroidetes* in HS/NAFLD patients. Adolescent patients with HS were also found to have upregulation of ethanol metabolism (39), and according to that, Jobira B et al. found a higher %RA *Bifidobacterium* (alcohol producing bacteria) in their adolescents with HS and PCOS. This suggests that bacteria taxa involved in ethanol production may contribute to endogenous ethanol production in NAFLD in PCOS (38).

One of the pathogenic mechanisms between NAFLD and PCOS women has to do with the accumulation of lipids in the liver (40). The high level of androgens, due to hyperandrogenism in these patients, suppress low-density lipoprotein receptors (LDLR) gene transcription to prolong the half-life of very low-density lipoproteins (VLDL) and LDL, thus causing the mentioned accumulation of lipids.

Furthermore, as indicated previously, it is essential to note that IR can increase the risk of NAFLD, since a higher prevalence of IR has been observed in women with PCOS and NAFLD compared to women with the same endocrinopathy but without steatosis (41).

Therefore, given the everyday higher prevalence of NAFLD in young women with PCOS and the high risk of developing long-term liver complications, it would be a must to recommend a systematic screening of NAFLD in patients with PCOS (42).

On the other hand, one of the factors influencing the development of NAFLD is the increased dietary fructose (sugar especially found in fruit, honey, and processed foods such as juices and other beverages (43)). This kind of diet led to an increase in the uptake of fatty acids to the liver and a reduction in the fat transport of VLDL-triglycerides. As a result, we can find a lot of metabolic and cardiovascular comorbidities related to NAFLD, such as metabolic syndrome (present in 41% of NAFLD and 71% of HS patients) (44).

The scientific evidence available to date suggests that chronic and excessive fructose intake is likely a major contributor to NAFLD pathogenesis ***[Figure 3 near here]***, figure 3 describes the mechanisms of NAFLD development and the influence of nutritional factors (44). So, in light of this, it could be crucial for patients with or at risk for NAFLD to improve their diet by reducing fructose intake.

2.6. Specific fecal and urinary metabolites

The study carried out by Ling Zhou et al (45) was the first in relate obese patients diagnosed with PCOS with the use of fecal metabolomics combined with gut microbiota.

They compared obese patients with and without PCOS, finding a linear relationship between gut microbiota, characteristic fecal metabolites and serum sex hormones. PCOS obese patients have characteristic intestinal species that correlate with fecal metabolites such as: arachidonic acid, dehydroepiandrosterone (DHEA) sulphate and taurocholic acid. At the same time, a correlation was also revealed with serum sex hormones (DHEAS and testosterone) ***[Appendix 1 near here]***. Thus, it is finally determined that fecal metabolites play a key role in the phenotypic changes caused by hyperandrogenism.

Another study (46) characterized urinary steroid hormone metabolites of 107 women (41 diagnosed with PCOS and 66 healthy controls). The results of this study showed a specific urinary steroid profile in PCOS patients. The urinary steroid profiling reveal androstanediol, estriol, 20βDHcortisone, and cortisol as promising diagnostic markers for PCOS. Another function of the microbiota is to interpose in the metabolism of steroid hormones. Therefore, even more research is required, having a specific microbiota will trigger on specific metabolites.

Between the latest findings associating PCOS and urinary metabolites we found the work of Wei Zhou et al. (47). In this study, a variation of metabolic states was found in different PCOS subtypes (healthy, with hyperandrogenism and with IR). A panel of 8

biomarkers was discovered from PCOS with hyperandrogenism vs IR: ribitol, trans-aconitic acid, 4-hydroxycyclohexanecarboxylic acid, tartronic acid, glycolic acid, myo-inositol, oxalic acid and ribonic acid. The area under curve (AUC) for the metabolic biomarkers was 0.8363. The AUC reached 0.9065 (with high sensitivity and specificity percentages) when combining biomarkers with 2 clinical markers (homeostasis model assessment-insulin resistance (HOMA-IR) and free androgen index).

All in all, it seems that the intestinal microbiota and its respective metabolites could be used to diagnose PCOS (in a noninvasively way) and better clarify the pathological metabolism of the disease. Thus, metabolomics should be considered as an essential science for knowing better about the ethology of PCOS. In connection with this, the study of metabolites could be crucial, because PCOS diagnosis by exclusion may no longer be warranted, and what is more, therapies could be more targeted for every phenotype and therefore, be more efficient.

3. COVID and PCOS

Nowadays, the whole world is facing a pandemic, it is for this reason that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most current topic. Some recent studies have already point out not only the link and implications with gut microbiota, but also the connection with PCOS.

