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Metabolic and hormonal aspects of polycystic ovary syndrome: the impact of diet

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Polycystic ovary syndrome (PCOS) is a common, chronic endocrine condition affecting young women of reproductive age. It is characterised by hyperandrogenaemia, and profound menstrual and ovulatory dysfunction with consequent sub-fertility. A clustering of metabolic aberrations is commonly associated with this condition and these include insulin resistance, disordered lipid metabolism and chronic low-grade inflammation. Overweight and obesity, as well as a degree of adipose tissue dysfunction, are present in a large proportion of women with PCOS, and where present, magnify the inherent hyperandrogenaemia characteristic of the condition, in addition to worsening the metabolic profile. Diet and lifestyle interventions are among the first-line treatments for PCOS, and weight reduction through energy restriction has been shown to exert positive influences on both metabolic and hormonal aspects of this condition. Alterations in carbohydrate amount and type have also been investigated, and more recently, dietary fatty acids, with a particular emphasis on PUFA, have been shown to have a positive impact within this population group. Although it is likely that diet is not the root cause of PCOS, it represents a modifiable variable with the potential to improve the health of women with this condition. Work to date has provided insights into the role of diet in PCOS; however, further work is required to determine the role of nutrients specifically within the context of PCOS, in order to develop more effective, evidence-based dietary guidelines for this condition.

Polycystic ovary syndrome: Diet

Polycystic ovary syndrome (PCOS) is the most common endocrine condition among women of reproductive age, affecting 5–10% of this population[1]. The condition is characterised by hyperandrogenaemia, which can be ovarian and/or adrenal in origin and which leads to profound menstrual and ovulatory dysfunction, manifested as oligomennorheoa or amennhorea, and consequent sub-fertility. Furthermore, PCOS is the most frequent cause of anovulatory infertility among young women[2]. Secondary to hyperandrogenaemia, PCOS is associated with hirsuitism, acne and male pattern balding. These symptoms, coupled with the frequently observed presence of overweight and obesity, as well as infertility and menstrual unpredictability, have a significant impact on the lives of women with this condition. As a result, PCOS has been linked with higher levels of psychological distress and depression than would be expected in a free-living population[3,4].

Over the past two decades, there has been increased interest in the clustering of metabolic abnormalities that occur in PCOS. Over the past 30 years, mounting evidence has linked insulin resistance to the PCOS population. Since

Abbreviations: LC, long chain; PCOS, polycystic ovary syndrome; T2DM, type 2 diabetes.

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the initial reports of hyperinsulinaemia[5] and impaired insulin sensitivity[6], insulin resistance is now recognised to play a pivotal role in PCOS. Insulin resistance has been reported in 30% of lean and 75% of obese women with PCOS. Where present, the degree of insulin resistance is more severe than would be expected for a given age and body weight[7].

Disordered lipid metabolism characterised by decreased HDL-cholesterol concentrations, with or without increased levels of TAG and/or LDL-cholesterol[8], has been identified within the PCOS population. Greater VLDL concentrations, in addition to a more atherogenic non-A LDL pattern, have also been reported[9]. It has been demonstrated that this pro-atherogenic lipid profile extends into the postprandial phase, and both lean and overweight women have elevated postprandial TAG concentrations[10,11], with a corresponding decrease in HDL-cholesterol[10]. These postprandial abnormalities are likely to be especially detrimental, as impaired lipid clearance is a significant risk factor for CVD[12,13]. Additionally, some evidence of sub-clinical carotid atherosclerosis exists within the PCOS population[14].

