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Overview of the Current State of the Art in Nutritional Interventions for Polycystic Ovary Syndrome

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RESUMO

O Síndrome do ovário poliquístico (SOP) é um complexo transtorno endócrino e metabólico frequentemente associado a infertilidade, obesidade e a insulinorresistência. A etiologia da doença permanece mal compreendida, apesar de causas poligenicas e multifatoriais parecerem estar envolvidas, incluindo, em particular, elementos ambientais, onde se destaca a dieta e outros fatores do estilo de vida. Neste contexto, a terapêutica da SOP deve ser ajustada de acordo com a gravidade das manifestações e necessidades da doença e, partindo da modificação do estilo de vida, conjuntamente com potenciais abordagens farmacológicas e/ou cirúrgicas.

A presente revisão tem por objetivo fornecer uma revisão compreensiva do estado atual da investigação no que respeita ao tratamento e gestão de mulheres com SOP, focando-se, em particular, nas intervenções nutricionais, pois são estas consideradas opções de tratamento de primeira linha.

A dieta e proporção relativa de macronutrientes ideal, em situações de SOP, ainda não foi passível de ser identificada e globalmente reconhecida, já que a heterogeneidade dos resultados encontrados é significativa. Não obstante, uma distribuição de macronutrientes similar à descrita na dieta Mediterrânica mostrou impactos positivos e revelou-se adequada para manutenção a longo prazo. A suplementação de certos elementos em défice pode também desempenhar um papel de interesse na abordagem destes doentes sendo que a vitamina D e os ácidos gordos polinsaturados n3 mostraram os resultados mais consistentes. Mais ainda, a disbiose da microbiota intestinal parece não só influenciar a patogénese da doença como também a sua correção, através de agentes prébióticos e probióticos, parece constituir uma intervenção terapêutica promissora.

Apesar da constante publicação de novos dados, esta área de investigação carece ainda de mais estudos de qualidade, visto que devem ser contempladas intervenções com maior duração e com grupos em estudo de maior dimensão e mais diversificados, especialmente no que se refere à etnicidade dos mesmos.

Palavras-chave: Síndrome do ovário poliquístico, desarranjo metabólico, intervenção nutricional, microbiota intestinal, disbiose.

ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder frequently associated with infertility, obesity and insulin resistance. The etiology of the disease remains poorly understood, despite polygenic and multifactorial causes are thought to be involved and from which environmental elements should be emphasised, these including diet and lifestyle factors. In this regard, treatment of PCOS should be adjusted accordingly to the severity of manifestations and necessities of the patient. The approach should be, ideally, based on lifestyle modifications, these eventually in conjunction with a pharmacologic and/or surgical approach.

This article aims to provide a comprehensive review of the current state of research in what regards the therapeutic strategies and management of women with PCOS, however, focusing, particularly, in dietary interventions as these are considered first line treatment options.

The optimal diet and macronutrient balance, in the PCOS setting, is yet to be identified and globally recognised, as heterogeneity in the available data is still significant. Nevertheless, a macronutrient distribution similar to the one found in a Mediterranean diet showed positive impacts and suitable for long-term maintenance. Supplementation of specific lacking elements may also play an important role in the approach of these patients, expressly vitamin D as well as omega-3 polyunsaturated fatty acids having the most consistent evidence-based results. Furthermore, dysbiosis of the gut microbiota may influence the pathogenesis of the disease and its correction, through prebiotic and probiotic agents, a promising treatment option.

Despite the fact that new data is constantly published, this area of research is still in lack of further good quality studies, as broader time of intervention as well as broader and more diverse intervention groups should be contemplated, especially in what refers to ethnicity variety.

Keywords: Polycystic ovary syndrome, metabolic dysfunctions, nutritional interventions, gut microbiota, dysbiosis.

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INTRODUCTION

DEFINITION, CLASSIFICATION AND PHENOTYPES

The polycystic ovary syndrome (PCOS) is an heterogeneous endocrine disorder, affecting women, most significantly of reproductive age, and which prevalence, when considering the 2003 Rotterdam diagnostic criteria, ranges from 5.5% to 19.9% (Azziz et al., 2016).

The core characteristics of this syndrome include hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology (PCOM) (Figure 1) (Azziz et al., 2016). Hyperandrogenism is considered when clinical manifestations such as hirsutism, acne, alopecia or seborrhoea are present and/or biochemical hyperandrogenism is determined. Ovulatory dysfunction comprises menstrual dysfunction, as found with oligomenorrhea or amenorrhea, subfertility and endometrial hyperplasia and/or carcinoma. PCOM, on its turn, is defined by an excessive number of preantral follicles in the ovaries, namely 25 follicles or more per ovary, and/or ovarian volume equal or higher than 10 ml.

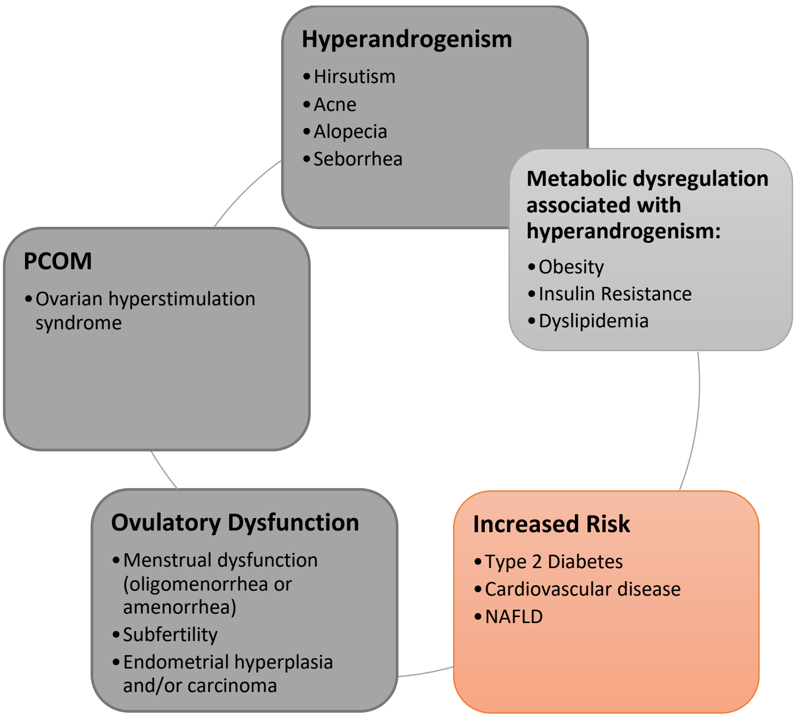

Figure 1: PCOS Characteristics

It is also pertinent to emphasise that despite the fact that most signs and symptoms are more marked in women of reproductive age, they are also beginning to be acknowledged in pre and postmenopausal women (Azziz et al., 2016).

Three alternate definitions (Figure 2) for PCOS remain valid, though the 2003 Rotterdam definition is the most widely used and supported by most scientific societies (Escobar-Morreale, 2018). According to these criteria, diagnosis implies the presence of two of the three core characteristics previously mentioned.

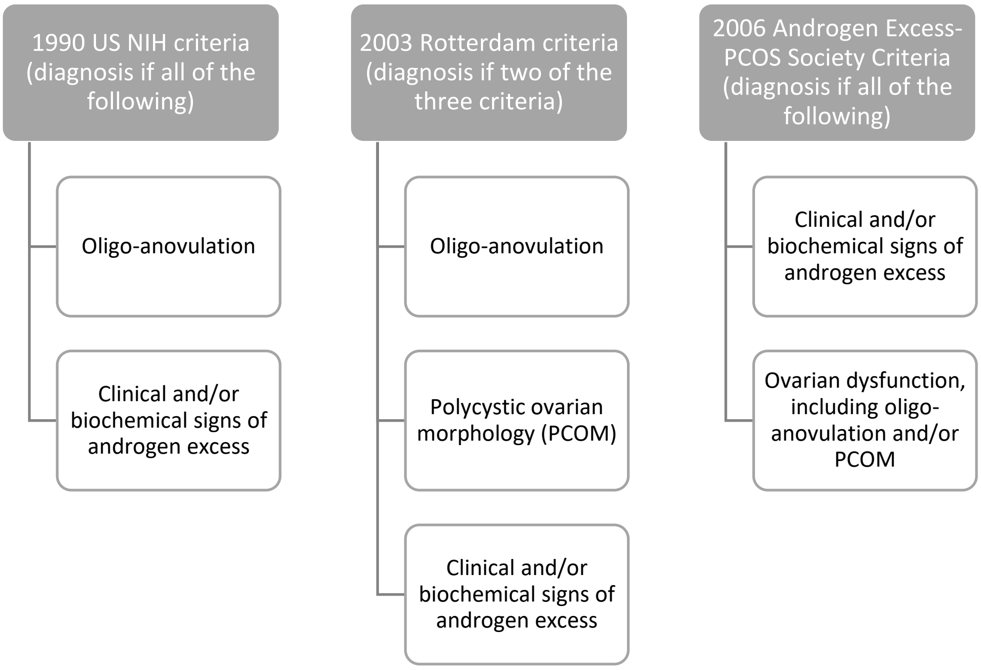

Figure 2: Diagnostic Criteria of PCOS

Multiple phenotypes can be defined based on these features and heterogeneity in presentation is often implied among the affected patients, not all of them having the same criteria met nor being exposed to the same health risk factors (Figure 3). It is worth of mention that the most severe phenotypes are also the predominant and the more strongly associated metabolic comorbidities, as are insulin resistance (IR), glucose intolerance and higher incidence of diabetes mellitus (Azziz et al., 2016; Escobar-Morreale, 2018; S. Patel, 2018). These metabolic comorbidities are independent of body mass index (BMI) (Azziz et al., 2016). Obesity is possibly more prevalent in PCOS patients, even though strong evidence supporting this data is lacking. Furthermore, obesity may play a role in the development of the disease but the evidence is conflicting (Azziz et al., 2016).

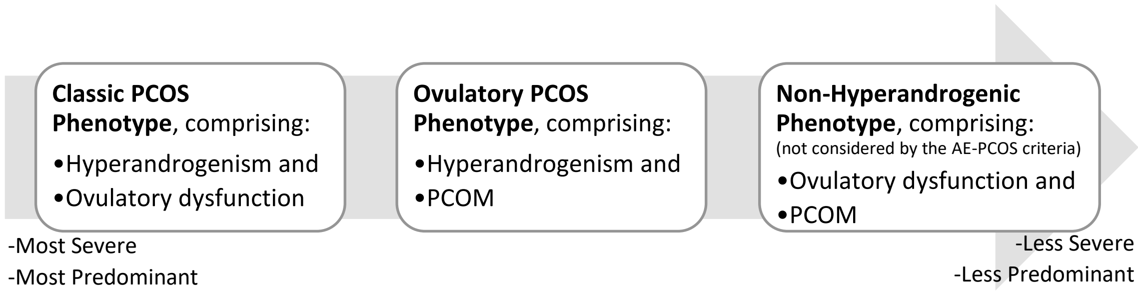

Figure 3: PCOS Phenotypes

As other extra-reproductive characteristics, PCOS is associated with a low-grade chronic inflammation, higher risk of cardiovascular (CV) disease and higher incidence of non-alcoholic fatty liver disease (NAFLD) (S. Patel, 2018; Rocha et al., 2019). Additionally, the syndrome is interlinked to higher incidence of cancer, respectively endometrial cancer, expected due to the hyperandrogenism, insulin resistance and its consequences (Azziz et al., 2016).

Psychological manifestations are also part of PCOS presentation. Depression and anxiety related disturbances are more prevalent and more severe in women with the disorder, regardless of the patients' phenotype or the presence of obesity. Factors like hirsutism or infertility are linked to the emotional distress but did not fully explain the high prevalence of mental disorders by themselves. Remarkably, insulin resistance degree seems to be significantly correlated with depression scores (Azziz et al., 2016).

