Dysbiosis of Gut Microbiota (DOGMA) – A novel theory for the development of Polycystic Ovarian Syndrome

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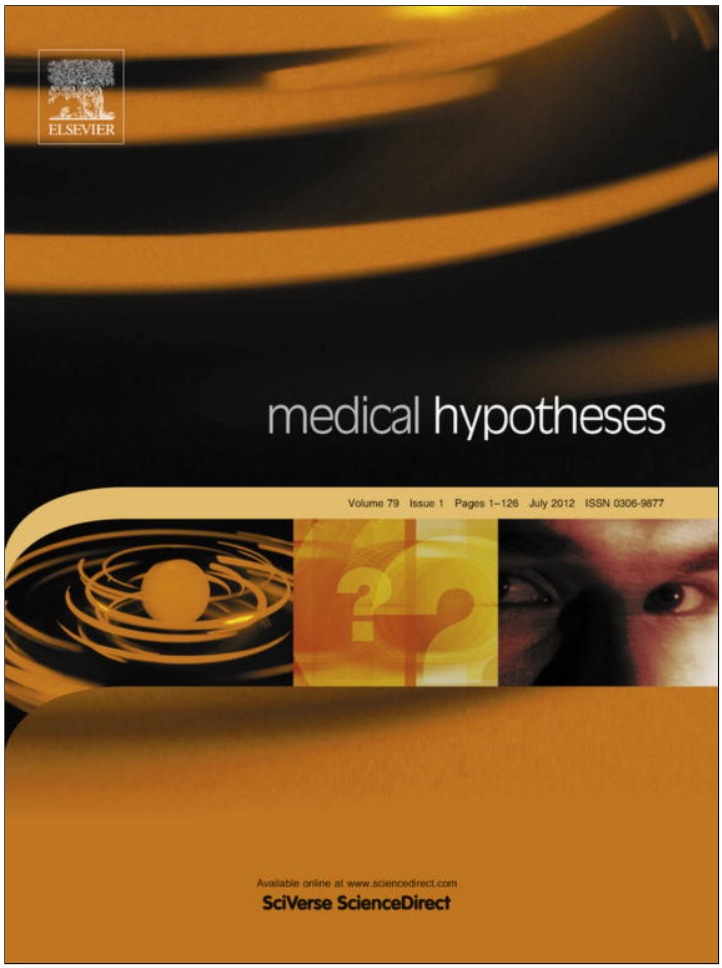

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Dysbiosis of Gut Microbiota (DOGMA) – A novel theory for the development of Polycystic Ovarian Syndrome

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is the most common cause for menstrual disturbance and impaired ovulation, effecting one in twenty women of reproductive age. As the majority of women with PCOS are either overweight or obese, a dietary or adipose tissue related trigger for the development of the syndrome is quite possible. It has now well established that PCOS is characterised by a chronic state of inflammation and insulin resistance, but the precise underlying triggers for these two key biochemical disturbances is presently unknown. In this paper we present support for a microbiological hypothesis for the development of PCOS. This novel paradigm in PCOS aetiology suggests that disturbances in bowel bacterial flora (''Dysbiosis of Gut Microbiota'') brought about by a poor diet creates an increase in gut mucosal permeability, with a resultant increase in the passage of lipopolysaccaride (LPS) from Gram negative colonic bacteria into the systemic circulation. The resultant activation of the immune system interferes with insulin receptor function, driving up serum insulin levels, which in turn increases the ovaries production of androgens and interferes with normal follicle development. Thus, the Dysbiosis of Gut Microbiota (DOGMA) theory of PCOS can account for all three components of the syndrome-anovulation/menstrual irregularity, hyper-androgenism (acne, hirsutism) and the development of multiple small ovarian cysts.

Introduction

Polycystic Ovarian syndrome (PCOS) affects between 4% and 8% of reproductive aged women and is the most common cause of menstrual irregularity and anovulatory infertility [1,2]. The syndrome, as defined in 2003 by the Rotterdam Consensus statement [3], 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. Biochemically PCOS is characterised by disordered gonadotrophin (LH and FSH) secretion from the anterior pituitary, high free androgen levels (increased testosterone, decreased SHBG), insulin resistance and chronic low grade inflammation [4–6]. It is believed that insulin resistance and inflammation are responsible for the increased risk of diabetes, metabolic syndrome and cardiovascular disease observed in long term PCOS patients [7,8]. In addition, the majority of women with PCOS are overweight or obese, yet this is not universally the case [2,9,10].

The exact patho-physiology behind PCOS is presently unknown, although genetic, neuroendocrine and metabolic causes have been suggested [7,11–14]. It is possible that no single pathological process can account for all cases of PCOS since the disorder is somewhat heterogeneous, with many patients not exhibiting all three cardinal features of the PCOS ''triad'' [14,15]. This view is supported by the conflicting definitions of PCOS generated by various reproductive medicine societies before the publication of the Rotterdam consensus [3]. For example, the American National Institutes of Health (USA) 1990 definition of PCOS [16] placed emphasis on the presence of hyperandrogenism and menstrual irregularity, disregarding polycystic ovarian morphology. Conversely, the European view considered the presence of polycystic ovarian morphology on ultrasound as being paramount for making a diagnosis of PCOS [17]. Even since the publication of the Rotterdam consensus statement there is still considerable debate on what exactly constitutes PCOS [18]. However, there is agreement

Abbreviations: PCOS, Polycystic Ovarian Syndrome; LH, Lutenizing Hormone; FSH, Follicle Stimulating Hormone; SHBG, Sex Hormone Binding Globulin; DOGMA, Dysbiosis of Gut Microbiota; LPS, lipopolysaccaride; CFU, colony forming units; SCFA, short chain fatty acids; IBS, Irritable Bowel Syndrome; CFS, Chronic Fatigue Syndrome; DEXA, Dual-Energy X-Ray Absorptiometry; TNFα, Tumour Necrosis Factor alpha; CRP, C-reactive protein; IL-6, interleukin 6; IGF-1, insulin like growth factor 1; GLP-1, Glucagon Like Peptide 1.