Obesity and overweight are also prevalent, and are estimated to affect 38–88% of women with this condition[15]. Within this, evidence exists for an altered body composition, including central adiposity[16], a factor that is evident even in lean women with the condition[17]; an increased intra-abdominal visceral fat accumulation is also evident[18,19]. Additionally, an altered adipokine secretion profile has been reported[15,20,21]. Although it is uncertain what drives this altered secretion profile, our group has recently demonstrated that high-molecular-weight adiponectin is selectively reduced in women with PCOS independent of BMI and severity of insulin resistance, with the reduced concentrations being substantially associated with central adiposity and androgenic status[22]. Adipocyte hypertrophy[23] has been reported in PCOS cases compared with controls of a similar BMI, accentuating the adipose tissue dysfunction present within this group. Furthermore, transcriptomic analysis of visceral adipose tissue in PCOS revealed substantial defects in gene expression relating to insulin signalling, oxidative stress and immunological function[24].

Although visceral adipose tissue is intimately linked with metabolic dysfunction[25], subcutaneous adipose tissue too is a substantial determinant of metabolic health. Several carefully executed studies have indicated that subcutaneous adipose tissue is more robustly associated with clamp-derived estimates of insulin sensitivity, due to it being the larger of the two depots, even in severely obese states[26,27]. Ongoing work within our research group has revealed that subcutaneous adipose tissue of women with PCOS exhibits a hypoxic and pro-inflammatory gene expression profile. Importantly, this level of dysfunction within adipose tissue was linked with the biochemical environment of PCOS subjects. Key differentially regulated pathways involved in inflammation and the cellular response to hypoxia are positively associated with central markers of metabolic function including plasma insulin and high-molecular-weight adiponectin, in addition to the circulating androgen free-testosterone, thus highlighting the impact of adipose tissue dysfunction in PCOS (A. O'Connor, M. Morine, N. Phelan, G. Boran, J. Gibney and H. M. Roche, unpublished results).

Aetiology and pathogenesis of polycystic ovary syndrome

To design effective treatments, the aetiology and the trajectory of disease pathogenesis must be fully understood. However, despite decades of knowledge and research, the exact cause of PCOS remains unclear. It is likely that the origins of PCOS are multi-factorial, and abnormal ovarian steroidogenesis, hyperinsulinaemia, and increased luteinising hormone drive, all make complementary and synergistic contributions, which will vary from individual to individual.

Central to the pathogenesis of PCOS is hyperandrogenism, and several groups have investigated this increased ovarian androgen production in PCOS, with clear evidence for an intrinsic ovarian defect emerging[28–33].

The concept of androgen excess in early life leading to epigenetic changes has led to the development of the theory of early origins of PCOS[34–37]. Additionally, the familial aggregation of PCOS strongly supports the role of a genetic component in the development of this complex disorder[38–40]. Several candidate genes involved in ovarian and adrenal steroidogenesis[41–44] have been proposed, as have key genes involved in insulin signalling[44,45]. However, conflicting findings and lack of replicate studies make interpretation difficult[46]. It is likely that PCOS is another oligenic disorder in which the interaction of a small number of genes with each other and with environmental factors such as diet results in a typically heterogeneous clinical and biochemical presentation[47].

Overweight and obesity, particularly abdominal adiposity, and subsequent insulin resistance are heavily involved in the pathogenesis of PCOS and appear to act as triggering factors that, where present, aggravate the inherent dysregulation of steroidogenesis, in addition to enhancing the cardio-metabolic risk associated with PCOS. This may occur through environmental factors such as the obesogenic environment, in addition to the effects of high plasma androgens that can drive abdominal fat deposition[48,49], leading to the central adiposity that is characteristic of PCOS[17]. Escobar-Morreale and San Millán[50] have proposed a unifying hypothesis highlighting this interaction, in which excessive androgens secondary to an intrinsic ovarian defect promote abdominal and visceral adiposity. This in turn exacerbates the hormonal abnormalities characteristic of the condition, through the enhancement of insulin resistance, altered adipokine secretion, and potentially, steroid hormone metabolism in the periphery. The authors describe a domino effect, in which the androgen excess leading to body composition changes promotes a further enhancement of the metabolic and hormonal aberrations[50]. Furthermore, similar to that suggested by Barber *et al.*[15], it is possible that the metabolic and hormonal implications of this deleterious fat accumulation are sufficient to unmask and enhance the symptoms associated with PCOS, even in women in whom the intrinsic ovarian defect is small.