AETIOLOGY AND PATHOPSHYSIOLOGY

PCOS aetiology and pathophysiology is still poorly understood and, as it stands, considered a complex multigenic disorder, in which different genetic variants interact with numerous environmental factors, including lifestyle factors and diet types to, ultimately, manifest in the diverse phenotypes formerly exposed.

Escobar-Morreale hypothesized that PCOS results from a vicious cycle in which hyperandrogenism favours abdominal adipose tissue deposition and visceral adiposity by inducing insulin resistance and consequent hyperinsulinism. These, in turn, facilitate androgen secretion by the ovaries (acting as a co-gonadotropin) and adrenal glands and modulate luteinizing hormone (LH) pulsatility in the patients with PCOS (Escobar-Morreale, 2018). As so, it appears that women with more severe androgen excess and ovarian dysfunction have also more severe insulin resistance. Androgen excess is, therefore, not only a major mechanism in the oligo-ovulation and cutaneous manifestations but also critical in the metabolism dysfunction of the disease. Moreover, the author suggests that in some cases the hyperandrogenic abnormality alone is sufficient for the syndrome to manifest, as it presents in lean women without any evidence of visceral adiposity or insulin resistance. In other cases, PCOS is only evident when obesity, abdominal adiposity, insulin resistance and hyperinsulinism trigger a mild alteration in steroidogenesis (Escobar-Morreale, 2018).

Besides elevated androgens, estradiol level is also high in PCOS women, whereas progesterone level is low. Anti-Müllerian hormone (AMH), on its turn, is elevated inhibiting folliculogenesis, leading to anovulation (S. Patel, 2018). This may be explained by the fact that AMH increases GNRH-dependent LH pulsatility which plays a role in PCOS pathophysiology as already mentioned (Rocha et al., 2019).

DISEASE MANAGEMENT

Interestingly, as a poorly understood disease a specific drug has never been approved for the treatment of PCOS. On one hand, this means that limited research resources are allocated to this disease. On the other hand, this could also indicate that the potential in this field of study is still immense, giving plausible purpose to this review.

According to the American Society for Reproductive Medicine (ASRM) 2018 Guidelines, lifestyle intervention is the first line treatment, including diet control and increased amount of exercise (Teede et al., 2018). The focus of the present review will fall back on the first component specified. Small lifestyle changes can improve metabolic dysfunction, ovulation, fertility and mood.

Lifestyle intervention may, consequently, improve free androgen index (FAI), weight and BMI (Lim et al., 2019). In addition, improvement in hyperandrogenism, both biochemical and clinically, as well as in the lipid profile, specifically in total cholesterol (TC) and LDL were identified, these effects despite modest weight loss. Moreover, lifestyle intervention might reduce surrogate markers of insulin resistance compared to minimal treatment, as evident from smaller waist circumference and lower oral glucose tolerance test (OGTT) insulin. This is an important finding considering the role of IR in the pathophysiology and comorbidities of PCOS (Lim et al., 2019). Minimal body weight reduction (at least 5%) can improve ovulation, suggesting that calorie restriction and fat distribution alterations are essential for these results. Finally, lifestyle interventions improves both depressive and quality of life scores specific to PCOS, except those for the body hair domain (Azziz et al., 2016).

In this review, it is pursued the intention of studying the implication of different nutrition interventions on different markers of the disease and their potentiality to ameliorate patients' symptoms and overall quality of life. In context of these interventions, different diet patterns will be addressed as well as potential specific elements supplementation. Microbiota as an impeding new approach to the disease treatment will also be discussed.

BODY COMPOSITION AND METABOLISM DERANGEMENT IN PCOS

BODY COMPOSITION

The body composition of women with PCOS has been a recurrent subject of study. In that setting, their mean BMI has frequently been encountered as higher (Chitme et al., 2017). Moreover, nonobese and obese PCOS women when in comparison with age and BMI-matched controls, had higher total body fat, truncal fat (and resultant higher central obesity prevalence) and estimated visceral adipose tissue, assessed by DXA scan. Nonobese PCOS women had similar amount of visceral adipose tissue as that of the obese PCOS women when body weight was adjusted, which indicates higher and similar risk of metabolic complications in both groups (Satyaraddi et al., 2019).

The metabolic complications, when analysed in the context of body composition, showed that the insulin resistance is about four times higher in obese PCOS patients comparing with the nonobese. Despite that fact, nonobese PCOS women were metabolically worse than nonobese healthy controls and had higher corrected insulin resistance than the obese PCOS patients compared with respective obese control group (Satyaraddi et al., 2019).

Besides the mentioned, and considering lean body mass, a significant negative correlation between total skeletal muscle and the incidence of PCOS has been encountered as well as a lower total skeletal muscle percentage in the affected patients, which is, in turn, associated with infertility and can reduce insulin sensitivity (Chitme et al., 2017).

METABOLISM DEARAGEMENT

Metabolic dearangement is another key feature in PCOS. In comparison with control heathy groups, a higher prevalence of metabolic syndrome has been identified, and more specifically within its componentes a low HDL and a high waist circumference are the most frequent implied alterations found (Hallajzadeh et al., 2018). The metabolic impairment, for its part, is is not exclusively dependent on the total fat mass content and body weight and might be also attributed to the androgen excess as discoursed in the introduction (Condorelli et al., 2018).

As other contributing factors for the alteration of normal metabolic profile are adipose tissue-derived hormones, commonly mentioned as adipocytokines, and gut hormone ghrelin, which function is altered and thus leading to PCOS. Leptin, crucial for the regulation of energy balance, and frequently known as the "satiety" hormone is elevated in PCOS patients' serum. Likewise, its elevation may be associated with insulin resistance, metabolic disorder, infertility and higher cardiovascular disease risk (S. Patel, 2018; S. H. Zheng et al., 2017). Ghrelin, the "hunger" hormone, with an opposite function to leptin, but both acting upon receptors in the hypothalamus, is independently associated with AMH levels in obese PCOS patients (S. Patel, 2018). Serum ghrelin levels have been found to be lower in PCOS patients than in healthy controls, irrespective of BMI (Baldani et al., 2019; Gao et al., 2016). Adiponectin serum levels are lower and irrespective of obesity, and are probably related to IR but not to testosterone (Baldani et al., 2019; Toulis et al., 2009). Remarkably, secretion of leptin, adiponectin and ghrelin may be affected by other factors independent of obesity, IR and hyperandrogenism, unique to PCOS and may, as a result, serve as independent biomarkers for the diagnosis of PCOS (Baldani et al., 2019).

Altogether, the altered components here identified alert for the need of them being addressed in prevention and treatment strategies, as will be furtherly discussed.

DIETARY INTERVENTIONS

WHAT DO PCOS PATIENTS' EAT?

Diet is a key determinant of excessive body weight in PCOS. Different studies in diverse populations have been trying to identify a particular eating pattern associated to PCOS women.

Tendentiously, PCOS patients' seem to show poorer dietary intake, especially in what refers to consumption of whole grains and fibre, showing, consequently, a higher dietary glycaemic index (GI) and glycaemic load as well as a total fat and overall higher energy intake (Altieri et al., 2013; Barr et al., 2011; Barrea et al., 2018; Eslamian et al., 2017). More particularly, and relevant as subsequent mention to the Mediterranean diet will be considered, women with PCOS seem to consume less extra-virgin olive oil, legumes, fish/seafood, and nuts, unsaturated fatty acids like n-3 PUFAs (Barrea et al., 2019).

As a matter of fact, diets rich in glucose and saturated fat can promote inflammation in women with PCOS and stimulate ovarian androgen production, independent of excess adiposity or IR and, as a result, worsen PCOS features (Barrea et al., 2018, 2019; Rodriguez Paris et al., 2020). This unhealthy pattern is also associated to IR, its severity increasing along with the raise in BMI (Barrea et al., 2019; X. Zheng et al., 2021). Moreover, PCOS women have been reported to have a decreased basal metabolic rate (Barrea et al., 2018).

Studies may be, however, controversial as others found no significant differences between the dietary habits, macronutrient intake and resting metabolic rate from women with or without PCOS (Altieri et al., 2013; Barrea et al., 2018; Rodriguez Paris et al., 2020).

WHAT DO WE KNOW SO FAR?

The ASRM 2018 Guidelines state that a variety of balanced dietary approaches can be recommended to reduce dietary energy intake and induce weight loss in women with PCOS whom are also overweight or obese. Furthermore, overall healthy eating principles should be followed, as per general population recommendations and unduly restrictive and nutritionally unbalanced diets should be avoided (Teede et al., 2018). As stated, one could question the usefulness of these directives, as their formulation is so unspecific. Additionally, the guidelines endorse that no or limited evidence that any particular energy equivalent diet type is better than another, or that there is any differential response to weight management intervention, compared to women without PCOS. By contrast, an energy deficit of 30% or 500 to 750 kcal/day (totalizing 1200 to 1500 kcal/day) is clearly mentioned to achieve weight loss, attending individual energy requirements, body weight and physical activity levels (Teede et al., 2018).

The optimal dietary composition for both weight management and improvement of clinical features of PCOS is still controversial. Several reasons may justify this fact, such as lack of well-controlled studies in this area and heterogeneous results of the ones until the present moment published (Moran et al., 2013; Rodriguez Paris et al., 2020). A wide range of different diet types have been analysed, some focusing on the macronutrient content, as high-protein diets, low-carbohydrate diets, lipid profile modifying diets or even low GI diets. Other focus on overall healthy ingredients instead as found in a Mediterranean diet or the DASH diet. Yet again, no particular diet seemed to prove distinctly superior to the others beyond energy restriction per se.

DIET PATTERNS

DASH diet:

The **DASH diet** (dietary approaches to stop hypertension), is a diet low in carbohydrates, concretely a low-glycaemic index diet, as also high in protein and low-energy-dense, that was firstly developed to lower the blood pressure in patients with hypertension. However, its potential usefulness has also be reported in the induction of weight loss and reduction in IR in overweight and hyperinsulinaemic individuals (Z. Asemi & Esmaillzadeh, 2015; Azadi-Yazdi et al., 2017). Other than that, it is characterised by higher amounts of dietary fibre, phyto-oestrogens, potassium, magnesium, folic acid and other beneficial nutrients. As so, it is naturally rich in fruits and vegetables, whole grains and low in saturated fats, cholesterol, refined grains and sweets. The macronutrient composition of this eating plan contains, typically, 50-55% carbohydrate, 15-20% protein and 25-30% total fat (Z. Asemi & Esmaillzadeh, 2015; Azadi-Yazdi et al., 2017).

RCTs using the DASH diet allowed studying its effects on multiple relevant parameters in overweight and obese PCOS women, through a calorie restricted designed eating plan which calorie deficit varied from 300 to 700 kcal adjusted to the computed energy requirement for each participant.

In regards to anthropometric indices, beneficial effects on weight, BMI and abdominal fat mass were encountered. Besides that, a significant decrease in waist and hip circumference was also seen when compared with the control group. (Z. Asemi & Esmaillzadeh, 2015; Azadi-Yazdi et al., 2017; Kazemi, Jarrett, et al., 2020).

Asemi & Esmaillzadeh RCT studied the impact on insulin resistance and serum hs-CRP. The DASH diet was able to demonstrate reduction in serum insulin levels, HOMA-IR score and serum hs-CRP levels. Relevantly, the effect on insulin resistance was shown to be independent of its effect on body weight loss. It is hypothesised that high arginine, magnesium and calcium content of the diet might explain its favourable effects on serum insulin levels and resistance. Low content in simple sugars as well as anti-inflammatory effects of dietary calcium, magnesium and high fibre content may explain lower levels of hs-CRP (Z. Asemi & Esmaillzadeh, 2015).