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that two key biochemical features, insulin resistance and chronic inflammation, appear to be present in the vast majority of women with PCOS and are likely to be central to the patho-physiology underlying the syndrome [6,7,14]. This paper will attempt to link these two cardinal features into a novel "microbiological" paradigm that may better explain the pathophysiology behind PCOS, while also opening up the possibility of new treatment approaches.

It is the author's hypothesis that imbalances in gut microbiology, often referred to as "Dysbiosis of Gut Microbiota", can result in the activation of the host's immune system, triggering a chronic inflammatory response that impairs insulin receptor function and initiates a state of insulin resistance. The resulting hyper-insulinaemia interferes with follicular development, while driving excess androgen production by the thecal cells of ovary – thereby producing all three classical features of the PCOS. We propose to call this novel microbiological paradigm for PCOS the DOGMA theory – Dysbiosis of Gut Microbiota.

This paper will outline the scientific background for the DOGMA theory in detail, but an initial brief overview may help the reader better follow the scientific arguments put forward supporting a microbiological cause for PCOS (Fig. 1). The key pathophysiological links in the DOGMA theory of PCOS are twofold. Firstly, a diet high in saturated fat and refined sugars, a common observation in overweight PCOS patients [19–22], is known to favour the growth of "bad" Gram negative bacteria within the gut; while reducing the growth of beneficial "good" bacteria such as Bifidobacteria and Lactobaccilus [24–28]. The cell wall of Gram negative bacteria contains a powerful immuno-stimulant called lipopolysaccaride (LPS), which can cause profound activation of the innate immune system if it is allowed to traverse the gut wall and enter the systemic circulation [24,29]. Secondly, the high saturated fat-sugar/low fibre diet, as well as obesity *per se*, causes an increase in gut mucosal permeability [29–32], facilitating the transfer of LPS from the gut lumen into the circulation, initiating a state of "metabolic endotoxaemia". The resultant chronic activation of hepatic and tissue macrophages produces impaired insulin receptor function and resulting insulin resistance [24,30,33]. Hyper-insulinaemia then drives an increase in the ovaries production of androgens and halts normal ovulatory processes [34–36], producing a "blockade" in follicle development from small to medium size antral follicles (2–10 mm) onto the mature "ovulatory" follicle [37]. As such, a "metabolic endotoxaemia" derived state of insulin resistance can produce all three classical features of PCOS; an increase in the number of small antral follicles seen on ultrasound scan, impaired ovulation with menstrual irregularity and hyper-androgenism. This paper will take the reader through the available human and animal evidence supporting each of these key steps in the development of PCOS and will propose a novel alternative treatment for the syndrome-prebiotic/probiotic therapy.

Scientific evidence supporting the DOGMA hypothesis

Colonic flora and the maintenance of mucosal integrity

The human gut is home to 10^{14} bacteria, a population which outnumbers the bodies own eukaryotic cells by a 10-fold order of magnitude [38]. At birth the gut of a neonate is sterile, but quickly becomes colonised by bacteria derived from its mother's birth canal, the external environment and food. The adult bowel microbiota harbours approximately 1000 different bacterial species, with Bacteroides (e.g. Prevotella and Bacteroides), Firmicutes (e.g. Clostridia, Enterococcus, Lactobacillus) and Actinobacteria (e.g. Bifidobacterium) being the dominant bacterial phyla [39,40].

The numbers of bacteria in the gastro-intestinal tract range from low numbers in the hostile low pH of the stomach, with approximately 10^3 colony forming units (CFU) per ml of gastric