Wambier CG and Goren A (48) study and conclude that SARS-CoV-2 it is androgen mediated. We come across the fact that the activity of the androgen receptor plays a crucial role in the transcription of the transmembrane protease serine 2 (TMPRSS2).

Following this path, the priming of the spike proteins by TMPRSS2 it is a basic required step for potential infectivity of SARS-CoV-2. This could be one of the reasons why males are more vulnerable to the disease, as reported in some studies (48,49).

PCOS women had greater androgen receptor activity than non-PCOS, therefore, a greater transcription of TMPRSS2 (50). In summary, the fact of having a hyperandrogenic PCOS phenotype might worse the severity of the currently known SARS-CoV-2. Even so, another risk factor for both the susceptibility to and the severity of the SARS-CoV-2 is obesity (and associated IR) (51). In this context, this is another PCOS feature that could worsen the severity of the disease.

4. Alternative treatments for PCOS involving the gut microbiota

The pathogenesis of this disease is not fully understood, it is still a syndrome with an exclusion diagnosis, for this reason, therapies are poorly targeted and possibly ineffective. The actual chosen treatment after PCOS diagnostic is the use of oral contraceptives. Even so, it is worth bearing in mind the undoubtedly growing interest about how to treat PCOS with lifestyle changes. It is for this reason that PCOS patients are interested in a more functional and integrative treatment.

As it has already been mentioned, it exists a relation between dysbiosis and intestinal inflammation, IR and all that this entails (hyperandrogenism, diabetes…). One of the bases for the human health is the diet (total energy intake, macronutrient and micronutrient composition), which has an important impact on the composition of gut microbiota (52).

Now, what our field refers to, we will take into account the modulation of the gut microbiome through diet as a potential PCOS treatment. Different studies show that it can be very useful to control inflammation through the intake and / or supplementation of:

- Omega-3: unsaturated fatty acid rich diets decrease the risk of insulin resistance and its related complications; in this way, intake of omega-3 reduces the risk of PCOS in women with IR (53,54).

- Vitamin D: this vitamin has a role in different pathways, including insulin signalling pathway and ovarian function. Vitamin D supplementation can alleviate inflammatory pathways causing IR and ovarian function, therefore, alleviating PCOS (53,54,55).

- Curcumin: this polyphenol may improve glycaemic control and lipid metabolism in patients with PCOS and metabolic abnormality. The anti-inflammatory property of curcumin may also play a role in glucose and lipid metabolisms and may mitigate hyperandrogenism. Regarding to this property, it also could inhibit the activity of cytochrome P450c17, and thereby reducing the synthesis of DHEA (56).

- Green tea: it has been seen that this beverage with high amount of antioxidants due to its polyphenols content promotes insulin sensitivity and lowers testosterone levels (57).

- Isoflavone: a recent study (58) found an improvement in fasting glucose and insulin sensitivity in PCOS patients, but not in controls after a short-term isoflavone intervention. This flavonoid could also be used as a prebiotic, supporting like this the growth of beneficial microbial strains in PCOS women (59).

- Probiotic: consumption of the probiotic *Bifidobacterium lactis V9* promotes the growth of SCFA-producing microbes, such as *Faeca libacterium praus-nitzii*, *Butyricimonas*, and *Akkermansia*. These microbes, as well as *Bifidobacterium*, produce increased levels of SCFAs, impacting the secretion of gut-brain mediators, including ghrelin and PYY, and regulating like this the levels of sex hormones (60). This increase in the production of SCFAs contributes to the barrier function of the gut and reduce the translocation of endotoxins across the gut wall, which promotes improvements in inflammation levels and IR (14). In connection with this, Jamilian et al. (61) prove that co-administration of probiotic containing *Bifidobacterium*, *Lactobacillus* and selenium to women with PCOS resulted in decreased levels of testosterone and hirsutism and had also beneficial effects on mental health parameters.

- Myo-inositol: the effectivity of supplementation with 4g of myo-inositol/day on PCOS females has been studied by many authors. Effects of myo-inositol treatment in females with PCOS increases insulin sensitivity, decreases hyperandrogenism and improves the menstrual cycle (normalizing ovarian function) (62).

By and large, improving all these parameters could result in an improved gut microbiota, as hyperandrogenism, IR or inflammation could modify gut microbiota leading to dysbiosis.

Instead of lifestyle interventions (diet, physical activity, good sleeping habits, stress management…), the treatment of PCOS has been approached from many different alternative therapies.