In what respects to the lipid profile, DASH diet led to decreased serum triglycerides and VLDL-C levels. However, no significant differences were encountered respecting serum TC, HDL- and LDL-C. These results may be explained, once more, by the high arginine content of DASH diet as it has been attributed to shifting nutrient portioning to promote muscle over fat gain in turn providing a useful tool improving the metabolic profile. Furthermore, also the high dietary calcium and magnesium levels affect positively the triglyceride levels (Zatollah Asemi et al., 2014).

In regards to the biomarkers of oxidative stress increased concentrations of plasma total antioxidant capacity (TAC) and total glutathione (GSH) were found in the DASH group when compared to the control group. Importantly, the effects on the antioxidant capacity seemed to be independent of the weight loss. Along with that, these results might be attributed to high content of antioxidant-rich fruit and vegetables, rich in vitamin C. Likewise, calcium, magnesium and arginine levels might play a role in decreasing oxidative stress (Zatollah Asemi et al., 2014; Azadi-Yazdi et al., 2017).

Finally, Azadi-Yazdi et al. also mentioned beneficial impact of this dietary pattern on reproductive hormones, respectively androstenedione and SHGH (Azadi-Yazdi et al., 2017).

Mediterranean diet:

One of the most researched diet patterns is the **Mediterranean diet**. It is recognised as a health-promoting dietary pattern due to its peculiar features. Those include the regular consumption of unsaturated fats, low GI carbohydrates, fibre, vitamins and antioxidants, and moderate amount of animal-derived proteins. In addition to weight loss, a well stablished anti-inflammatory activity has been identified, which may be mainly due to the microbiota-derived production of short chain fatty acids (SCFAs). SCFAs production may, therefore, be induced by dietary fibre and the high intake of PUFA omega 3 and antioxidants contained in fruits, vegetables, extra-virgin olive oil, and wine. The Mediterranean diet is inversely associated with adiposity, IR, and risk of type 2 diabetes mellitus and cardiovascular disease. In particular, extra-virgin olive oil has phenolic compounds, such as oleocanthal, recognised as a potent anti-inflammatory agent (Barrea et al., 2019). Additionally, polyphenol stilbene derivative resveratrol, a dietary biomolecule widely found in Mediterranean nutritional approach, particularly in foods like grape seed, red wine and some berries, is thought to improve the hyperandrogenic traits of PCOS indirectly by inducing the reduction of IR (Mirabelli et al., 2020).

On top of everything previously discussed, the relationship between diet quality and ovarian morphology is deserving of mention. The adherence **either to the Mediterranean diet as well as to the DASH diet** have been suggested to be indirectly associated with improved ovarian morphology, as evidenced by lower follicle populations and ovarian size. Obesity, IR and hyperandrogenism were identified as probable key factors to explain the indirect relationship between lower diet quality and ovarian dysmorphology, (Kazemi, Jarrett, et al., 2020). These factors may be improved by the diets in analysis, as has been previously discoursed. In a complementary manner, and as per example, both DASH and Mediterranean indices emphasize the increasing consumption of protein from fish, seemingly beneficial in improving ovarian steroidogenesis and modulating serum FSH concentrations as well as from dairy products, which are associated with a decrease on antral follicle counts (Kazemi, Jarrett, et al., 2020).

Pulse-based diet:

On an interesting note, and not mutually exclusive with the already presented diet patterns it is worth of mention a more specific impact of a **pulse-based diet** on cardio-metabolic risk profile in PCOS women. (Kazemi et al., 2018; Rodriguez Paris et al., 2020).

Pulses include dried edible seeds of legume family including split peas, dry beans, lentils and chickpeas, are high in fibre, contain complex carbohydrates with a low GI, are low in fat, contain high-quality protein and are a source of vitamins and mineral such as iron, zinc, folate, calcium, magnesium, and potassium (Kazemi et al., 2018).

When compared with an healthy control diet, namely the Therapeutic Lifestyle Changes diet (TLC), and without differences in the total energy intakes during the 16 weeks interventions, a low-GI-pulse-based diet is likely more effective at decreasing levels of LDL-C, serum triglyceride (TG), TC/HDL-C ratio and increasing the concentration of HDL-C. Furthermore, a decrease in total insulin area under the two-hour response versus time curve (AUC) and in diastolic blood pressure was noted. This results obtained in a multi-dimensional lifestyle change program. Both diets improved glucose regulation, BMI, waist circumference (WC), trunk fat mass, percentage body fat, systolic blood pressure, and TC. During a 12-month follow up period, however, both groups tended to regain weight and revert to baseline values for fasting insulin levels.

Hypocholesterolemic effects of pulses are multifactorial and can be attributed primarily to their high fibre content. Pulses have both insoluble and soluble fibre, the latest being capable of binding bile acids in the intestine and increasing the excretion of cholesterol. Moreover, more fermentable dietary fibre can lead to improvement of the gut microbiota composition, regulation of hepatic gluconeogenesis, lipogenesis, and lipid storage through mechanisms mediated by short-chain fatty acids, as signalling metabolites with prebiotic effects. It is also relevant to mention that the reduction of diastolic blood pressure found in this study is estimated to reduce the risk of myocardial infarction by 9 to 13%. The increased insulin sensitivity encountered, on its turn, was also attributed to the consumption of low GI foods and to the modest weight loss encountered (Kazemi et al., 2018).

As other beneficial effects of pulse based diet, decreased risk of endometrial carcinoma was mentioned due to adequate micronutrient intakes, in particular of folate. (Kazemi et al., 2018).

Ketogenic Diet:

Even though the evidence available is scarce and still very recent, **a Ketogenic diet (KD)** is another diet pattern worth recognising. It consists of a nutritional protocol in which carbohydrates are lower than 30g per day or 5% or less of total energy intake and, thus, normally, described as high-fat, adequate-protein and low-carbohydrate diet. In response to limitation of carbohydrates, a nutritional ketosis (blood ketones >0.5 mmol/L) is maintained over time, but different to fasting ketosis as it ensures adequate protein and preservation of tissues, especially the muscle (Paoli et al., 2020).

More importantly, the reduction of the amount of circulating glucose and insulin produces a reduction of the oxidation of glucose and an increase of the fat oxidation. KD also activates the AMPK and SIRT-1, even in the absence of caloric deprivation, which produces beneficial effects on glucose homeostasis and improve insulin sensitivity. This diet has been shown to be useful in other pathological conditions, such as epilepsy, neurological diseases, cancer, DM2, and amelioration of respiratory and cardiovascular disease risk factors (Paoli et al., 2020).

In a study with fourteen overweight women with PCOS, a Mediterranean eucaloric ketogenic protocol with phytoextracts was tested. In terms of anthropometric and body composition parameters, weight loss was achieved as well as significant decrease in BMI, FBM, VAT and WC. In regards to metabolic biomarkers, glucose, insulin and, consequently, HOMA-IR reduced. The lipid profile ameliorated as reduction in TGs, TC, LDL and increase in HDL was registered. Concerning endocrine biomarkers, reversal of LH/FSH ratio was verified, and a decrease in LH levels, total and free testosterone and DHEAS was noted. FSH, estradiol, progesterone and SHBG levels increased. Hirsutism scores did not reduce, however, 12 weeks of the intervention were assumed insufficient to observe differences (Paoli et al., 2020).

For all the above mentioned, KD may be considered as safe, at least, for short cycles and, then, followed or alternated with a balanced dietary regimen. Finally, it could be regarded to increase insulin sensitivity, contributing to normalize the clinical features of PCOS (Paoli et al., 2020).

DIETARY MACRONUTRIENTS BALANCE

Dietary macronutrients include carbohydrates, proteins and fat. The balance amongst these components has been recurrently proposed as having an impact in PCOS features' and promising regarding its applicability as a therapeutic tool as will be presently discoursed.

The macronutrient balance: what do animal models say?

For defining the impact of dietary macronutrient balance on PCOS traits, Rodriguez Paris et al. study combined a PCOS mouse model with the Geometric Framework, which is a nutritional approach. As relevant findings, it demonstrated that control mice are able to maintain ovulations on a wide range of dietary intakes, while PCOS mice are only able to ovulate under a narrower nutritional niche. Diet alteration, by itself, was capable of restoring the ovulatory function, as indicated by the presence of corpora lutea in their ovaries, pointing to occurrence of recent ovulations and by the presence of complete oestrous cycles.

Furthermore, and regardless of dietary intervention, PCOS mice displayed a significant increase in body weight with the same raise pattern independently of the macronutrient responsible for the higher caloric intake. Despite that fact, PCOS mice were more sensitive to macronutrient intake, with PCOS mice gaining more weight at lower caloric intakes than control mice and, in particular, PCOS mice gaining more weight at low carbohydrate and fat intakes. The study affirmed not to exist differences between the groups, a week prior to the dietary choice study, while consuming a standard chow diet, in what came to eating pattern, caloric intake and activity and therefore implying post-ingestive responses to diet intake and concluding that the androgen excess was the underlying pathogenic cause of the weight gain variances. What is more, changes in macronutrient balance had minimal beneficial effect on ameliorating metabolic dysfunction observed in PCOS mice.

Moreover, the study observed serum cholesterol levels to be affected by fat intake in PCOS mice, in a direct manner, observation not revealed in control mice and thus, implying that the androgen excess pathogenesis of PCOS caused the PCOS mice to have a reduced ability to regulate their cholesterol levels in response to changes in macronutrient intake. Adiponectin, typically decreased with PCOS, did not change in relation with the relative macronutrient composition of the diet. Notwithstanding weight loss is capable of increasing its circulating levels, which indicates, once more, hyperandrogenism is at the root of adipose tissue dysfunction (Rodriguez Paris et al., 2020).

Also worth of mention, the combination of letrozole and a **high fat diet** is capable of inducing PCOS-IR rat model which has characteristics of ovarian polycystic changes and endocrine and metabolic disorders (R. Patel & Shah, 2018; M. X. Wang et al., 2020). Of notice, for an intervention period of 27 days, weight gain, augmented volume and number of follicles in ovaries was registered. Increased serum level of fasting plasma glucose, fasting insulin, HOMA-IR and TG were also noted. Finally, abnormal sex hormone secretion was encountered, with higher LH, LH/FSH and testosterone levels measured (M. X. Wang et al., 2020).

Additionally, hyperandrogenism significantly reduced the capability of PCOS mice to modulate glucose levels in response to diet, this parameter being significantly increased in PCOS mice (Rodriguez Paris et al., 2020).

Overall, in a PCOS mouse model, a macronutrient balance of **low protein and medium carbohydrate and fat dietary consumption** can ameliorate ovulatory dysfunction observed, proving that the macronutrient balance does affect the development of reproductive PCOS traits. Interestingly, the macronutrient ratio identified, 14% protein, 47% carbohydrates and 39% fat, is reasonable similar to the one classically recognised in a Mediterranean diet, which has been associated with numerous health benefits as formerly discussed. As other key finding, hyperandrogenism decreases the capacity of the metabolic system to cope with a poorer diet (Rodriguez Paris et al., 2020).

The macronutrient balance: what do human models say?

Carbohydrate macronutrient is probably the most extensively studied in what concerns hypothesized benefits of its reduction as a diet intervention. Lower carbohydrate intake in PCOS should, in theory, lead to lower levels of insulin and subsequently improve alterations in androgens, increase weight loss and improve satiety (Frary et al., systematic review, 2016). As per definition, low carbohydrate diet (LCD) implies a reduction of the intake of carbohydrates to less than 45% of the total daily calorie intake (X. Zhang et al., 2019).