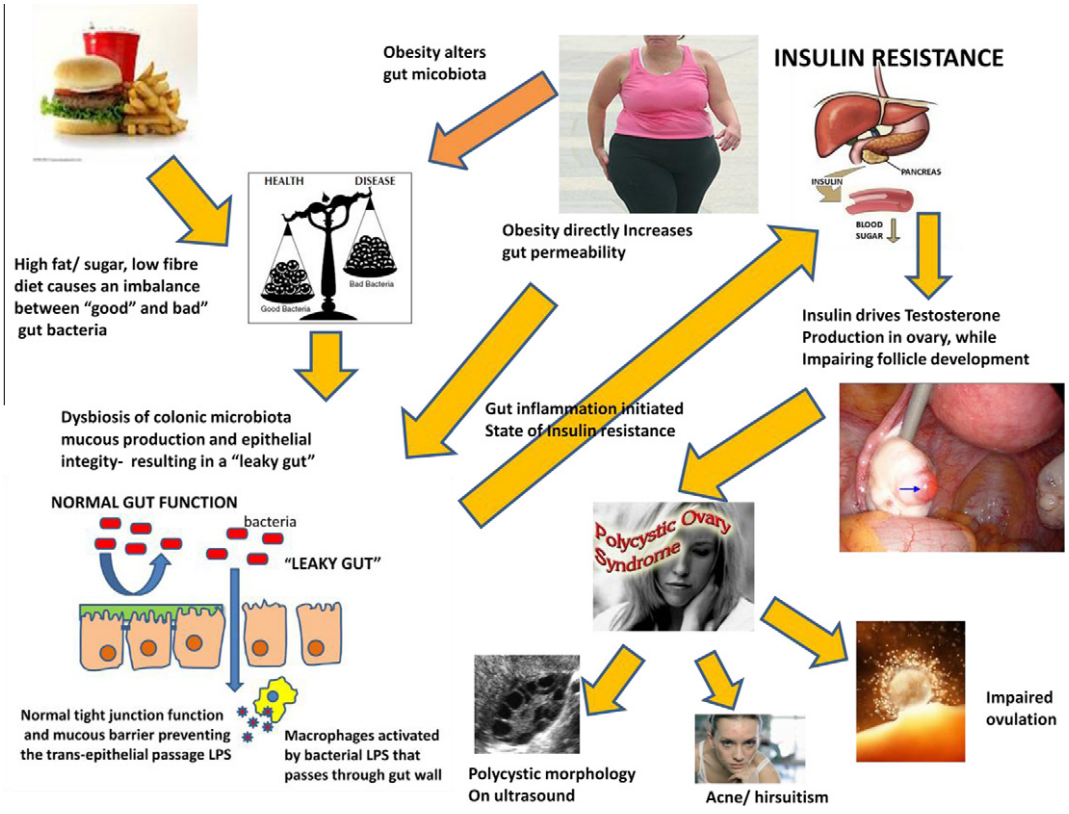

Fig. 1. The DOGMA theory for creation of PCOS.

juice being present, to increasing numbers of bacteria in the large colon where bacteria number 10^{12} CFU per gram of faecal material. Indeed, 60% of the wet weight of colonic material is composed of viable bacteria or the breakdown products of dead bacteria [38,41]. The ileo-caecal valve helps prevent reflux of large bowel contents and their associated bacteria into the small intestine, resulting in relatively low numbers of bacteria in the ileum/jejunum. Therefore, when discussing gut bacteria, we are primarily referring to the vast number of bacteria present in the large intestine (colon).

The gastrointestinal tract constitutes a large body surface area, comparable in size to a tennis court in area. This mucosal surface potentially could provide a large area of susceptibility for points of entry of gut bacteria into the systemic circulation, overwhelming the bodies immune system and creating a rapidly lethal state of systemic infection. Of course this does not normally occur as the colonic mucosa has developed an intricate selective "barrier" capacity which allows the transfer of useful nutrients and water across the bowel wall, while preventing the passage of potentially harmful bacteria [32]. The manner in which the colon achieves this barrier function is complex and has already been well described by several recent reviews [32,42]. However, in summary the colonic mucosal barrier consists of two key features. Firstly, goblet cells within the mucosa produce a thick mucous barrier that prevents colonic luminal bacteria from developing a close contact with the mucosal surface [43]. Although small molecules can pass through this heavily glycosylated mucous layer with relative ease, large molecules and bacteria are prevented free passage. Mice that lack specific genes encoding for the production of mucin have been shown to have defective colonic mucous barrier protection, resulting in spontaneous bacterial colitis, attesting to the importance of this mucous in maintenance of gut mucosal barrier integrity [43,44]. Secondly, cell to cell adhesion proteins that allow selective para-cellular passage of colonic luminal contents between mucosal epithelial cells provides an important barrier function [32]. This is because the mucosal epithelial cells lipid membranes are relatively impermeable to most hydrophilic solutes in the absence of specific trans-membrane transporter "shuttle" proteins. Tight junctions between epithelial cells, formed by the interaction of adhesive proteins such as Claudins and Occludins (zona Occludens 1 and 2) provides a tight seal between adjacent epithelial cells and thereby regulates the passage of solutes from the luminal space through the colon wall into the circulation. Large molecules such as whole bacteria cannot pass through these para-cellular pathways if tight junctions are functioning normally [32].

Intestinal bacteria such as Bifidobacteria and Lactobacilli are often referred to as beneficial "good bacteria" as they play an important role in helping prevent the passage of potentially harmful "bad bacteria" (Enterococcus, Clostridia, *Escherichia Coli*, Proteus, Pseudomonas) across the colonic mucosal barrier and into the systemic circulation [45]. This symbiotic relationship between beneficial "good bacteria" and their human host is mediated by these bacteria's ability to produce nutrients and regulatory substances that enhance the function of the colonic epithelium. Short chain fatty acids (SCFA) such as acetate, butyrate and proprionate are produced by "good" bacteria through the fermentation of carbohydrates such as soluble fibre delivered undigested to the colon [46–48]. These SCFA have been reported to enhance the colonic mucosal cells production of MUC-2 mucin, boosting this "front line" barrier against trans-mucosal passage of bacteria [49,50]. In addition, SCFA provide an essential energy source for the colonic epithelium, helping maintain tight junction integrity. The production of SCFA and lactic acid by Bifidobacteria and Lactobacillus also helps to reduce the pH of the colonic lumen, providing conditions which are hostile for the growth of a pathogenic "bad bacteria". Finally, competition between "good" and "bad" bacteria for a finite food supply helps the Bifidobacteria and Lactobacteria keep "bad bacteria" numbers in check, reducing colonic luminal endotoxin (LPS) formation [24,51].