Regarding alternative treatments, another therapy for treating gut dysbiosis in PCOS is the fecal microbial transplant (FMT) from healthy donors or representative microbes from a healthy gut to re-diversify the gut microbiome. Despite the fact that there are limited studies to support this idea, experimental studies show promise that adjusting the gut microbial community in PCOS patient through FMT could be a great alternative for treating hyperandrogenic-induced symptoms (3).

Discussion and conclusion

The bacteria that inhabit our intestines are responsible of many current diseases, as well as our health. Studies have shown that dysbiosis of gut microbiota occurs in PCOS: alpha diversity decreases, and the ratio or abundance of some bacteria related to obesity, IR, hyperandrogenism, neurological disorders or HS differs from healthy controls.

As explained earlier, we focused on the studies about gut microbiota in humans with PCOS, leaving aside studies with animal models, which other magazines have not done. Moreover, we summarized not only the alterations of the gut microbiota in humans with PCOS but also the correlations between the gut microbiota and different PCOS features.

At this time there is no cure for PCOS. However, it has been shown from this review that with proper treatment, many of the symptoms could be controlled. Having into account that every PCOS phenotype have different features and symptoms, more studies are required to find out the association between the causes of dysbiosis of the gut microbiota and how this affect to the different PCOS phenotypes.

Besides, the heterogenicity of the pathogenesis makes crucial the need for more studies to better understand the mechanisms and association between PCOS and gut microbiota, and how gut dysbiosis works as cause and/or consequence.

Taking everything into consideration, we can reach an agreement and say that diet modulation is not only an excellent, but also a low-risk strategy to help change the microbiome. As Hippocrates said once “Let food be the medicine, and let medicine be the food”. So, in light of this, it would be a must for females with PCOS to focus on the microbiota and live a healthy lifestyle, including an anti-inflammatory diet and probiotics.

Additionally, the data obtained from recent studies show us the relationship between androgens in PCOS and COVID-19. Hence, knowing about the possible crucial paper of gut microbiota in these endocrinopathy, it would be also engaging to investigate about the possible relation between PCOS, gut microbiota and the severity of COVID-19, further studies in humans are needed.

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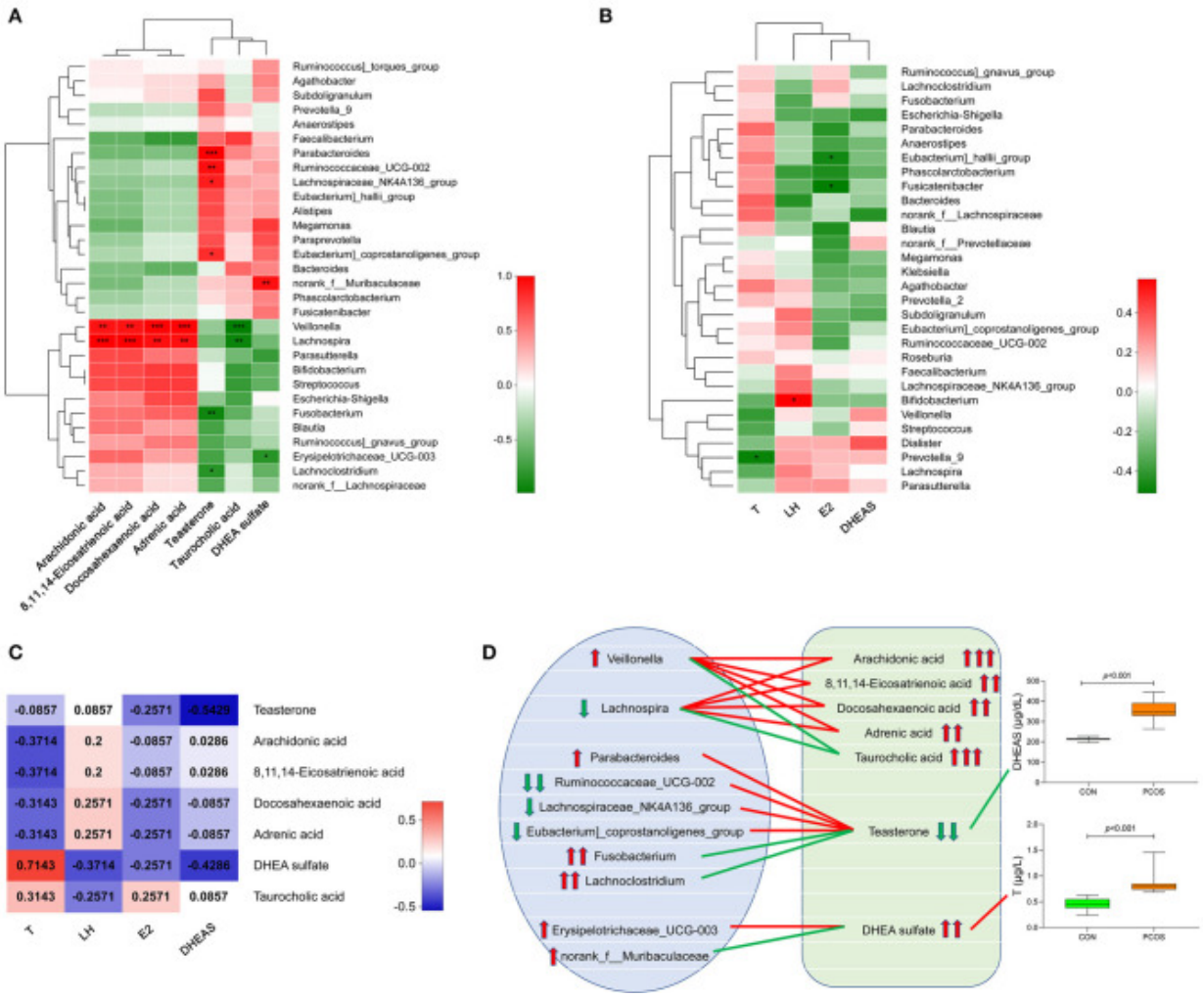
Appendix 1