The results forward presented are based on studies in which LCD intervention groups were subjected to an eating plan in which 39-45% or less than 20g/ day came from carbohydrates, 21-35% from protein and 20-40% from fat. The included studies had varying durations, up to 32 weeks (Frary et al.) (X. Zhang et al., 2019). Interventions with a **low fat** simultaneously with **low carbohydrate** content and for an period longer than 4 weeks seemed to show the more significant results (X. Zhang et al., 2019).

As relevant findings, **LCD** caused a reduction in BMI, and, in particular, 1-5% additional weight loss compared to caloric restriction alone in women with PCOS. Increased satiety and weight loss may be due to higher intake of protein (Frary et al.) (X. Zhang et al., 2019).

In terms of the lipid profile, lower serum levels of TC and LDL-c in PCOS patients was encountered (X. Zhang et al., 2019)

As concerns to the glucose metabolism, decreased circulating glucose, IR and consequent hyperinsulinemia improvements were seen in a short period as of 2 to 4 weeks, despite weight loss being considered as possible confounders (Frary et al.). Sørensen et al., on the other hand, reported that **higher protein and lower carbohydrate diet** improves glucose metabolism by an effect that seems to be independent of the weight loss (Sørensen et al., 2012).

The impact on the glucose metabolism factors are, nonetheless, critical and might counterbalance the hyperandrogenism, metabolic alterations, anovulation and irregular menstrual cycles of the PCOS patients, both the lean and the obese. The **low carbohydrate and low fat diet** might restore, even if only partially, the insulin sensitivity.

It is hypothesized that may be related with inositol metabolism regulation, a component of the vitamin B family, as there is an impairment in the conversion of myoinositol into D-chiro-inositol, interfering with insulin sensitivity (X. Zhang et al., 2019). What is more, the lower the percentage of carbohydrates in a diet, the more effective seems to be the lowering effect in IR (Porchia et al., 2020).

In respect of reproductive parameters, menstrual regularity tended to improve more during a LCD rather than a fat restricted diet but the results were not statistically significant. Moreover, significant increase of FSH and SHBG levels and decrease of testosterone levels were encountered, especially in long-term interventions and not only in **low carbohydrate diet** but also with simultaneous **low fat consumption**. These findings might be explained by the decrease in the circulating insulin and glucose levels. These reductions may improve ovarian function as well as reduce hyperandrogenism symptoms. (Frary et al) (X. Zhang et al., 2019). However, energy restriction and consequent weight loss seem to be the primary inducers of these results. (Frary et al.).

Despite of the previously stated results, the sustainability of weight loss diets in the long term have been debated, as some of LCD interventions reported high dropout rates. This indicates lack of compliance and points the need for a healthy diet composition so long term results can be attained (Frary et al.). In that regard, **low GI diets** have gained recognition in replacement or complementary to a LCD. By definition, The GI represents the effects of the carbohydrate portion (50gr) of a particular food on postprandial glucose concentrations compared with white bread or glucose as a reference containing the same amount of carbohydrate.

LGI diets, when compared to high GI diets, showed to improve glucoregulatory status as HOMA-IR and fasting insulin decreased, as well as whole-body insulin sensitivity (Barrea et al., 2018; Kazemi, Hadi, et al., 2020). No effects on fasting glucose were obtained, despite the lack of difference might have been obtained if longer interventions were studied. Ameliorated lipid profile was evidenced by decreased TC, LDL and TGs. Reduction in abdominal adiposity was also observed, proved by the decrease in waist circumference (Kazemi, Hadi, et al., 2020). In addition, improvement in levels of fibrinogen, an acute-phase protein of inflammation have been described (Barrea et al., 2018). Moreover, menstrual disorders and improvement in hyperandrogenism, as assessed by decreased total testosterone, were reported (Barrea et al., 2018; Kazemi, Hadi, et al., 2020).

The biological mechanisms that might explain the benefits of LGI diets on cardiometabolic and reproductive complications associated with PCOS are not fully elucidated. However, it is thought to be related to the complex carbohydrate profile and high dietary fibre content, particularly soluble fibre as well as with lower saturated fat and increased mono- and polyunsaturated fat content, more plant protein over animal protein; lower energy intake independent of calorie restriction and a favourable micronutrient composition (Kazemi, Hadi, et al., 2020).

In a continuum with the previously exposed but shifting the centre of attention to **protein rich diets**, the existent evidence is scarcer. However, one could argument that when lowering the carbohydrate load in a diet, tendentiously, the protein load will increase in compensation and, consequently, an overlap in the beneficial effects would be expected.

As example of the just mentioned, a RCT compared a conventional hypocaloric diet (15% of daily energy from protein, 55% from carbohydrates) with a modified hypocaloric diet with high-protein, low-glycaemic load (30% of daily energy from protein, 40% from low and medium-GL carbohydrates) during twelve weeks. The weight loss was significant and similar within the two groups and, additionally, androgen levels were reduced. The modified diet caused a more significant increase in insulin sensitivity and, equally, a decrease in hsCRP level when in comparison with conventional diet. However, it is not possible to differentiate the beneficial effects encountered, i.e, whether these were mainly caused by the higher protein content or to the lower GL in the diet (Mehrabani et al., 2012).

Interestingly, a RCT compared a high protein diet (>40% from protein, <30% from carbohydrates, 30% from fat) to a standard protein diet (<15% protein, >55% from carbohydrates, 30% from fat) for a period of 6 months. The intake of the ad libitum high protein diet resulted in higher weight loss and decrease in glucose and a trend to lower C-peptide concentrations than did the intake the ad libitum standard protein diet. The effect on glucose and C-peptide persisted after adjustment for changes in body weight.

It also suggested that energy restricted diets are not as essential in higher protein diets as this component induces satiety and, therefore, naturally causing a spontaneous reduction in energy intake. No effects were observed, however, on total and free testosterone concentrations (Sørensen et al., 2012).

Furthermore, diets higher in protein are more effective at lowering IR in PCOS women and the effect is as proeminent as the severity of the IR present Moreover, Mizgier et al. study revealed that protein intake is inversely related to inflammation and oxidative stress, the correlation noted, in particular in overweight or obese girls (Mizgier et al., 2021).

In relation to **lipids**, some beneficial effects of a low fat diet were formerly characterised when in addition to a low carbohydrate intervention. However, a limited number of studies explore the relationship between fat intake and endocrine abnormalities in PCOS (X. Zheng et al., 2021). Moreover, and as described initially, PCOS patients' basal diet is naturally rich in fat intake and for that reason the recommendation of other high fat diets might not resonate as an optimal intervention (Nybacka et al., 2017). Reinforcing this idea, Nybacka et al. RCT demonstrated that low saturated and trans fatty acids, as well as consequent higher fibre consumption, correlated with decreased BMI and an improved insulinogenic index. A positive effect on ovulatory function may also be achieved (Nybacka et al., 2017).

As a final remark, while regarding the multiple possible dietary interventions, a change in the lifestyle must aim at achieving specific metabolic goals, such as IR improvement, adipokines secretion and reproductive function. As a transversal finding, weight loss seems to have a positive effect on PCOS outcomes regardless of dietary composition. Short-term advantages of energy restriction, and especially with low GI diets, include increased insulin sensitivity, improving glucose regulation, and inducing weight loss in overweight and obese women. However, long-term sustainability and applicability across the different PCOS phenotypes is limited and scarce evidence exists in what comes to optimal dietary composition without energy restriction on cardio-metabolic alterations associated with PCOS (Kazemi et al., 2018). Lack of compliance and high dropout rates are some of the obstacles encountered when highly restrictive diets are implemented. Nonetheless, a balanced and nutrient-dense diet, rather than a single macronutrient-focused approach, as recognised and discussed earlier with the DASH and the Mediterranean diet is, potentially, the best approach to the adherence and maintenance in the long term of healthy habits in women with PCOS.

AND MICRONUTRIENTS - WHAT DO WE KNOW

Nutrients as vitamins and minerals may play a role in the pathophysiology of PCOS and, hence, may be useful as a therapeutic tool. PCOS has been associated with multiple deficiencies that, in turn, may lead to PCOS associated complications. In this regard, providing sufficient nutrients and energy is pivotal for proper cell growth and reproduction as well as potentially having an impact in the comorbidities implied in the disease. In the next few pages, some of those elements that may be scant will be analysed.

Vitamin D:

Vitamin D is a steroid hormone that regulates calcium, magnesium and phosphate homeostasis and plays a role ant proliferative and immunomodulatory mediator (Menichini & Facchinetti, 2020). It is mainly produced in the skin after sunlight exposure. In this case, it is formed vitamin D3 or cholecalciferol in the presence of ultraviolet B radiation. On the other hand, diet and dietary supplements constitute alternative sources of vitamin D for humans. Vitamin D2 or ergocalciferol is the form found derived from nutritional sources such as green plants, mushrooms, fish fat and cod liver oil. Independently of the source, vitamin D requires two hydroxylations in the liver and in the kidneys to become biologically active, in the form of 1,25(OH)2D3 or calcitriol (Voulgaris et al., 2017).

Vitamin D deficiency (VDD) might be a factor in the development of PCOS. This fact is unsurprising as it is estimated that 65 to 85% of women with PCOS have VDD (Łagowska et al., 2018). Moreover, VDD, on its turn has been associated with some of the comorbidities found in PCOS such as diabetes, insulin resistance, metabolic syndrome and cardiovascular disease (Figure 4).

In more detail, vitamin D supplementation, in the PCOS setting, may regulate glucose metabolism as a significant reduction of fasting plasma glucose as well as improvements in IR and increased serum fasting insulin were identified, therefore, enhancing insulin synthesis and release and increase insulin receptor expression (Łagowska et al., 2018; Menichini & Facchinetti, 2020; Mu et al., 2020). These results were identified with cholecalciferol supplementation in the intervention group with a dosage ranging from 200 IU/d to 60000 IU/weekly, for a supplementation period from 8 to up to 60 weeks and, in some cases, co-supplementation with calcium, vitamin K, zinc and magnesium was performed (Łagowska et al., 2018; Menichini & Facchinetti, 2020). Nonetheless, vitamin D alone taken every day, at lower doses (<4000 IU/ d) also lowered HOMA-IR index in the PCOS women (Łagowska et al., 2018).