Clinical conditions that have already been linked with Dysbiosis of the Gut Microbiota and a "leaky gut"

The importance of "good bacteria" in maintaining normal colonic mucosal barrier integrity is highlighted by the condition Irritable Bowel Syndrome (IBS), where it has been shown that gut microbiological balance is disturbed [52,53]. Irritable Bowel Syndrome (IBS) is a common chronic gastrointestinal disorder characterised by the triad of abdominal pain, bloating and change in bowel habit, with an absence of any overt mucosal abnormality, thereby differentiating it from classical inflammatory bowel disease (Crohn's Disease, Ulcerative Colitis). A consistent theme seen in IBS patients is a relative reduction in numbers of Lactobacilli and Bifidobacteria in faecal samples and a higher concentration of Enterobacteria, coliforms and Bacteroides [52,53]. This selective expansion in numbers of "bad bacteria" at the expense of "good bacteria" has been linked with an increase in colonic mucosal permeability due to a decrease in tight junction function [54]. It has been proposed that the breakdown of cell membranes from Gram negative bacteria results in the production of LPS and its subsequent passage across the "leaky gut" wall into circulation resulting in a systemic state of immune activation [52].

Chronic Fatigue Syndrome (CFS), a condition characterised by chronic fatigue, malaise, muscle aches, gastro-intestinal upset and cognitive impairment, is another condition recently linked with a "leaky gut" and chronic activation of the immune system [55]. Studies have identified significantly elevated levels of antibodies against endotoxins (LPS) of bowel Gram negative bacteria in patients with CFS [56], with clinical improvement in this condition being associated with normalisation of this "metabolic endotoxinaemia" [57]. Therefore, it is now becoming recognised that a disturbance in colon mucosal permeability ("leaky gut syndrome"), potentially triggered by perturbations in the balance between "good" and "bad" bacteria in the gut, can produce a systemic state of immune activation and associated symptomatology far removed from the gastroenterological tract.

Evidence for microbiological Dysbiosis and a "leaky gut" in PCOS

No study has directly investigated potential differences in bowel flora between patients with PCOS and normal controls. However, it is well established that the majority of PCOS patients are overweight or obese [2,9,10], with many of these women having quite poor diets. Previous dietary surveys have identified that a high energy intake, with abundant saturated fats and refined sugars, is more common in PCOS women than their normal or overweight ovulatory controls [19–22]. Both animal and human studies have shown that a high-fat/high-sugar diet favours colonisation of the intestine with "bad" Gram negative bacteria, at the expense of "good" Bifidobacteria [23–28], resulting in a metabolic endotoxaemia induced inflammatory state [24,27,29,30,58]. Likewise, a diet low in indigestible fibre (e.g. inulin, plant fructose-oligosaccaride prebiotic fibre) results in a decrease in food available to the Bifidobacteria in the colon, and a subsequent decrease in the numbers of these beneficial bacteria in the gut [59–61]. The net result of a high fat-sugar/low fibre diet is therefore likely to be a decrease in Bifidobacteria numbers, an increase in gut permeability and a resulting chronic activation of the immune system from colonic LPS derived metabolic endotoxaemia.

Obesity, a common co-morbidity in PCOS, has also been shown to result in significant changes in gut permeability. A recent prospective survey of 122 healthy normal to overweight female

subjects identified a positive correlation between waist circumference, visceral fat content on DEXA assessment and increases in colonic gut permeability [31]. While an obesity-related poor diet (high fat/refined sugar, low fibre) may account for some of these differences in gut permeability, it has also been proposed that adipose tissue itself may trigger an increase in gut permeability. The accumulation of visceral fat deposits has been linked with an increase in production of the pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNFα) by the adipose tissue macrophages [62], while also resulting in a reduction in the production of the adipocyte derived anti-inflammatory protein adiponectin [63]. The net pro-inflammatory state results in an increase in gut permeability due to a reduction in intestinal mucous production [64], and an increase in intestinal tight junction permeability mediated by TNFα's action on tight junction proteins [65]. The increase in gut permeability and resulting metabolic endotoxaemia would help explain the observed positive correlation between markers of immune activation such as CRP and increasing BMI [66].

No study to date has directly measured differences in colonic mucosal permeability between PCOS patients and normal controls, yet several lines of research suggest that such a difference is likely to exist. Firstly, Irritable Bowel Syndrome (IBS) and Chronic Fatigue Syndrome (CFS), two chronic conditions previously linked to increased gut permeability and metabolic endotoxaemia [54,56], have both been shown to be significantly more common in PCOS patients. A prospective survey of 65 reproductive aged subjects (36 PCOS, 29 healthy controls) from the USA identified a fourfold increase in the prevalence of IBS in the PCOS group compared to the healthy controls, with a staggering 41.7% of the PCOS cohort complaining of gastrointestinal symptoms of IBS [67]. Similarly, a retrospective case-control study of 227 women with Chronic Fatigue Syndrome identified a nearly fivefold increase in the odds ratio of them having PCOS as a co-morbidity [68]. Since both IBS and CFS have been conclusively linked with an increase in gut permeability, metabolic endotoxaemia and a systemic state of immune activation, it is highly probable that a very significant number of women with PCOS experience a similar "leaky gut" syndrome, based on the common co-morbidities of IBS, CFS and PCOS.

Chronic low grade inflammation as a cornerstone of PCOS pathology

While no study to date has investigated the correlation of PCOS with a state of "leaky gut" related metabolic endotoxaemia, there is extremely good evidence linking PCOS with a pro-inflammatory state. A recent meta-analysis of 31 studies has identified chronic low-grade inflammation in the majority of PCOS patients, with circulating C-reactive protein (CRP) levels being twice as high in PCOS than controls [6]. As the majority of PCOS patients are overweight or obese, it is likely that the presence of excess adipose tissue is causing some of this inflammation mediated by TNFα release from adipose macrophages [62,63]. However, the correlation between PCOS and elevated circulating CRP levels remains significant in this meta-analysis [6] even after controlling for mismatches in obesity or BMI between groups, suggesting that PCOS itself is associated with chronic activation of the immune system independent of obesity.