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The action of insulin on the ovary in PCOS is important, and it has been demonstrated that ovarian cells isolated from women with PCOS secrete significantly more androgens upon insulin stimulation than ovarian cells isolated from women without the condition; this can be further up-regulated by the action of insulin[51]. Additionally, increased insulin can have a direct effect on the liver, lowering the production of sex-hormone-binding globulin and thus leading to a situation of increased androgen bioavailability in the circulation. Insulin may also stimulate luteinising hormone production, resulting in a stimulatory effect on ovarian theca cell steroidogenesis, thereby inducing further androgen production. Direct *in vivo* evidence for this comes from intervention studies, whereby the administration of insulin sensitisers such as metformin to women with PCOS has resulted not only in a reduction in peripheral insulin resistance but also in a reduction in circulating androgen levels[52]. Through these reports, it has become increasingly clear that a significant interplay exists between the metabolic and hormonal components of PCOS, and environmental influences such as overweight or obesity, as well as insulin resistance, appear to play a role in the progression and severity of the syndrome.

Long-term health consequences of polycystic ovary syndrome

The clustering of potentially modifiable metabolic aberrations is frequently observed within the PCOS population, and these require amelioration if optimal health is to be maintained.

An increased risk of developing type 2 diabetes (T2DM), in addition to greater complications and higher than expected mortality rates secondary to T2DM, is associated with PCOS[53]. One report revealed that the risk of developing T2DM in a cohort of women with PCOS was 13·4 % compared with 5·8 % in the control population, with this risk increasing five-fold in obese women compared with age-adjusted controls, thus representing the interactive nature of obesity in PCOS. Treatment of T2DM and its consequences represent a significant health burden, with 40·5 % of PCOS-related health care spending in the USA pertaining to the treatment and management of T2DM[54]. These findings prompted a report from the Androgen Excess Society recommending that the biennial assessment of glucose tolerance by the oral glucose tolerance test forms part of the routine clinical management of women with PCOS[55].

Although there are numerous reports linking PCOS with a more deleterious CVD risk profile, to date, there exists no convincing evidence of increased mortality from CVD[53,55,56]. It should be noted, however, that existing studies have been retrospective or cross-sectional in design, and there is a distinct lack of prospective studies in this area[57]; thus, it is difficult to draw firm conclusions regarding the association between CVD-related mortality and PCOS. However, even taking the normalising effect of age into consideration, the longer exposure to the effects of several CVD risk markers (deleterious lipid profile, chronic low-grade inflammation and insulin resistance) is an important factor to consider. Over a 15–20 year period this could translate into an increased coronary artery and atherosclerotic risk[58] and so prophylactic management is prudent.

Treatment of polycystic ovary syndrome

Due to the chronic nature of PCOS and the young age at which both the hormonal and metabolic symptoms begin to manifest, lifelong strategies that improve the care of women with PCOS are essential. Identifying effective modifications to habitual diet or lifestyle can be advantageous in the treatment of any condition, as they are considered safe and are associated with few notable side effects. Additionally, lifestyle interventions are generally acceptable to patients; hence compliance may be increased. Furthermore, current widely used pharmacological treatments for PCOS are not without drawbacks. Gastrointestinal side effects are commonly experienced with metformin[59], and oral contraceptive pill usage is linked with increased metabolic irregularities[60], which further complicate the clinical picture. The efficacy of long-term lifestyle interventions has been highlighted by work conducted as part of a large-scale clinical trial aimed at preventing the onset of T2DM in high-risk individuals. This trial conducted in 3234 non-diabetic individuals compared the effects of metformin and an intensive lifestyle intervention (weight reduction through energy restriction and increased physical activity) and showed that although both lifestyle changes and metformin reduced the incidence of T2DM, the lifestyle intervention alone was significantly more effective[61].