In what comes to the lipid profile, four studies included in the Menichini & Facchinetti review, reported reduction of TG and one other demonstrated VLDL, LDL and total/HDL-cholesterol ration decreases. Further results presented in a 2020 narrative review also demonstrated a protective effect of vitamin D on lipid metabolism (Mu et al., 2020). (Menichini & Facchinetti, 2020). In terms of obesity, low vitamin D might contribute to accumulation of intra and inter-muscular adipose tissue. Higher BMI and total body fat mass were found to be strong predictors of low 25(OH)D (Mu et al., 2020). Beneficial effects on chronic inflammation and oxidative stress were also found, demonstrating a potential role of supplementation in supressing proinflammatory cytokines (Menichini & Facchinetti, 2020)

On a hormone profile level, Jamilian et al. demonstrated that high-dose vitamin D (4000 IU/ weekly) supplementation had beneficial effects on total testosterone, SHBG, free androgen index when compared to low-dose (1000 IU/ weekly) and placebo groups. A negative correlation between 25(OH)D levels ant total testosterone and androstenedione has also been reported (Menichini & Facchinetti, 2020; Mu et al., 2020). Moreover, vitamin D is, physiologically, an important factor in oestrogen biosynthesis and 1,25(OH)2D3 could induce the production of progesterone, estradiol and oestrone in vitro (Mu et al., 2020). In addition to this, vitamin D can possibly regulate the AMH expression, decreasing serum levels of this hormone in PCOS patients, and implying that a positive impact on the folliculogenesis, despite the number of studies reporting this finding being limited. The effects in question appear to be dependent on a woman's ovulatory status. Vitamin D supplementation appear to decrease AMH levels in anovulatory women with PCOS, while the same supplementation appear to increase AMH levels in ovulatory non-PCOS women (Moridi et al., 2020; Mu et al., 2020). Furthermore, a positive effect on menstrual cycle regulation and on endometrial alteration of PCOS patients is recurrently mentioned (Menichini & Facchinetti, 2020; Moridi et al., 2020; Mu et al., 2020)

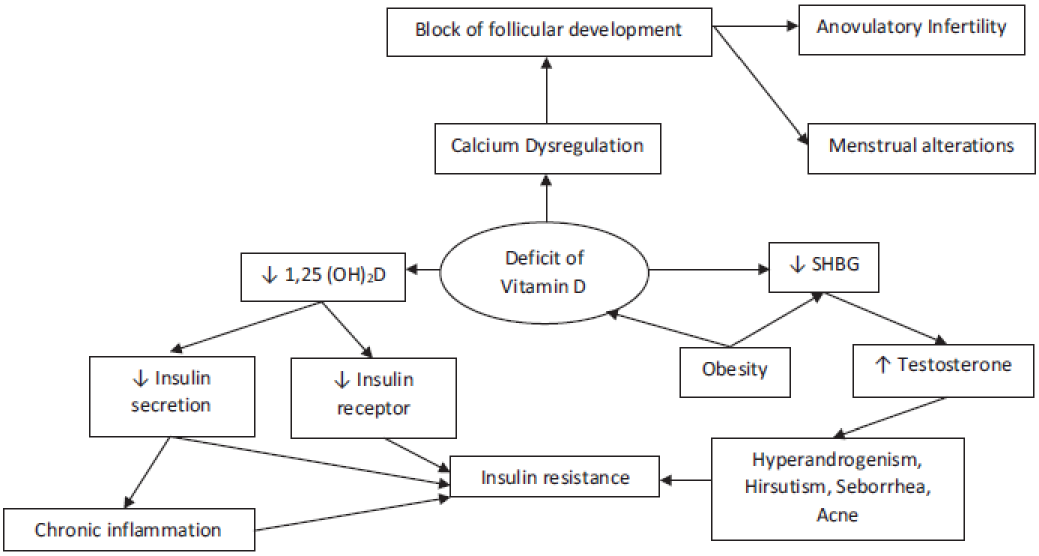

Figure 4: Possible role of vitamin D in the pathogenesis of PCOS, retrieved from Menichini & Facchinetti, 2020

In opposition to the findings previously presented, not all of the studies included in the previously mentioned reviews and meta-analysis were contentious, as not all of them showed integrally the potential benefits referred. As per example, not all studies showed reduction in LDL, HDL and TC, in what matters to lipid profile, nor all corroborated the beneficial decrease in testosterone levels (Menichini & Facchinetti, 2020; Mu et al., 2020). Additionally, 2019 RCT only found a decrease in plasma glucose after 60 min on an OGTT, result of unclear clinical relevance, especially since no significant effect was encountered in what comes to their primary outcome (plasma glucose under the curve) (Trummer et al., 2019). Finally, a 2017 meta-analysis stated that the potential benefit of vitamin D supplementation on hormonal and metabolic status could only be notorious in patients with initial lower serum levels of the vitamin (<12ng/ ml) and minimal in those with only mild deficiency, ergo not support the use of vitamin D supplementation (Pergialiotis et al., 2017).

Justifying the differences encountered, one should consider that doses and timing have significant impact on the outcomes and justify some level of heterogeneity. On this account, population characteristics such as BMI and ethnicity as well as basal levels of vitamin D and hormones are all variables that should be considered when supplementing vitamin D (Menichini & Facchinetti, 2020).

As easily perceived, further investigation is warranted ideally with larger sample sizes and longer intervention timings and follow up, so more conclusions that are significant can be uncovered. Despite the limitations and heterogeneous results presented, vitamin D supplementation may be a therapeutic approach with potential, as it positively influences metabolic and reproductive dysfunction, and at the same time constituting an accessible and inexpensive element.

Inositol:

Myo-inositol (MI) and D-chiro-inositol (DCI) are two stereoisomeric forms of inositol, belonging to vitamin B complex, which balance metabolic deregulations concurrent with IR. On the ovary, MI regulates glucose uptake and FSH signalling and DCI regulates the insulin-induced androgen synthesis. MI has been recognised as insulin-sensitizing supplement which could benefit women with PCOS (Unfer et al., 2017).

A recent meta-analysis on myo-inositol used as a supplementation alone or combined with DCI in PCOS highlighted its beneficial effects, i.e., in improving the metabolic profile of these patients, with fasting serum insulin and HOMA-IR being significantly decreased (Unfer et al., 2017; Zeng & Yang, 2018). In addition, serum SHBG was shown to be increased after a supplementation period of, at least, 24 weeks, which leads to lower availability of testosterone, ameliorating symptoms related to hyperandrogenism (Unfer et al., 2017). Other effects on hypothalamic-pituitary-ovarian axis include increased oestrogen and decreased LH levels (Zeng & Yang, 2018). As so, an improvement in ovulation rates in PCOS women maybe stipulated. The studies included used a daily dose of MI supplementation ranging from 0.55 to 4g, either given by itself or alongside folic acid or with DCI and duration of interventions ranging from 12 weeks to 6 months (Unfer et al., 2017; Zeng & Yang, 2018).

Additionally, MI can be converted into DCI by a specific NAD/NADH-dependent epimerase, which is unidirectional and stimulated by insulin (Roseff & Montenegro, 2020). In normal women, the plasma ratio of MI to DCI is 40:1, while in ovarian follicular fluid is close to 100:1. The conversion rate may be impaired in PCOS due to IR and might result in an overproduction of DCI and, simultaneously, a deficiency of MI in the ovary. As described in the “DCI paradox” hypothesis, DCI supplementation alone is not recommendable, as high doses of this element have been considered toxic to ovaries and oocyte maturation. In addition, DCI is not converted into MI and MI deficiencies are correlated with many IR conditions, which would, then, imply not benefiting from its effects if not supplemented (Unfer et al., 2017). Conversely, potential benefits of DCI at low dosage for refining the insulin sensitivity and ovulation frequency have been stated (Zeng & Yang, 2018).

Remarkably, a 2020 review article, considering not only these meta-analyses but also the latest studies in this area, alerts for the fact that inositol treatment in PCOS women should be science-based and no arbitrary. DCI was found to be an aromatase inhibitor, which increases androgens and may have harmful consequences for women and, thus, the 40:1 ratio between MI and DCI is the optimal combination to restore ovulation in PCOS women. Some treatment formulations are, however, randomly chosen, with counterproductive or useless elements, as seen when DCI and MI are simultaneously administered competing with each other to be absorbed or when MI absorption is jeopardized by the co-administration of certain sugars, both situations weakening the potential therapeutic efficacy (Roseff & Montenegro, 2020).

Zinc:

Zinc is an essential microelement present in all body tissues and fluids, mainly intracellularly. Approximately 2 to 3 g is present in the human body and 0.1% of that needs to be supplied daily, depending also on gender, age, physiological condition and diet. The richest sources of well absorbable zinc are those of animal-origin, including organs and flesh of mammals, fish, eggs and dairy products. Plant-based foods as cereals, grains, nuts and legumes also contain this element but in a smaller quantity and in a less absorbable manner. Protein intake increases its absorption, while phytate, found in some cereals and beans, inhibits its absorption. Zinc has a relevant role maintaining homeostasis, essential for proper functioning of cells, including their growth, differentiation and division, also in the endocrine and immune system and synthesis of proteins, RNA and DNA. It also has critical in maintaining the redox balance, having an antioxidant action and protective effects against reactive oxygen species. Zinc is also pivotal for the functioning of the reproductive system as cells involved in this system replicate extensively. Research indicates that zinc deficiency could induce impaired synthesis and secretion of FSH and LH, abnormal ovarian and oocytes development, disruption of menstrual cycle, abortion, and pregnancy and delivery complications (Nasiadek et al., 2020).

Women with PCOS may have reduced serum zinc levels compared to healthy controls (Abedini et al., 2019). Zinc, as a multidirectional effect may contribute to the development of many of the abnormalities found in PCOS. It is involved in insulin synthesis, release, action and storage (Nasiadek et al., 2020). Indeed, higher zinc intakes are associated with lower risk of diabetes mellitus type 2 in women (Abedini et al., 2019). As so, zinc supplementation seems to be beneficial for PCOS patients in decreasing insulin concentration and IR (Abedini et al., 2019; Nasiadek et al., 2020). Furthermore, it may play a role ameliorating the lipid profile, as the supplementation induces decrease in triglyceride, TC, LDL and VLDL concentrations. The effects of supplementation on hormonal balance, especially in testosterone, is still unclear, despite some beneficial mechanisms of its supplementation on this balance are beginning to be identified. PCOS is also characterized by a redox imbalance, which can be ameliorated by the zinc supplementation, measured through the increase in total antioxidant capacity and decrease in MDA concentration (Abedini et al., 2019; Nasiadek et al., 2020). The beneficial effects previously exposed were encountered in studies using a 220 mg zinc sulfate (50 mg zinc) supplementation, with the exception of one study, that only used a 4 mg zinc dosage. It is also relevant to mention that not all of the studies used zinc supplementation in isolation, which hinders the interpretation of the results found (Nasiadek et al., 2020).

Conversely, results from Yin et al. systemic review and meta-analysis did not stablish an association between zinc serum concentration on PCOS women and respective control groups. Indeed, only subgroup analysis in PCOS women with IR against healthy women showed the serum concentration of the element to be lower (Yin et al., 2020).

Magnesium:

Magnesium is an important intracellular cation in the human body, with a crucial role as a cofactor of many enzymatic processes, energy metabolic pathways and nucleic acid synthesis as well as in glucose and testosterone metabolism and in insulin secretion and function (Babapour et al., 2020; Hamilton et al., 2019). The normal blood serum concentration is between 0.76 and 1.15 mmol/L (1.85-3.65 mg/dL) and the recommended daily intake for women older than nineteen years old is 320 mg (Hamilton et al., 2019). This mineral is found abundantly in plant and animal sources, most foods containing dietary fibre also providing magnesium, such as leafy green vegetables, whole grains, legumes, nuts and seeds. Water is also a source of the mineral, its content depending, however, on its origin (Hamilton et al., 2019).

Magnesium increases insulin sensitivity and its deficiency is associated with IR and diabetes mellitus, therefore it may be implied in PCOS pathophysiology. Conversely, poor glycaemic control, seen in diabetes mellitus type 2 and in PCOS, is a risk factor for magnesium deficiency and some studies have reported that PCOS patients tend to consume less magnesium-rich foods.

Babapour et al. systemic review and meta-analysis showed that serum magnesium may be lower in women with PCOS particularly in overweight or obese objects when compared with lean women, even though the heterogeneity of included studies was high (Babapour et al., 2020). In contrast with these findings, Yin et al. systematic review and meta-analysis did not encounter statistically significant differences in magnesium levels between PCOS women and healthy controls, not even when only overweight or obese PCOS subgroup was taken into consideration (Yin et al., 2020).

These findings alert to the importance of screening for magnesium deficiency in women with PCOS also overweight or obese and for the need of more research on the role of this element in the disease as well as for the standardization of the type, dose and time of magnesium supplementation (Babapour et al., 2020). A upper intake level of 350 mg/d from supplemented magnesium for women with more than fourteen years old is suggested in order to reduce the risk of adverse reactions from excessive intake (Hamilton et al., 2019).