Molecular studies have identified a possible genetic co-factor responsible for the chronic state of immune activation seen in PCOS subjects, with variants in genes encoding for the pro-inflammatory cytokines TNFα and interleukin-6 (IL-6) being reported to be more common in the PCOS population [69,70]. An increase in activity of these pro-inflammatory cytokines is known to interfere with tight barrier function in the colonic epithelium, resulting in an increase in gut permeability, increased transfer of endotoxin across the mucosal epithelium and a resulting metabolic endotoxaemia-a clear potential positive feedback loop.

Chronic inflammation as a cause of insulin resistance

Numerous studies have documented that insulin resistance is common in both obese and lean women with PCOS, affecting up to 70% of the PCOS population [7,8,14]. This observation, together with the fact that there is a 5 to 10-fold higher prevalence and later conversion to frank diabetes in the PCOS cohort compared to the general population [7,8,11], underlines the strength of association between the PCOS condition and insulin resistance. While some studies support a genetic predisposition to insulin resistance [71], there is mounting evidence suggesting that the chronic state of inflammation seen in PCOS may be responsible for initiating insulin resistance.

An increased production of the pro-inflammatory cytokines TNFα and IL-6 by tissue macrophages and stromal cells contained in adipose tissue has been observed in the majority of overweight individuals [6,62,66]. Even in lean PCOS individuals, the presence of a "leaky gut" would generate an increase in serum TNFα and IL-6 mediated by endotoxin induced activation of macrophages. Both TNFα and IL-6 have been linked to the generation of insulin resistance. The cytokine TNFα is thought to activate JNK1 and NF-κB which results in phosphorylation of the serine residues on the insulin receptor substrate-1 (IRS-1) protein, thereby preventing its interaction with the insulin receptor beta subunit, impeding the insulin signalling pathway and generating a state of insulin resistance [72,73]. Furthermore, the administration of recombinant TNFα to mice renders them insulin resistant [72], while TNFα receptor-null mice have an increased sensitivity to insulin [74], emphasising the important role of TNFα in the maintenance of normal insulin sensitivity. Similarly, IL-6 has been identified to cause insulin resistance [75], but the molecular mechanisms for this impairment are presently unknown.

Several studies suggest that gut microbiota would be capable of initiating a state of insulin resistance if the passage of LPS from these bacteria into the systemic circulation is permitted by an increase in colonic mucosal permeability ('leaky gut"). The direct administration of LPS into the circulation of mice [24] and humans [76] has been shown to cause an increase in fasting glucose and insulin levels, confirming insulin resistance. Secondly, in animal models the administration of an antibiotic has been reported to decrease the caecal content of Gram negative bacteria, resulting in a reduction in systemic absorption of endotoxin, a reduction in systemic inflammation and an improvement in insulin sensitivity/glucose homeostasis [29,30]. Finally, human studies have reported that a beneficial adjustment in the balance between "good" and "bad" bowel bacteria using Lactobacillus and Bifidobacterium probiotics can result in improved insulin sensitivity [77–79].

Insulin resistance – the last frontier in PCOS pathophysiology

Insulin resistance, an abnormality seen in the majority of PCOS patients, is the final link in the generation of all three classical clinical features of PCOS-hyper-androgenism (acne, hirsuitism, high serum free androgens), the formation of multiple small follicular cysts within the ovary and impaired ovulation. The inflammation induced impairment of the insulin receptors function produces an increase in serum insulin concentrations, which increases serum free testosterone levels via two mechanisms. Firstly, insulin drives excess androgen production by the ovarian theca cells [34–36]. Secondly, insulin reduces the production of Sex Hormone Binding Globulin (SHBG) by the liver, producing an increase in free (bioavailable) testosterone [80]. The net effect of these changes is a marked increase in androgen availability within the skin, resulting in the acne and hirsutism features of PCOS.

The paradox of PCOS is that while soft tissue such as the liver and muscle exhibit impaired insulin action, this state of insulin

resistance does not extend to the ovary itself [81]. It has been suggested that insulin could act on the ovaries of insulin-resistant women with PCOS through either homodimeric IGF-1 receptors, or heterodimeric receptors having one insulin receptor subunit and one IGF-1 receptor subunit [81]. Furthermore, insulin inhibits the production of insulin like growth factor 1 binding protein (IGFBP-1), which in turn increase the amount of free IGF-1 available to stimulate thecal androgen production [82]. A local ovarian milieu containing high concentrations of androgens, insulin and IGF-1 activity may impair the normal development of follicles from the small antral follicles (2–10 mm), through to maturation [37]. This of course would provide the mechanism for the development of the last two cardinal features of PCOS-multiple sub-capsular small ovarian cysts and impaired ovulation/menstrual irregularity.