Correlation analysis of gut microbiota, fecal metabolites and serum sex hormones in obese PCOS group. (A) Correlation heatmap between seven important differential fecal metabolites and the top 30 genera in abundance. (B) Correlation heatmap between serum sex hormones and the top 30 genera in abundance. (C) Correlation heatmap between serum sex hormones and seven important differential fecal metabolites. Different colors represent correlation level; *p < 0.05; **p < 0.01, ***p < 0.001. (D) Correlation map among gut microbiota, fecal metabolites and serum sex hormones. The red line represents a significant positive correlation, while the green line represents a significant negative correlation; the up arrow represents an increase in abundance, while the down arrow represents a decrease in abundance; one, two and three arrows represents $p > 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Taken from Ling Zhou et al. 2020 (45).

Figure 1

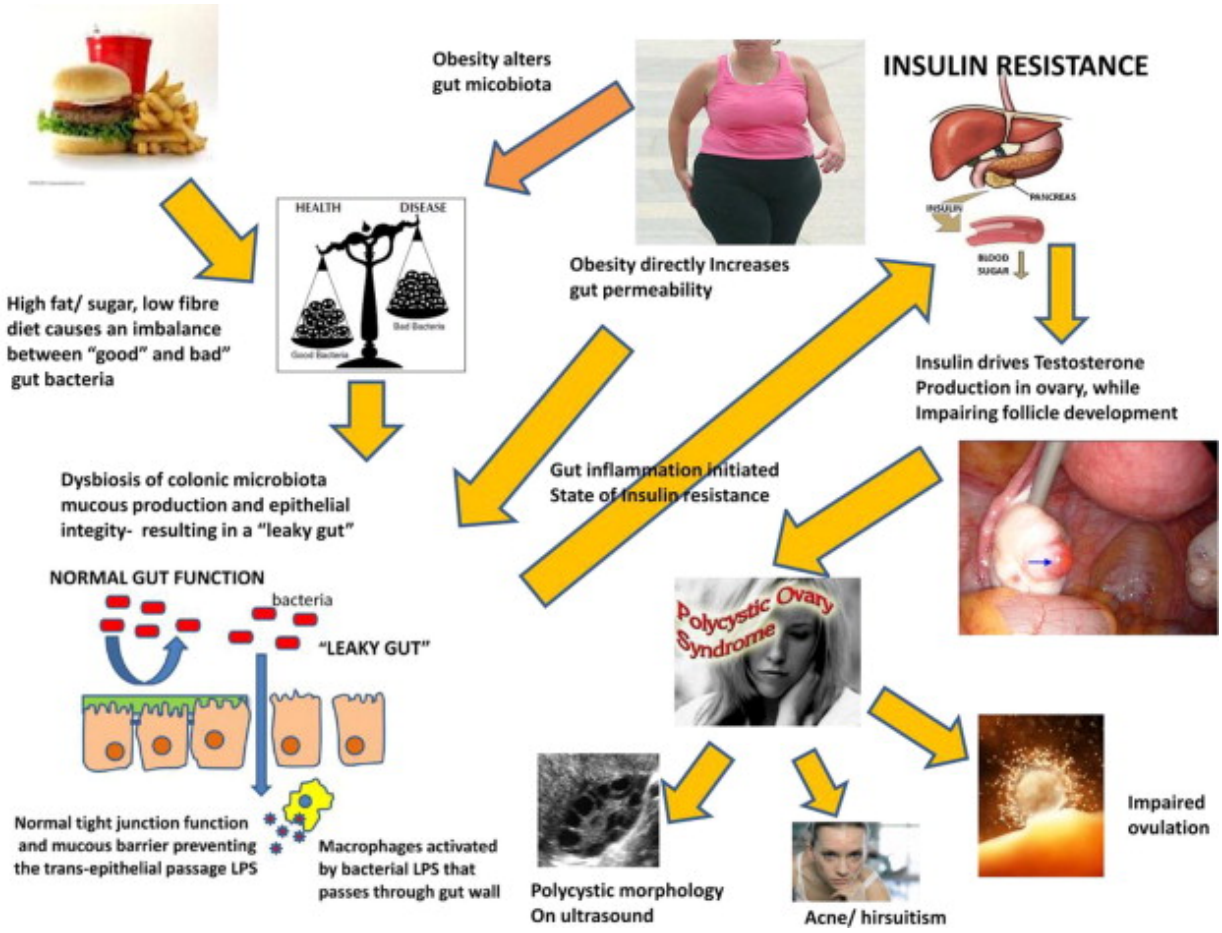


Figure 1. The DOGMA theory for creation of PCOS.

Taken by Tremellen and Pearce 2012 (2).