Magnesium supplementation may alleviate systemic inflammation, insulin sensitivity and diabetes symptoms in non PCOS population but that possess similar pathophysiological aberrations with PCOS women and present decline in serum magnesium concentrations (Babapour et al., 2020). However the effects of magnesium supplementation in PCOS women remain unclear, specifically in what respects to glycaemic improvements, only one study finding significant effect ameliorating IR, and only prominent when co-supplementation with vitamin E was considered (Hamilton et al., 2019). Magnesium may not be the only or best solution for improving glucose metabolism and insulin sensitivity, as more research is needed, and an individualized plan for the supplementation of micronutrients may be warranted to meet PCOS women micronutrient needs (Hamilton et al., 2019).

Omega-3 Polyunsaturated Fatty Acids:

Omega-3 polyunsaturated fatty acids (PUFAs) include alpha-linolenic acid (ALA; 18:3 omega-3), stearidonic acid (SDA; 18:4 omega-3), eicosapentaenoic acid (EPA; 20:5 omega-3); docosapentaenoic acid (DPA; 22:5 omeg-3) and docosahexanoic acid (DHA; 22:6 omega-3). These or part of these originate from certain plant sources or are modified in plants as well as from marine, algal and single-cell sources. Long chain (LC) omega-3 FAs such as EPA and DHA are found in the adipose tissues of fatty fish such as mackerel and salmon, the liver of white line fish such as cod and halibut, and the blubber of marine mammals such as seals and whales, and are frequently sold as fish oils supplements. Despite the fact that marine organisms are the major source of omega-3 PUFAs, some plant seeds and nuts also contain them, as flax, chia, hemp and canola seeds as well as walnuts. These are the primary source of ALA, a precursor to the synthesis of LC PUFAs in the human body, even though this conversion is limited to rates of less than 4%. Besides, soy and other plants have been genetically modified to contain higher amounts of omega-3 PUFAs. Moreover, numerous algal species are good sources of DHA. Omega-3 PUFAs are synthesized by phytoplankton and eventually via the food web deposited into the lipids of fish and marine mammals (Shahidi & Ambigaipalan, 2018). According to NIH, ALA daily-required intake varies between 1.1 and 1.6 g/day depending on age and gender (National Institutes of Health, 2019).

-What do human models say?

Over the past few decades, multiple studies have been identifying the health benefits of omega-3 PUFAs. Likewise, the impact of these FAs has already been studied to the particular case of PCOS. The most recent systematic review and meta-analysis, published in 2018, revealed that omega-3 supplementation is superior to placebo in improving insulin resistance, as well as in increasing adiponectin, and decreasing TC, TG and LDL-C. However, no statistical significance in reducing BMI, hyperinsulinemia or fasting glucose was found. Furthermore, no differences were encountered in what comes to HDL-C levels, or either in reproductive hormones, namely FSH, LH, SHGH and total testosterone (Yang et al., 2018). Relevant of mention, the studies included used omega-3 FAs dosages ranging from **900mg to 4000mg per day**. Notably, three out of the nine trials analysed used a combined supplementation of omega-3 FAs and vitamin E, one combined the omega-3 FAs with metformin, and other combined the three mentioned possible substances. In addition, the duration of the monitored interventions ranged between 6 to 24 weeks.

In contradiction with the previously mentioned, Salek et al. review article favoured the anti-obesity effects of omega-3 FAs, respectively mentioning the reduction of BMI and waist circumference (WC) in multiple studies with referred supplementation dosages of omega-3 FAs ranging from 1,5g to 2g/day. In what regards glycaemic and hormonal haemostasis, not only reinforced effects on IR, but also defended possible beneficial effects in insulin sensitivity check index and serum insulin levels. Furthermore, effects on adipokines production as well as on lipid metabolism and reproductive parameters were mentioned as limited. Notwithstanding, the results presented were coherent with Yang et al., except for a possible reduction in total testosterone and increase in SHGB that was pointed. In addition, ameliorated chronic inflammation was noted as another potential benefit of omega-3 FAs supplementation (Salek et al., 2019).

-What do animal models say?

As results presented within clinical trials are limited, here are also presented some of the most recent research in what comes to animal model studies.

Wang et al. studied the beneficial effects of dietary alpha linolenic acid-rich flaxseed oil (FO) on PCOS induced rats. Each animal received 1ml/kg of FO daily for 8 weeks and the disease simulated through letrozole administration. They demonstrated improvement in the disrupted oestrous cycle and ovulatory function. In more detail, abnormal elevated plasma testosterone and LH/FSH ratio, as well as reduced levels of plasma FSH, oestrogen, SHBG in PCOS were rectified by the intervention. Furthermore, FO supplementation promoted increase in follicular cell layers.

On other hand, metabolic parameters were also analysed. In that context, TC, TG, and LDL decreased, and HDL increased. The IR improved as well. These metabolic parameters were, as so, (bar the HDL findings) coherent with the findings of Yang et al. meta-analysis.

Moreover, and to be further detailed in this article, **gut microbiota** and their metabolites may be a related to the incidence of PCOS and its features. The study confirmed gut dysbiosis with the decrease in Ruminococcaceae and increase in gram-negative bacteria. The gut dysbiosis ameliorated with FO supplementation, attenuating, therefore the disease, as observed by the Firmicutes/Bacteroiedetes elevated ratio being rectified with the intervention. Moreover, abnormal LPS was restored, decreasing its translocation from the intestines to the systemic circulation and contributing, in consequence, to the suppression of systemic and ovarian inflammation. Finally, SCFAs elevated after FO administration, enhancing the intestinal mucosal barrier as well as reducing gut inflammation (T. Wang et al., 2020).

What is more, Komal et al., also using a rat model, studied the influence of omega-3 PUFAs and, additionally took in consideration the different food sources, flaxseed oil (rich in ALA) and fish oil (rich in EPA and DHA), and intended to study their possible distinct influence. Each group received either synthetic (ALA+EPA+DHA), flaxseed oil or fish oil in a dosage of 300 mg/kg/orally/daily during 16 weeks. The study confirmed, once more, that supplementation with omega-3 PUFAs sources had a positive impact on hormonal profile, respectively reduced testosterone, LH, and insulin, as well as on the lipid profile, blood glucose levels, and body weight loss. No differences were found depending on the type of supplementation with the only exception of the body weight parameters, as the group supplemented with fish oil seemed to lose more weight. Additionally healthy follicles, corpus luteum, and visible granulosa cell layers were observed. FSH, progesterone, prolactin, and estrogen remained non-significant (Komal et al., 2020).

Succinctly, omega-3 fatty acids benefits may be explained by their capability of increasing insulin sensitivity by producing anti-inflammatory adipokines, as it is adiponectin, as well as reducing inflammation and proinflammatory cytokines, as reinforced by the results of the studies presented (Salek et al., 2019; Yang et al., 2018). These fatty acids can also reduce cholesterol absorption and LDL-C synthesis, improve LDL receptor activity in the liver and increase the catabolism of LDL-C. Hence, these outcomes expose a promising positive impact on cardio metabolic risk factors present in PCOS patients (Yang et al., 2018).

In light of the formerly discoursed, omega-3 fatty acids supplementation may be a plausible recommendation for the treatment of PCOS with insulin resistance or/and high

TC, especially high LDL-C and TG. However, caution should be accounted due to possible selection bias and small samples used in the different trials analysed. Nonetheless, omega-3 fatty acid supplementation seems to offer a safe and affordable treatment option.

Other elements: Cinnamon, a curious herb

Cinnamon is a medicinal herb adopted by different cultures and multiple health benefits are attributed to it, such as blood glucose and serum lipid-reduction and it might also beneficial for regulating menstrual cycle and improving gynaecological, respiratory and digestive disorders. Furthermore, it is thought to decrease IR, through the promotion of insulin action, stimulating insulin-signalling pathways.

Heshmati et al. systematic review and meta-analysis is the most recent study assessing the effects of this herb on glycaemic control in PCOS women. It included five trials with a duration between 8 and 52 weeks, cinnamon used as the only supplement and dosages varying between 500 to 1500 mg/day. The results found a significant reduction on HOMA-IR and fasting serum insulin (FSI), although the results must be interpreted with caution due to limited number of studies existent. Nevertheless, multiple mechanisms are thought to be involved, among these, the fact that cinnamon contains several potential bioactive components such as trimeric, tetrameric and type A polyphenols with proven effects on insulin sensitivity. In addition, cinnamon supplementation elevates glucose uptake by cells, through the improvement of insulin sensitivity via enhanced phosphorylation of signalling proteins and/or activation of PPARs. Another mechanism thought to be involved is related to digestion and absorption processes, with reduction of the serum glucose to oral glucose challenge, reduced insulin response to glucose, and increased insulin sensitivity as the supplementation may significant delay gastric emptying (Heshmati et al., 2020).

Complementing the previous findings**,** Heydarpour et al. systematic review and meta-analysis, focusing on the effects of oral cinnamon supplementation on metabolic parameters, found no significant difference on body weight and BMI either of normal, overweight or obese PCOS women. In respects to glucose metabolism, not only HOMA-IR and FSI were significantly reduced, but also fasting blood sugar decreased.-In addition, significant reduction of serum LDL, TC and TG was encountered, as well as improved HDL. The five studies included in the systematic review varied in duration of intervention between 6 and 24 weeks and cinnamon supplementation consisted of 1500 mg/day either in capsules or in an extract form except for one study that only used 336 mg/day and in combination with metformin (Heydarpour et al., 2020).

Cinnamon may be, considering the results presented, of interest as a therapeutic tool as it is inexpensive, easily accessible and, as for as noted, safe.

MICROBIOTA IN PCOS AND ITS THERAPEUTIC APPLICABILITY

As previously exposed, genetics, the environment, neuroendocrine, metabolic as well as lifestyle factors contribute to the physiopathology of PCOS. However, the etiology of the disease has not yet been fully explained, and gut microbiota has recently grown in importance as a possible key factor. Nonetheless, it should be noted that is not clear if the gut microbiome is the one causing the syndrome or whether it is the syndrome causing the alterations documented in the gut microbiota (Yurtdaş & Akdevelioğlu, 2020).

The microbiota composition (Figure 5), on its turn, is influenced by a number of factors such as age, type of birth, diet, lifestyle, genetics, and use of certain drugs such as antibiotics (Thackray, 2019). It comprises more than 100 trillion microorganisms and human gut microbiota is rich in five main bacterial phyla: Firmicutes (e.g. *Clostridium, Lactobacillus, Eubacterium, Ruminococcus, Butyrivibrio, Anaerostipes, Roseburia, Faecalibacterium*, among other gram-positive species), Bacteroidetes (e.g *Prevotella* and *Bacteroides*, among other gram-positive species), Proteobacteria (*Enterobacteriaceae*, etc. gram negative species), Acinobacteria (e.g *Bifidobacterium gram-negative species*) and Verrucomicrobia (*Akkermansia*, etc. gram-negative species). 90% are attributed to the first two phylum, and the remaining 10% mainly to the third and fourth groups mentioned (Yurtdaş & Akdevelioğlu, 2020).