Potential novel treatments for PCOS – the DOGMA approach

Current treatments for PCOS

Traditionally in PCOS patients three different approaches have been used to help initiate normal ovarian function, with variable success. The ideal initial approach is "lifestyle" modification to produce weight reduction through a combination of diet and exercise [11,83,84]. It has now been well established that decreasing energy intake, especially when combined with exercise, results in improvements in insulin sensitivity and may lead to a resumption of normal ovarian function with ovulation [83,84]. While this "lifestyle" approach has the advantage of improving overall health, at a minimal cost, it is successfully achieved by only the minority of PCOS patients. This may be due to a lack of "willpower" on the part of PCOS patients to change life-long poor habits, or a reticence to try diet and exercise because of poor physical health and multiple past failures in "healthy living". A second approach to managing PCOS is the use of insulin sensitizing medications such as Metformin, which normalise insulin sensitivity, leading to an improvement in ovarian function [83,84]. Finally, the direct application of recombinant Follicle Stimulating Hormone (FSH) injections, or the triggering of the pituitary's own release of FSH by estrogen antagonists (Clomiphene Citrate) or aromatase inhibitors (Anastrazole) have all been shown to successfully induce ovulation in PCOS patients. However, these "ovulation induction" agents have the disadvantage of often producing multiple mature oocytes in each menstrual cycle, with a significant risk of higher-order pregnancies (twins, triplets), as well as having no effect on the hyperandrogen symptoms of PCOS (84–85). Therefore, there is a significant unmet demand for effective new treatment approaches to PCOS.

Probiotics and Prebiotics – potential new treatment for PCOS

Introduction

Modification of the colonic bacterial balance through the use of prebiotics and probiotics has the potential to be an effective treatment of PCOS, with several advantages over traditional treatments since it targets the proposed initial pathological insult in the condition – microbiological Dysbiosis and the resulting "leaky gut".

A probiotic, as defined by the WHO (2002), is a "live microorganism which, when administered in adequate amounts, confers a health benefit to the host" [85]. Therefore, three main criteria needed to be met by a microorganism in order for it to be considered a probiotic. Firstly, the organism must be resistant to gastric acidity and bile acid toxicity so that it may transit through the upper gastrointestinal tract and still be viable once it reaches the lower regions of the gut. Secondly, the probiotic organism must adhere to the intestinal epithelial cells or mucous coat so that it is not rapidly lost in the faecal material, but instead has a chance to replicate in the intestinal lumen. Finally, the probiotic organism must convey a benefit to the host human – implying it is non pathogenic (safe) and must produce antimicrobial substances that favour a healthy gut microflora composition. The two most commonly used probiotic organisms are Bididobacteria and Lactobacillus, since they meet all of these desired criteria for a positive effect on the host [85].

Prebiotics are best described as a selective food source for beneficial "good" bacteria. The concept of prebiotics was first introduced by Gibson and Roberfroid [45] as an alternative way of boosting the number of "good" bacteria within the gut, rather than by direct application of these bacteria (prebiotic approach). Prebiotics are defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and heath" [85,86]. Therefore, two key criteria are required to be considered a prebiotic. Firstly, the substance cannot be digested by the host, instead being delivered relatively intact to the colonic lumen where it can act as a bacteria food source. Secondly, the prebiotic substance must selectively support the growth and function of beneficial bacteria such as Bifidobacteria and Lactobacteria, while not facilitating the growth or function of non-beneficial "bad" bacteria [86]. Currently, only fructo-oligosaccharides (FOS), inulin (a long chain form of FOS), galactooligosaccharides and lactulose meet the European Union's criteria for bioactive prebiotics [85]. Probiotic oligosaccarides are found naturally in several different foods such as leek, asparagus, artichoke, garlic, onion, wheat, oats and banana [86]. However, the amounts of FOS in these plants is relatively low, making artificial supplementation with soluble fibre inulin derived from chicory roots a more attractive probiotic therapy. To date, over 20 studies have shown that inulin and FOS prebiotic have the capacity to selectively increase the number/proportion of beneficial Bifidobacterium in the colon or faecal material, with some studies showing a similar effect on Lactobacillus numbers [86]. In addition to increasing the number of beneficial bacteria, some studies have also confirmed a reduction in the number or proportion of harmful "bad" bacteria in the gut with prebiotic use [86].

Symbiotics is a type of combinational therapy consisting of a prebiotic and a probiotic, with the obvious target synergy being that the prebiotic will selectively stimulate the growth and/or activity of both the endogenous and exogenously applied (prebiotic) beneficial bacteria [85]. This type of symbiotic therapy is likely to have an advantage over a pure prebiotic approach if the host has a diet that has insufficient dietary fibre to support the optimal growth and function of the colonic microbiota.

Current evidence supporting the use of probiotics and prebiotics in PCOS

To date, no study has been conducted using prebiotics, probiotics or symbiotic therapy as a treatment for PCOS [87]. One large prospective study of 2165 women with infertility identified a very significant reduction in the incidence of anovulatory infertility (primarily PCOS mediated) if the subject consumed high amounts of whole milk, compared to those subjects drinking skim or low fat milk [88]. While the authors of this study were uncertain on the underlying cause for this observation, a prebiotic effect of whole milk is possible. Bovine whole milk is known to contain probiotic oligosaccarides, often bound to milk-fat globules [89]. The ingestion of oligosaccarides in human milk has been shown to enhance the growth of colonic Bifidobacteria in infants [90], raising the possibility that bovine whole milk may have a similar bifidogenic effect. The removal of oligosaccarides bound to milk-fat globules in the production of low fat milk may help explain why this

type of milk had no protective effect on the incidence of anovulatory infertility in the Chavarro study [88].