Weight reduction as a treatment for polycystic ovary syndrome

Dietary and lifestyle interventions, with a focus on weight management through increased physical activity and overall energy restriction, are considered among the first-line treatments of women with PCOS. The importance of adipose tissue as a central organ involved in the storage of fatty acids has been well documented, and there is mounting evidence highlighting the role of adipose tissue in the development of the systemic inflammatory state that contributes to obesity-associated vasculopathy and CVD risk. White adipose tissue was traditionally considered to play a minor role in glucose uptake and glucose homeostasis, accounting for not more than 10–15 % of post-meal glucose uptake[62]. However, white adipose tissue is now thought of as a highly dynamic endocrine organ and a central contributory player in whole-body glucose[63]. The aberrant effects of adipose tissue dysfunction have profoundly negative effects on insulin sensitivity, with a differential expression of pro- and anti-inflammatory factors observed with increasing adipocyte size. This shift in expression patterns and the consequent pro-inflammatory environment can affect insulin signalling within the adipose tissue[64].

Within the context of PCOS, maintenance of a healthy body weight is of prime importance. Weight reduction

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leads to improvements in clamp-assessed insulin sensitivity[65], decreased insulin resistance as assessed by homeostasis model assessment[66], as well as an improved lipid profile[66]. Importantly, weight reduction improves hyperandrogenism and increases menstrual function, ovulation and fertility[66–68], with reductions in adiposity from the truncal–abdominal area appearing to exert particularly positive benefits[67].

However, overweight and obesity, although commonly associated with the condition, are not globally present[17], suggesting that body weight is not the only issue. Additionally, although overall weight management is important for the metabolic health of any individual, the macronutrient composition of the diet itself can actively contribute, regardless of body mass. However, despite the focus that dietary practices receive, little is known about the optimal diet for women with PCOS beyond those designed for weight reduction through energy restriction.

Dietary modification in the treatment of polycystic ovary syndrome

Carbohydrate- and protein-based interventions in polycystic ovary syndrome

Considering the intimate link with insulin resistance, low-glycaemic-index diets have been promoted for women with PCOS[69] and have recently been demonstrated to result in significant improvements in both insulin sensitivity and menstrual cyclicity within the context of PCOS[70]. Additionally, low-carbohydrate diets have been examined, with energy substituted with either protein[66,71], or MUFA[72], with improvements in insulin sensitivity reported following both interventions. The acute effects of carbohydrate compared with protein consumption in women with PCOS revealed that carbohydrates (glucose) resulted in significantly greater postprandial excursions in plasma glucose, androsteindione, dehydroepiandrosterone sulphate, ghrelin and insulin[73]. Although short-term positive effects of these diets were demonstrated, the long-term suitability is questionable. Whereas low-carbohydrate diets will lead to a short-term decrease in glucose levels, they can in the longer term result in increased hepatic glucose production and a reduction in peripheral glucose utilisation[74].

Alterations of dietary fat content in polycystic ovary syndrome

Dietary fats have traditionally been regarded as important energy dense nutrients, and are increasingly recognised as key biological regulators, influencing various aspects of metabolic health[75]. Considering the important role of dietary fat in this regard, it is surprising that only a handful of studies have investigated the effects of dietary fat modulation specifically within the PCOS population. That differences in fat consumption can have an effect was highlighted in a cross-sectional study in which women of Caucasian origin with PCOS living in the USA were compared with those residing in Italy. Women in the USA had higher SFA intakes and in addition were significantly more overweight, with lower HDL-cholesterol concentrations, despite reporting similar energy intake[20]. Mai *et al.*[76] demonstrated an increase in the androgen precursors dehydroepiandrosterone sulphate and androsteindione following lipid infusion (Abbolipid 20%; safflower oil and soya oil) in healthy men[76], an observation independent of changes in circulating insulin. More recently, this finding was pursued further by investigators within the same group in a cohort of women with PCOS, and similar results were observed following administration of the same lipid preparation[77], further highlighting the role of fatty acids in androgen synthesis *in vivo*. Further support for the role of fatty acids in reproductive health comes from data obtained as part of the Nurses' Health study, in which it was clearly demonstrated that with each 2% increase in energy intake from *trans* fats when substituted for unsaturated fats or carbohydrates, the risk of ovulatory infertility increased by 50–73%[78]. Although suggestive of a link between fatty acids and reproductive health, this study was not conducted with women for whom a definitive diagnosis of PCOS had been made, making it somewhat difficult to apply this with certainty within the context of PCOS.