Figure 2

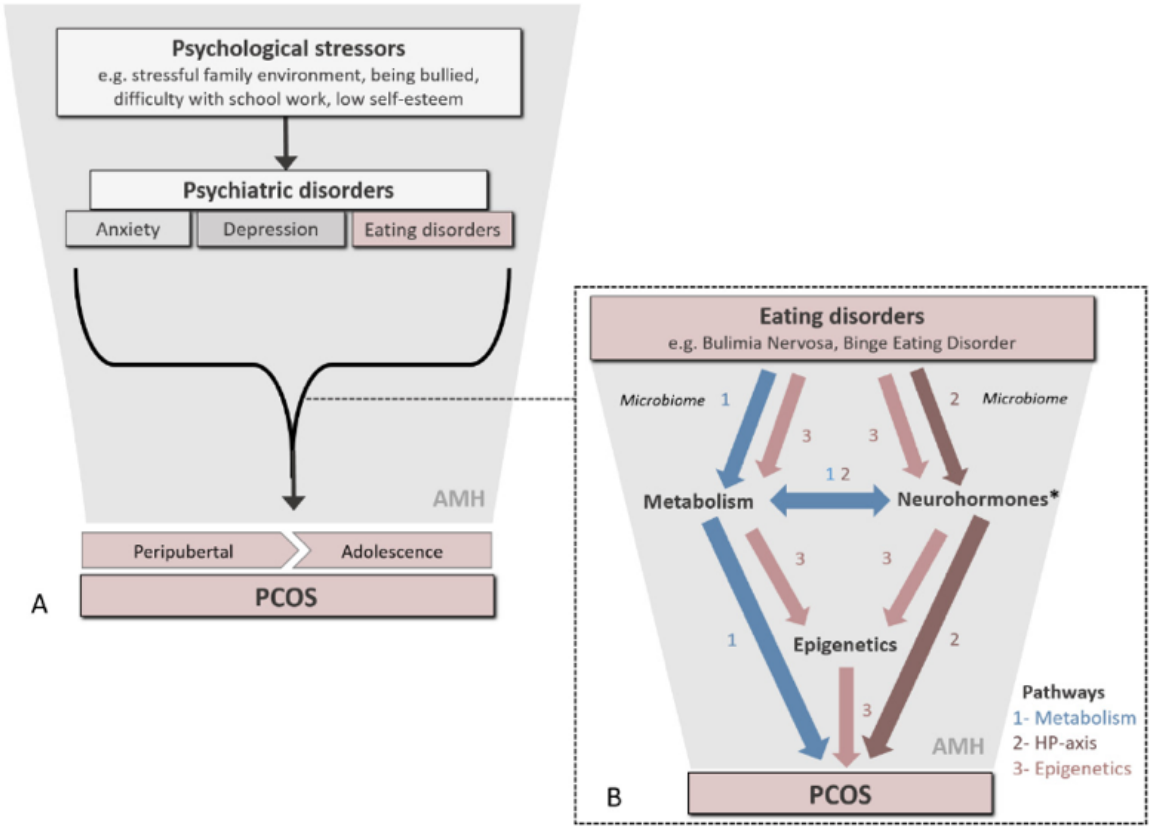


Figure 2. Psychological distress and its associated eating disorders could lead to the epigenetic dysregulation of neurohormones, which contributes to the development of PCOS.