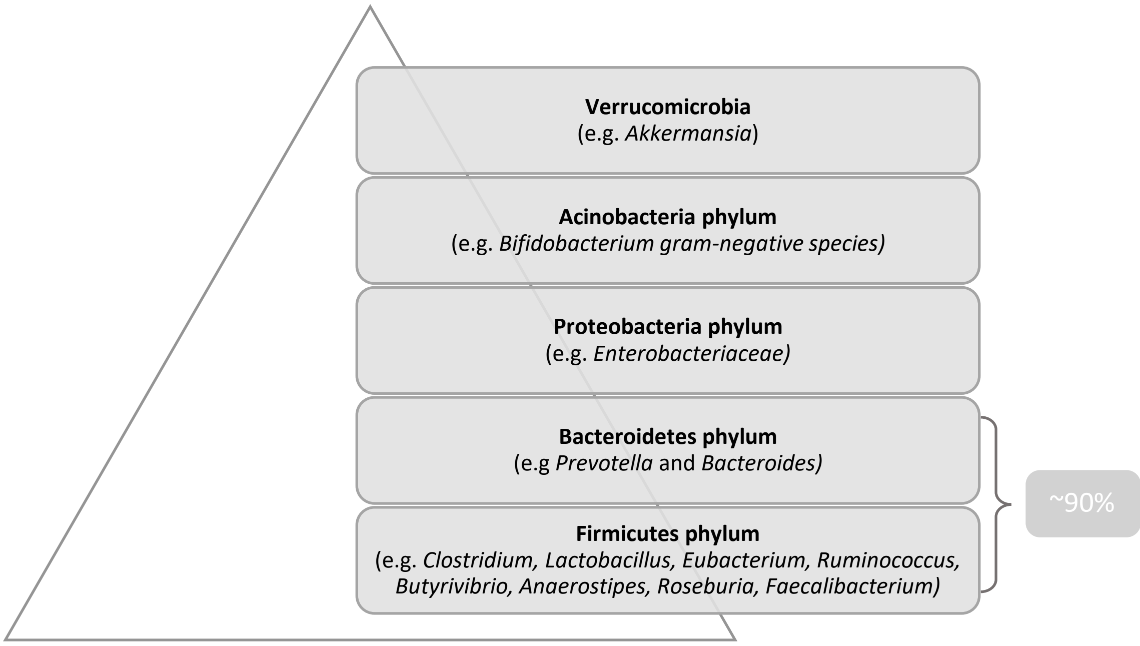

Figure 4: Human Microbiota Composition

The microbiota affects multiple physiologic functions of its host, in a metabolic, protective, structural and histological level. Metabolic functions include the production of short-chain fatty acids (SCFAs), acetate, butyrate and propionate being the major ones produced through fermentation. These have an anti-inflammatory, anticarcinogenic and immunomodulatory effects. The microorganisms are also capable of secreting antimicrobial components, which inhibit the production of lipopolysaccharides (LPS) and peptidoglycan, which are harmful to the host. In what respects to the modulation of the immune system, reports show that signals from the gut are effective in the development of T-cells, respectively TH1, TH2, and TH17. In terms of structural and histological functions, the gut microbiota can induce the formation of the mucus layer and the secretion of mucins that reinforce the colonic barrier. In addition, it can maintain the epithelial integrity through regulation of thigh junction permeability (Yurtdaş & Akdevelioğlu, 2020).

Dysbiosis is the term used when there is a disruption of the composition of intestinal microbiota, and results from it a decrease in the ratio of beneficial/harmful bacteria and it associated with many diseases, PCOS being one of them.

Multiple mechanisms through which gut microbes are associated with PCOS have been studied, and a recent review from L. Wang et al. summarizes six different pathways

(Figure 6). Firstly, *Bacteroides vulgatus* appears to be significantly increased in PCOS patients, which, in turn, causes reduction in specific bile acids, through higher production of its hydrolases, resulting from there a decline in IL-22 levels. IL-22 is a cytokine capable of correcting PCOS phenotypes, aggravated by the dysbiosis, once it mitigates IR and ovary dysfunction. Secondly, researchers observed reduction of beneficial microbes such as *Faecalibacterium, Bifidobacterium* and *Blautia* in PCOS patients, while *Parabectoides* and *Clostridium* were increased. Additionally, PCOS patients showed lower levels of SCFAs in their faecal samples, the most important metabolites of intestinal microorganisms as already mentioned. Moreover, ghrelin and PYY levels were observed to be lower, both being peptides with crucial roles in functions concerning the pituitary gland-gonad axis. Remarkably, the administration of *Bifidobacterium lactis* V9 augmented the abundance of SCFA-producing bacteria such as *Faecalibacterium* and *Bifidobacterium*, which stimulated the release of gut-brain mediators like ghrelin and PYY, these being known to influence sex hormones secretion and, consequently, ameliorating PCOS symptoms. More SCFAs also contribute to the barrier function of the gut, reduced translocation of endotoxins resulting in reduced inflammation and IR. Thirdly, and accordingly to another study, an increase in *Bacteroides* and *Escherichia/Shigella* and a decrease in *Akkermansia* both downregulate ghrelin production, possibly exacerbating PCOS symptoms. Fourthly, abundance of *Prevotella* is increased which leads to an increase in testosterone and androstenedione production. At the same time, *Lactobacilli* is decreased in these patients, leading to a decrease in oestradiol and estrone levels. Consequently, both findings might contribute to the PCOS pathogenesis, demonstrating the interplay between gut microbes and sex hormones. Fifthly, LPS-producing bacteria increased, i.e., gram-negative bacteria, while protective bacteria like *Akkermansia* decreased. LPS is capable of inducing metabolic disorders that induce inflammation, obesity and IR (L. Wang et al., 2021). Finally, decreased alpha, which is the number of different species that can be found in a specific sample, and altered beta diversity, i.e., the similarity amongst the different samples examined, is observed. These changes may lead to altered intestinal function and, as a result, aggravate PCOS(L. Wang et al., 2021; Yurtdaş & Akdevelioğlu, 2020b).

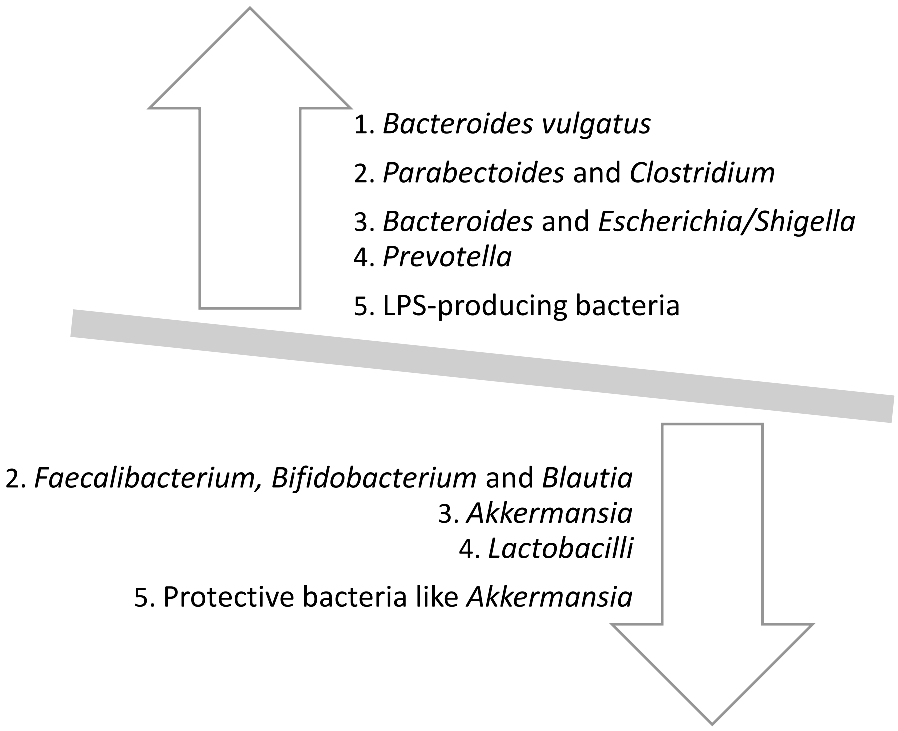

Figure 5: Gut Microbiota Influences PCOS. Adapted from L. Wang et al., 2021

Taking into consideration these findings, **modulation of intestinal microbiota**, such as through the use of **probiotics, prebiotics or synbiotics** as well as through the transplantation of faecal microbiota might be of great potential for clinical application in the improvement of PCOS features (L. Wang et al., 2021). Relevantly, microbiota can exert an impact on drug and therapy response as well as act as a source of new therapy itself.

A **probiotic** is a live microorganism which, when administered in the correct amounts confers a health benefit to the host. The most commonly used probiotic organisms are Bifidobacteria and Lactobacillus (Tremellen & Pearce, 2012). Probiotic microorganisms are naturally found in fermented foods such as yogurt, kefir, ayran, pickles or tarhana. (Yurtdaş & Akdevelioğlu, 2020). As example of its benefits and as already referred, J. Zhang et al. study demonstrated that the administration of *Bifidobacterium lactis* V9 augmented the abundance of SCFA-producing bacteria which stimulated the release of gut-brain mediators, influencing sex hormones secretion and ameliorating PCOS symptoms (J. Zhang et al., 2019).

Prebiotics are selectively fermentable ingredients that allow specific changes, both in the composition and/or activity of the gastrointestinal microflora and that confers benefits upon host wellbeing and health. These oligosaccharides are naturally present in different foods such as leeks, asparagus, artichoke, garlic, onion, wheat, oats and banana. However, the levels found in these are low and artificial supplementation with soluble fibre inulin derived from chicory roots, for example, is a more attractive therapeutic option (Tremellen & Pearce, 2012).

Synbiotics consist of combinational therapy of pre- and probiotics, so that the prebiotic will selectively stimulate the growth and/or activity of both the endogenous and exogenously applied beneficial bacteria (Tremellen & Pearce, 2012).

Cozzolino et al. systematic review and meta-analysis summarizes the evidence on the effects of probiotic/synbiotics on metabolic, hormonal and inflammatory parameters of PCOS. From the nine studies included, four compared the administration of synbiotics twice a day versus placebo and five the effects of probiotics twice a day versus placebo. The administration timing varied between 8 and 12 weeks. The supplementation most frequently used was *Lactobacillus acidophilus* and *Lactobacillus casei* except one study that administered *Lactobacillus rhamnosus* and *Bacillus koagolans*. As results, probiotics and synbiotics showed to significantly reduce fasting plasma glucose (FPG), fasting blood insulin (FBI), HOMA-IR and TGs. Furthermore, reduced serum testosterone was found, without effect on SHBG, DHEAS, and free androgen index (FAI). Moreover, increased serum hs-CRP, nitric oxide (NO), total antioxidant capacity (TAC), total glutathione (GSH) and malondialdehyde (MDA) was noted. No statistically significant effect was showed on LDL, HDL and TC. Additionally, a decrease in BMI was documented (Cozzolino et al., 2020).

Shamasbi et al., conversely, found significant reduction on FAI and increase in SHBG and DHEAS. MDA and NO increased significantly and coherently with the previous meta-analysis presented. It also showed increased serum GSH and TAC and reduction in CRP, although these were not found in a statistically significant manner, the same applying for reduction in testosterone (thought to be due to short term interventions). Furthermore, in what respects the clinical symptoms outcomes, reduction in hirsutism score was noted, however not significant, as short term consumption of the supplements may be insufficient when improvement of signs may take a long time to appear after the regulation of male sex hormones. As far as the contents of interventions is concerned, supplementation used consisted of different combinations of: *Lactobacillus (acidophilus, casei, rhamnosus, bulgaricus, delbruekii, fermentum; reuteri), Bifidobacterium (bifidum, breve, longum)* and *Streptococcus thermophiles* (Shamasbi et al., 2020). Similar results to the already discoursed were also advocated in Tabrizi et al. meta-analysis (Tabrizi et al., 2019).