PUFA dietary interventions in polycystic ovary syndrome

Reports presented so far have detailed the effects of SFA or MUFA; however, PUFA have significant potential to impact the metabolic and hormonal environments of PCOS.

Kasim-Karakas *et al.*[79] examined the effects of habitual diet enrichment with walnuts, which provided significant amounts of the *n*-3 PUFA α-linolenic acid, in addition to the *n*-6 fatty acid linoleic acid. The authors postulated that increased amounts of dietary PUFA would benefit the metabolic and hormonal profiles of women with PCOS. Following the 3-month intervention period, there was a trend towards improvements in fasting TAG, HDL- and total-cholesterol, although these changes were not substantial. Furthermore, contrary to the group's hypothesis, no changes in androgens were observed. The use of plant-derived *n*-6 PUFA may have precluded the observation of positive effects of these fatty acids, as all forms of *n*-3 PUFA do not have identical biological effect. In a recent systematic review, it was revealed that there is currently no high-quality evidence to support the use of α-linolenic acid for CVD risk reduction[80]. The author's hypothesis that increasing the parent fatty acid α-linolenic acid would result in downstream increases in the longer chain, more metabolically active EPA and DHA is also erroneous. Whereas chronically high intakes of α-linolenic acid will result in increased concentrations of EPA[81], the overall conversion is low, and despite the heterogeneity in results reported, the overall consensus is that the conversion rate in human subjects is <10%[82]. Additionally, the presence of linoleic acid in the diet will result in a further reduction in this conversion rate due to the competition for the required desaturation enzymes[83]. Hence, the most effective way of increasing plasma EPA and DHA is to supplement with these fatty acids directly[84].

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The long chain (LC) *n*-3 PUFA EPA and DHA have emerged as particularly potent biological regulators, with distinct and unique properties compared with other fatty acids. These notable differences may be due to the longer chain length or the higher number of double bonds, both of which will alter the chemical properties of these molecules[85]. LC *n*-3 PUFA are suggested to play a role in cognitive development, learning and visual function, immune-inflammatory response, neurological degeneration and cancer[85]. LC *n*-3 PUFA are also associated with CVD risk reduction[80,81,86], with hypolipidaemic effects both in the fasted[87,88] and postprandial[89] state. LC *n*-3 PUFA also have a role in various aspects of reproduction[90], and are involved in oocyte fertilisation[89], as well as fetal and infant development[91,92]. Fatty acids and their derivatives are also involved at various stages of folliculogenesis, including during steroidogenesis and the activation of steroid acute regulatory protein[93,94], as well as during ovulation when luteinising hormone-driven *COX-2* expression leads to the production of prostaglandins, essential components in the processes of cumulus oocyte complex expansion and ovulation[95].

The pathology of PCOS is linked with many of the metabolic aberrations for which *n*-3 PUFA have been shown to exert a positive effect. These include abdominal adiposity[17], chronic low-grade inflammation[95] and postprandial dyslipaemia[10,11].