Taken from RPM Steegers-Theunissen et al. 2020 (31).

Figure 3

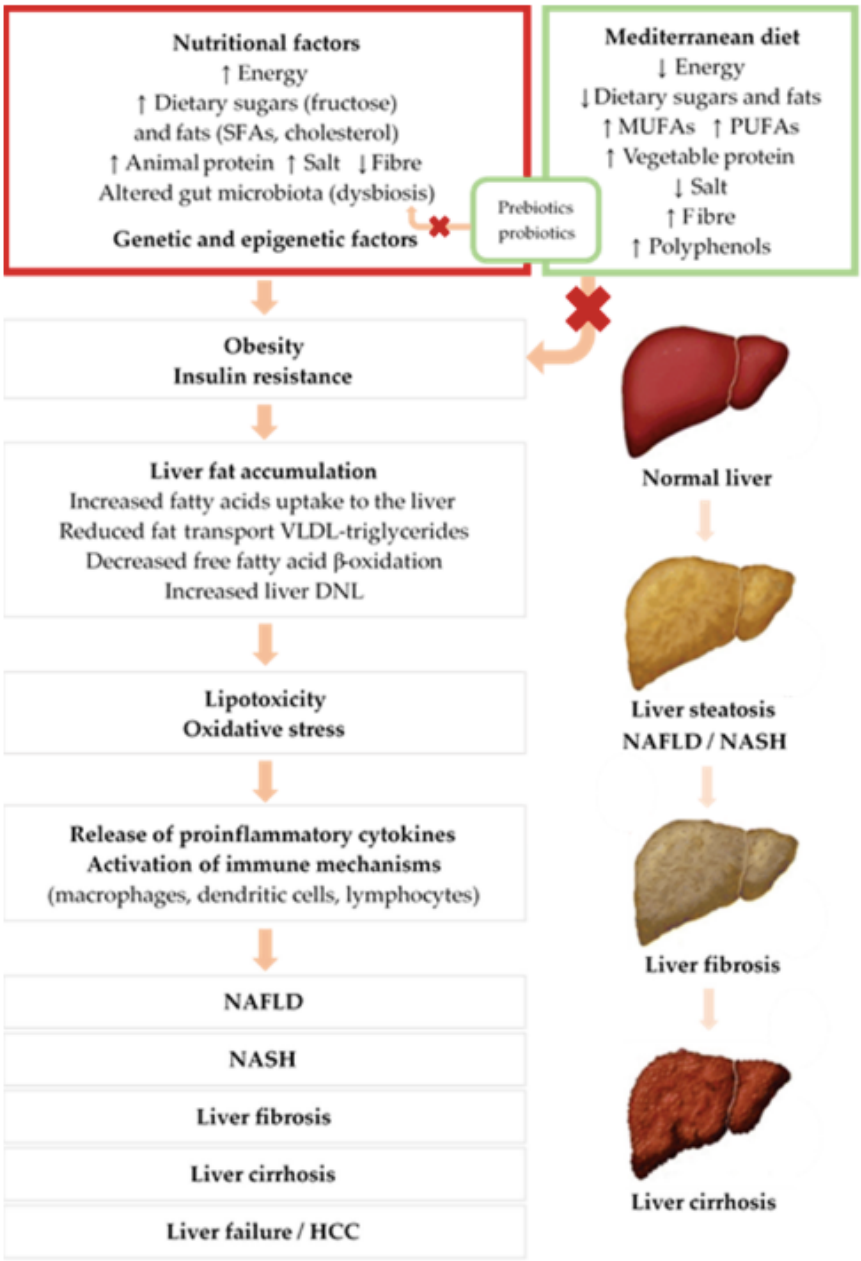


Figure 3. Influence of nutritional factors in the pathogenesis of NAFLD.

Taken from Pau Vancells et al. 2021 (44).