The beneficial effects encountered may be explained by attenuated systemic inflammation through regulation of inflammatory signalling pathways, producing of antioxidant metabolites and downregulating of reactive oxygen species (ROS). In women with PCOS oxidative stress biomarkers such as TAC, MDA, GSH are often increased. Moreover, increased oxidative damage is associated a hyperandrogenism, IR, CV events and DM in PCOS. As so, the supplementation of probiotic or synbiotics may restore the balance in the gut microbiome (Cozzolino et al., 2020). In addition, significant increase in NO may be, on one hand, beneficial as NO can kill microorganisms and has a protective effect on the body but, on the other hand, it can damage normal tissue cells to generate the pathogenic effects. (Shamasbi et al., 2020). Furthermore, beneficial effects on BMI may be due to positive modulation of energy balance, supported by a reduction of leptin levels after the intervention. These effects were shown with prolonged administration (for twelve or more weeks) of probiotics therapy with Lactobacillus rhamnosus or Lactobacillus sallivarius in obese women (Cozzolino et al., 2020). This anthropometric parameter effect was not established in previous meta-analysis (Hadi et al., 2020; Heshmati et al., 2019). As seen, the meta-analysis also documented a restored glucose homeostasis, probably through modification of the absorption of micronutrients in PCOS patients (Cozzolino et al., 2020). Indeed, uptake of prebiotics, probiotics and synbiotics may balance the colony of intestinal microbes and intestinal pH, improve intestinal decomposition and metabolism of lipids and starch, intestinal digestion and absorption of nutrients such as iron, magnesium, calcium and zinc. (Shamasbi et al., 2020). Furthermore, improvement of TG was also registered, as administration of probiotics may improve the gut microbiome in a dietary lipid content-dependent manner (Cozzolino et al., 2020). The modulation of the genes that control appetite is probably due to enhancement of fatty acids produced by microbiota and also due to probiotics capability of inducing entero-endocrine cell proliferation, thus increment and decrement gut metabolic peptide production and secretion (Cozzolino et al., 2020).

Relevant and recent data is published almost daily, being from this example, the Karimi et al. randomized controlled trial. It further reinforces the potential use of synbiotics. The intervention group received a capsule containing 500 mg of seven strains beneficial bacteria (Lactobacillus and Bifidobacterium variants and Streptococcus thermophilus) and prebiotic Inulin during 12 weeks. Results showed increase in HDL and decrease LDL. No significant between-group difference was found for anthropometric indices such as BMI, WC, HC and waist-to-hip ratio (WHR) in women with PCOS. Different composition of probiotic and prebiotic supplements is mentioned as an argument for the differences amongst other studies and the one presently being mentioned. (Karimi et al., 2020).

Esmaeilinezhad et al. trial evaluated the effect of synbiotic pomegranate juice (SPJ) on cardiovascular risk factors in PCOS patients. The intervention lasted 8 weeks and PCOS patients were divided in four groups, receiving one of the following: synbiotic pomegranate juice, only pomegranate juice, symbiotic beverage or placebo beverage. 300 mL/day of the beverage was given, SPJ, in particular, consisting of the juice with inulin and Lactobacillus strains. Metabolic outcomes, as TG, LDL, HDL, and TC, as well as oxidative stress, evaluated through MDA and TAC, as well as blood pressure improved with SPJ consumption (Esmaeilinezhad et al., 2020).

One other interesting trial, from Ostadmohammadi et al. studied the effects of vitamin D and probiotic supplementation. The intervention group received 50000 IU vitamin D every 2 weeks as well as a daily capsule with Lactobacilus and Bifidobacterium (8x10^9 CFU/day) during 12 weeks. Beneficial effects on mental health parameters, seen in depression, anxiety and stress scale scores, on serum total testosterone, hirsutism, hs-CRP, plasma TAC, GSH and MDA levels were verified when compared to placebo. No effects were demonstrated in serum SHBG, plasma NO levels, acne and alopecia. (Ostadmohammadi et al., 2019).

Overall, microbiota modulation is a novel field with a lot more to be explored, and although the potential is significant, more plausible data is still to be documented.

IS IT WHAT YOU EAT THE ONLY THING THAT MATTERS?

"Chrononutrition" is a newly proposed discipline that addresses meal timings and its interaction with the circadian clock and metabolism. This is relevant since increasing evidence indicates that the meal timing has a significant role in the metabolic regulation, as it may induce a misalignment between the peripheral circadian clocks present in all body cells and the central biologic clock, the suprachiasmatic nucleus in the hypothalamus and, thus increase the risk of cardiometabolic diseases (BaHammam & Almeneessier, 2020).

Hereof, Jakubowicz et al. studied the effect of caloric intake timing in lean women with PCOS over a period of 90 days. Two isocaloric diets divided over three main meal with different caloric distribution were put into test, one with the highest caloric intake at breakfast followed by lunch and dinner and the other with the exact opposite distribution. The study revealed reduced fasting serum glucose and insulin concentrations and reduction of insulin resistance in the group with high calorie intake at breakfast. Moreover, reduction of the ovarian cytochrome P450c17alpha, characteristically increased in PCOS women, was noted and accompanied by a decline in the serum ovarian androgen concentrations, respectively total testosterone, free testosterone, DHEA-D and androstenedione. Additionally, meal composition appears to influence satiety as showed with greater initial and sustained satiety and decreased levels in serum of ghrelin lead by higher protein content at breakfast. Besides, increasing carbohydrate intake at breakfast could be protective against long-term development of the metabolic syndrome. Further, the improved insulin resistance indices in the breakfast group may suggest that a high caloric intake in the morning represent a schedule more synchronized with the circadian pacemaker. (Jakubowicz et al., 2013).

Regardless of the fact that this is a single study, meal timing and distribution should be considered as therapeutic option for women with PCOS, as resetting the circadian clock with scheduled meals, thus resulting in ameliorated insulin resistance and hormone secretion may have beneficial effects.

On another point of note, and focusing exclusively on the meal timing component, time restricted feeding (TRF), as a form of intermittent fasting, has also been reported as having beneficial effects on weight loss and cardiometabolic parameters. A non-randomized trial with a five-week intervention period tested an eight-hour time restricted feeding in women with anovulatory PCOS. The participants were allowed to eat their usual diet within a period comprehended from 8 am to 4pm daily. As relevant results, TRF improved menstrual gonadal profiles (evaluated through total testosterone, SHBG and FAI), body weight, BMI, body composition profiles (assessed with body fat mass/percentage and visceral fat area), hyperinsulinemia and insulin resistance (fasting insulin, HOMA-IR, AUC). Beneficial effects on chronic inflammation were also noted. No significant differences, however, were found in TC, TG and LDL-C levels (Li et al., 2021).

Supplementarily, a randomised crossover trial studied the effect of meal frequency on glucose and insulin levels in women with PCOS. The participants followed a weight maintenance diet in either the form of a three or six isocaloric meal-eating plan for a period of 12 weeks, then switching to the other meal pattern for more 12 weeks. Body weight remained stable during the intervention period. As relevant results, the six meal-eating plan decreased, in a significant manner, fasting insulin and post-OGTT insulin sensitivity when compared to the three-meal pattern. No significant differences were found in serum glucose or HbA1c levels, blood lipids, hepatic enzymes, and neither in the subjective desire to eat and satiety. The beneficial effects on glucose levels have underlying unclear mechanisms but it is thought to be related to nutrient spreading, which produces lower postprandial insulin concentrations, reduces hunger and supresses the inhibitory effects of free fatty acids on glucose uptake. The study favours, ultimately, the six meal pattern considering it as a possible non-pharmacologic adjunct to PCOS treatment (Papakonstantinou et al., 2016).

Notably, and despite current evidence is limited and further studies are required, these new insights may indicate that not only the diet composition or caloric intake are of relevance but also the approach to its distribution is pertinent.

FINAL THOUGHTS

PCOS is a multisystem disease that affects not only the sexual hormones balance and fertility parameters, but also increases the susceptibility to diverse comorbidities, from metabolic syndrome to higher cardiovascular morbidity and certain neoplasia incidence. In that context, reviewing the impact of first line therapies as lifestyle modifications and, concretely of nutritional intervention seemed not merely reasonable but also crucial considering the potential positive outcomes.

In the present review, the dietary habits of women with PCOS were identified and showed a higher GI and glycaemic load as well as a total fat and overall higher energy intake. Despite the fact that the mentioned range of consumption is, unequivocally, not beneficial for the general health or for the specific features of the women with PCOS, this also implies that there is scope for research intervention in the nutrition area.

On one hand, caloric restriction, with the objective of creating an energy deficit, and consequent weight loss, is frequently stated as a general consensual approach to women with PCOS. However, understanding if more specific interventions may add further benefits in the management of these patients, and not exclusively in weight control parameters, is still a controversial discussion. Toward that end, a broad assessment from a larger spectrum comprising the analyses of different diet patterns to a progressively narrower scale with the evaluation and impact of macronutrients and finally the review of micronutrients was addressed.

The impact of these modifications was measured through clinical parameters as well as objective parameters such as anthropometric indices, insulin resistance, lipid profile, biomarkers of oxidative stress and reproductive hormones plasma levels. Whether a wholefood with its intrinsic macronutrient content is analysed or whether the macronutrient in isolation is studied, these will direct or indirectly influence the disease mechanism and, thus, can be of use as treatment strategies. Overall, a low protein and moderate carbohydrate and fat dietary consumption ameliorated the development of PCOS features, being this balance similar to the one discussed both in a Mediterranean diet as well as in the DASH diet. Indeed, wholesome and less restrictive diets seem to have the best outcomes as long-term maintenance is also, in those cases, facilitated.

What is more, giving primacy to low GI foods within the carbohydrate component appears to be an attractive and accessible measure to apply.

Moreover, micronutrients imbalance was repeatedly identified. In that regard, a balanced nutrient-dense diet may play a beneficial role just as well through the supplementation of these particular elements. In this area of study in particular, the results encountered are particularly conditioned by the supplementation composition, i.e., whether and, ideally, the element in study is given in isolation, or, alternatively, in co-supplementation with others, as well as by the dosage utilised, highly variable amongst studies and, therefore, influencing the results obtained. Further than that, also the duration time of the interventions affects the achievement of certain benefits and whether they are sustained over time.

Furthermore, other determinants have gained importance in the latest years, as found with not only the impact of the gut microbiota in the pathophysiology of the disease but also in its utility as a treatment option. The imbalance of these microorganisms in the gut of women with PCOS may alter the severity of the disease presented and its correction may potentiate clinical and laboratorial improvement using either prebiotics, probiotics or synbiotics.

Apart from the content inherent to all of these specific modifications, how it is applied may also play a significant role as discussed with different meal timings or different restriction feeding schedules. This alerts to the need of understanding diet interventions as of the highest complexity, importing not exclusively the diet content but also the way it might be metabolised and absorbed, as per example, influenced by the microbiota composition, to the time of day food is consumed.

Overall, the review here presented is inevitably conditioned by the studies used as basis of justification. In that context, the heterogeneity of the findings in the different was a constant limitation to the establishment of meaningful conclusions and formulation of evidence-based practical recommendations. As major limitations is worth of mention the reduced number of studies concerning the subjects discussed, as well as their limited sample pooling, diversity in the populations studied in terms of ethnicity variety and the short-term intervention times. Apart from that, not all the studies included had the same data quality, as they were not always randomized controlled trials, these providing, naturally, more reliable and reproducible results. Aside from this, nutrition as a general area of intervention is, by its nature, difficult to approach as it is highly susceptible to patients' compliance to the diet that is intended to be studied. What is more, single nutrient testing, independently if it is a micro or a macronutrient, is, most probably, impossible to be studied in complete isolation and, as a consequence, benefits or harmful effects identified cannot be fully attributed to it.

In summary, the identification of an optimal dietary intervention for PCOS would be of the highest importance, as it would constitute a feasible and cost effective therapeutic measure for women with the disease. Truthfully, the potential of this area of study is indubitable as more data is upturning and being widely divulgated. However, it also unquestionable that more good quality studies are warrantied. In spite of everything, the available evidence and the promising findings already identified establish a precedent and an opening to further research and discussion.

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