The effect of LC *n*-3 PUFA was examined recently within the context of PCOS[96]. In this double-blind, randomised, cross-over study, 4 g LC *n*-3 (2·24 g DHA and 1·08 g EPA) followed by 4 g olive oil (67 % oleic acid) were administered for 8 weeks, with each treatment period separated by an 8-week wash-out period. High field magnetic resonance spectroscopy measurement of liver fat was the primary assessment objective following each treatment arm, in addition to fasting lipid profile. Overall there was a decrease in fasting TAG and liver fat; however, these results were observed in those individuals with a high baseline liver fat content only. In addition, the use of an anti-androgen oral contraceptive pill did not preclude participation in the study and hence limited the investigative potential of the effect of *n*-3 PUFA on this aspect of PCOS.

Ongoing work within our research group has suggested that supplementing the diet of women with PCOS with LC *n*-3 PUFA may have an anti-androgenic effect. However, further analysis of this group, in addition to cross-sectional analysis of plasma fatty acids in an independent cohort of women with PCOS, suggests that this anti-androgenic effect appears to be mediated by a decrease in the plasma *n*-6:*n*-3 ratio rather than a direct functional effect of *n*-3 PUFA, suggesting a potentially direct effect of *n*-6 fatty acids on circulating androgens within this population. That *n*-6 fatty acids may directly impact steroidogenesis was corroborated by treatment of bovine theca cells with the *n*-6 fatty acids arachidonic acid, resulting in increased androgen secretion (N. Phelan and A. O'Connor, unpublished results).

Dietary fatty acids can be assimilated into adipose tissue where they can alter the function and fatty acid profile of the adipocyte. Evidence taken from the literature[20,23,24,97] as well as from our group (A. O'Connor, M. Morine, N. Phelan, G. Boran, J. Gibney and H. M. Roche, unpublished results) suggests that a degree of adipose tissue dysfunction is present within the PCOS population. Therefore the therapeutic role of *n*-3 PUFA supplementation in the adipose tissue was considered of prime concern. These effects of *n*-3 PUFA on the adipose tissue were assessed by transcriptomic profiling as gene expression changes within this organ may indicate the extent as well as the nature of changes driven by an increased dietary consumption of *n*-3 PUFA. This comprehensive phenotyping may add to our knowledge of the impact of *n*-3 PUFA on metabolism in women with PCOS.

Conclusions

PCOS is a complex, multi-faceted condition encompassing aspects of reproductive and metabolic health, with an appreciable level of interplay between these hormonal and metabolic environments.

Numerous physiological and behavioral mechanisms link reproduction and energy metabolism. From an evolutionary standpoint, the link between adiposity and reproductive capacity would ensure optimal nutritional status for conception and pregnancy and would help ensure successful propagation of the species[15]. Additionally, fuel-sensing pathways such as PPAR and the AMP activated kinase pathway are active within the ovary[98]. Recent evidence suggests that pharmacological agents commonly used for the treatment of PCOS act directly on the ovary itself to impact steroidogenesis through nutrient-sensing pathways[99,100]. Considering the emerging role of fatty acid derivatives in aspects of reproduction, it may be of interest to determine whether nutrients such as fatty acids may impact circulating androgen levels or may have a direct effect on steroidogenesis in the ovarian theca cells.

Many of the current dietary recommendations for women with PCOS are extrapolated from data obtained from insulin-resistant populations or are based on studies conducted within the general population, a sensible approach considering the appreciable overlap that exists between these groups and PCOS. Whereas traditionally nutrient requirements and recommendations were based on the concept of 'essentiality' and aimed at preventing nutritional deficiencies as assessed by the appearance of clinical lesions, we are now moving towards the idea that nutrients can modulate chronic disease susceptibility and are important in maximising health. This has resulted in a broadening of requirement endpoints and a general widening of the recommended intake ranges to facilitate the goal of optimal health outcomes[101]. In order to achieve this, a deeper understanding of the potentially specific functional roles of nutrients in PCOS is required. Considering the prevalence of this disorder, the relative dearth of controlled dietary intervention studies conducted within a PCOS population therefore represents a missed opportunity to understand how nutrients act specifically within the context of PCOS to influence overall metabolic and reproductive health.

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