While direct evidence supporting the use of prebiotics/probiotics in PCOS is presently very weak, two indirect lines of evidence suggest that such an approach may be beneficial. Firstly, several animal studies have now shown that prebiotic [91] and probiotic [76,77,79,92–94] supplements can help prevent impaired insulin resistance, a central driver of PCOS pathology. These animal studies are backed up by a large trial of 256 pregnant women who were randomised to probiotic treatment (Lactobacillus rhamnosus GG, Bifidobacterium lactis Bb12) or placebo in their first trimester of pregnancy and then followed for the development of gestational diabetes/insulin resistance during the pregnancy and postnatal period [77,79]. Prebiotic treatment significantly reduced the incidence of gestational diabetes (36% v 13%, $p = 0.003$), while also improving insulin sensitivity in both the pregnancy and 12-month post natal period [79]. We therefore propose that if prebiotics can improve insulin sensitivity in reproductive age women, they certainly have the potential to drive down insulin levels in women with PCOS, thereby reducing the anovulatory and hyperandrogenism symptoms triggered by hyperinsulinaemia.

It has been well established that a reduction in body fat stores brought about by a combination of diet and exercise has the potential to initiate normal ovulation in PCOS patients, as well as normalise androgen, glucose/insulin and lipid profiles [11,83,84]. There is now mounting evidence that probiotics/prebiotics may help with weight loss and therefore treat PCOS via a weight loss mechanism. The proposed manner in which probiotics and prebiotic may enhance weight loss is threefold. Firstly, studies suggest that prebiotic fibre increases faecal energy and fat excretion [95], effectively reducing energy intake. Secondly, the production of SCFA by beneficial bacteria has been shown by *in vitro* [96] and *in vivo* animal studies [97] to increase the colonic mucosa's production of the "satiety" hormone Glucagon-Like Peptide-1 (GLP-1). This GLP-1 peptide is known to have a central action on the brain reducing appetite and therefore energy intake. The prebiotic oligosaccharide has also been shown to increase serum GLP-1 levels in humans [98], supporting a role for prebiotics in controlling human nergy intake. Finally, research now suggests that changes in bowel flora may even be directly responsible for obesity by increasing the energy harvest from the colonic luminal contents. Gut microbes process indigestible dietary polysaccharides by fermentation, producing the SCFA's butyrate, acetate and propionate. While the majority of butyrate is used as an energy source for colonic epithelial cells, acetate and propionate are primarily absorbed by the gut and delivered to the liver where they are used for *de novo* lipidogenesis [99]. Since obese humans have been reported to have increased concentrations of SCFA in their faeces due to an imbalance in bowel flora [100], they are predisposed to further excessive weight gain. While still a developing area, a recent randomised-controlled trial (RCT) has confirmed the ability of a Lactobacillus gasseri containing probiotic to cause a significant reduction in abdominal adiposity and body weight, without the need for an increase in exercise or change in energy intake [101]. If future RCTs confirm this observation, one would expect that probiotic assisted weight loss would be an effective treatment for PCOS, given the currently well established beneficial effects of weight loss through diet and exercise.

Overview and future research directions

Summary of the DOGMA theory of PCOS

A diet high in fat and sugar, yet low in fibre, alters the normal balance of beneficial and harmful bacteria in the gut lumen. "Good" bacteria such as Bifidobacteria and Lactobacteria limit the growth of "bad" bacteria by reducing colonic pH and competing for nutrients, while also producing beneficial SCFA that act as a food source for the colonic epithelium. Any decline in beneficial bacteria numbers will result in impairment of the colonic epithelial cells production of essential factors required for maintenance of the intestinal barrier (mucins, tight junction proteins), and a reduction in the colon's production of the satiety hormone GLP-1. When serum GLP-1 levels decline, hunger increases, resulting in an increased energy intake and the potential for a positive feedback loop. The increase in gut permeability ("leaky gut") produced by poor diet related changes in gut bacteria, together with a direct inflammatory effect of excess adiposity, combined with a possible increase in numbers of Gram negative "bad" bacteria, results in an increase in the translocation of immuno-stimulatory LPS molecules from gut lumen into the systemic circulation. This state of "metabolic endotoxaemia" activates macrophages in the fat, liver and muscle, leading to the release of high levels of TNFα and the initiation of insulin resistance. This state of hyper-insulinaemia interferes with normal follicle development in the ovary, causing a halt in follicular development with the generation of multiple small follicles (typical polycystic morphology on ultrasound), and impaired ovulation with its associated menstrual irregularity. High serum insulin also drives excessive androgen production by ovarian thecal cells, while depressing hepatic production of SHBG, resulting in a net increase in free androgen availability and the development of acne and hirsuitism. Fig. 1 summarises these proposed steps in the patho-physiology behind the generation of PCOS according to the DOGMA theory.

The symbiotic application of a probiotic containing Bifidobacteria and Lactobacterium, together with an appropriate prebiotic "food source" (inulin, fructose oligosaccharide) is likely to improve intestinal barrier function (increased mucin production, better tight junction integrity), while possibly reducing the colonic Gram negative bacterial load. These changes should result in a reduction in transfer of LPS across the mucosal wall, reducing metabolic endotoxaemia. A probiotic mediated increase in colonocyte production of the satiety hormone GLP-1 will reduce energy intake, producing a drop in adipose tissue mass and a decrease in inflammation, with a resulting further improvement in gut mucosal barrier function. The net reduction in colonic mucosal permeability and resulting metabolic endotoxaemia will lead to an improvement in insulin receptor function, a drop in serum insulin and a normalisation of ovarian function. These beneficial changes brought about by prebiotic/probiotic use are summarised pictorially in Fig. 2.

Future research directions

While we have outlined abundant circumstantial evidence supporting a link between gut microbiota dysbiosis, "leaky gut" and PCOS, this association needs further study before a causal link can be proven. It is proposed that future prospective studies should be conducted measuring differences in intestinal permeability and serum endotoxin (LPS) levels between women with PCOS and ovulatory controls (both normal and increased BMI). If our Dysbiosis Of Gut Microbiota/leaky gut theory is correct, the PCOS cohort should have increased gut permeability and serum endotoxin (LPS) levels compared to their lean or BMI matched ovulatory controls. Furthermore, studies analysing differences in colonic microbiology between PCOS and normal ovulatory women would be helpful to establish the voracity of the DOGMA theory for creation of the PCOS clinical state.

While the available animal and human evidence suggest that prebiotic/probiotic use should result in an improvement in gut mucosal barrier function, a drop in metabolic endotoxaemia and

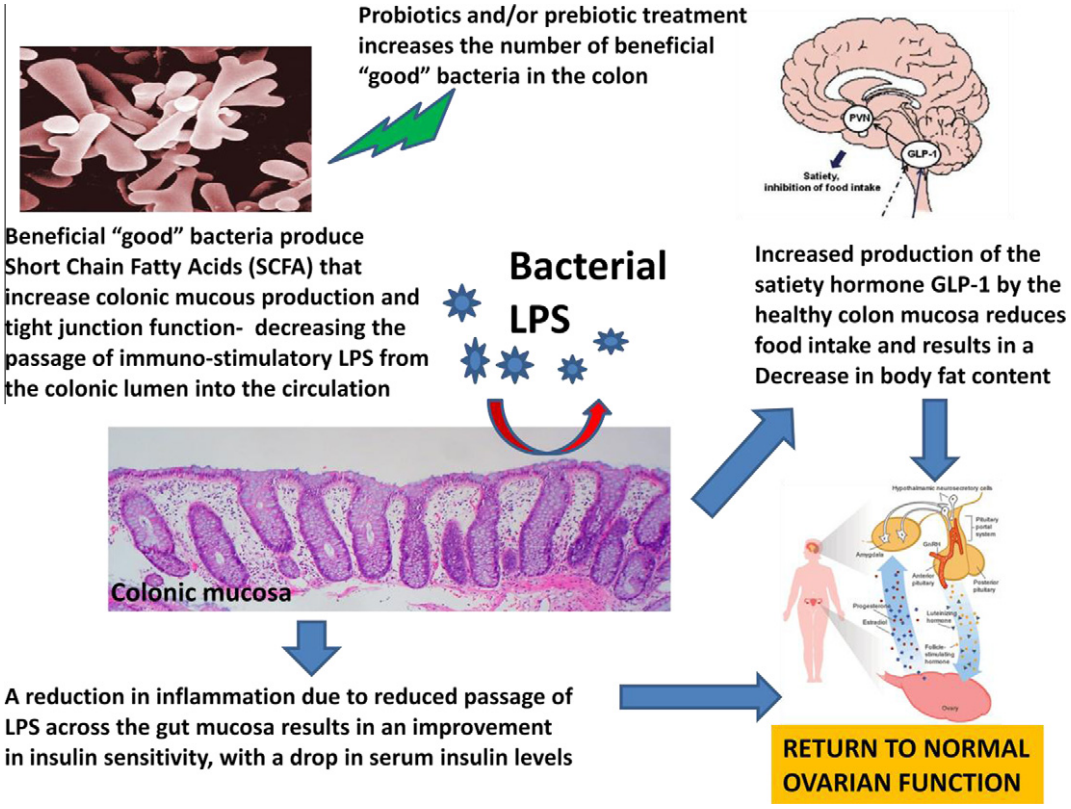

Fig. 2. A symbiotic approach to the management of PCOS.

an improvement in insulin sensitivity, no study to date has examined if prebiotic/probiotic supplementation can normalise ovarian function in PCOS women. If such a trial was to be conducted, preferably in a randomised placebo-controlled manner, we suggest that a good first-line choice for a probiotic would be the bacteria Lactobacillus rhamnosus GG, Lactobacillus gasseri (LG2055) and Bifidobacterium lactis Bb12, as these strains have been confirmed in human RCTs to improve insulin sensitivity and reduce body fat stores [77,79,101]. An appropriate probiotic would be inulin or FOS, in sufficient dosage to increase Bifidobacteria numbers [86], without producing adverse symptoms such as bloating, "wind" pain and flatulence commonly seen with excessive prebiotc use. If short-term trials do confirm that prebiotic/probiotics can normalise the clinical and biochemical features of PCOS, a longer term study could be conducted to analyse if prebiotics/probiotics can reduce some of the adverse long term complications of PCOS, namely increased rates of cardiovascular disease (hypertension, ischemic heart disease, CVAs), diabetes and cancer [11,84].

We believe that there is excellent circumstantial evidence supporting the conduct of these types of experimental studies in PCOS women. Furthermore, a recent survey of 648 women with PCOS were asked the question, "If your PCOS could be safely and effectively helped by something besides fertility drugs or birth control pills, would that interest you?" An overwhelming 99% of respondents stated that they would be interested in such a Complementary and Alternative Medicine (CAM) approach [102]. Therefore, there is likely to be a high patient driven demand for a prebiotic/probiotic approach to management of PCOS. The public generally perceive CAM to be safe and with minimal side effects, thereby explaining their popularity. While probiotics are known to be safe, it is presently unknown if PCOS women contemplating pregnancy perceive a live bacterial probiotic "medicine" as being safe, especially given the high profile concerns of miscarriage and intra-uterine fetal death associated with the accidental ingestion of Listeria bacteria during pregnancy. Future surveys of the community regarding their perception of the safety of probiotics in women contemplating pregnancy are therefore also required.

Conflict of Interest Statement

None.

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