MANAGEMENT OF ENDOCRINE DISEASE

Secondary polycystic ovary syndrome: theoretical and practical aspects

Renato Pasquali¹, Evanthia Diamanti-Kandarakis² and Alessandra Gambineri¹

¹Division of Endocrinology, Department of Medical & Surgical Sciences, University Alma Mater Studiorum, S. Orsola-Malpighi Hospital, Bologna, Italy and ²Department of Endocrinology and Diabetes Center of Excellence, EUROCLINIC, Medical School University of Athens, Athens, Greece

Correspondence should be addressed to R Pasquali
Email
renato.pasquali@unibo.it

Abstract

PCOS is a clinical heterogeneous entity of female androgen excess diagnosed by exclusion of other disorders responsible for androgen excess. The concept of secondary PCOS implies that there is a primary well-defined cause leading to the PCOS phenotype with underlying androgen overproduction, regardless of the origin. In these cases, we presume the term of 'secondary PCOS' could be used. In all these conditions, the potential complete recovery of the hyperandrogenemic state as well as the remission of the PCOS phenotype should follow the removal of the cause. If accepted, these concepts could help clinicians to perform in-depth investigations of the potential factors or disorders responsible for the development of these specific forms of secondary PCOS. Additionally, this could contribute to develop further research on factors and mechanisms involved in the development of the classic and the nonclassic PCOS phenotypes.

European Journal of Endocrinology
(2016) **175**, R157–R169

Introduction

The polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women in reproductive age. PCOS is largely heterogeneous, including both classic and nonclassic forms, characterized by major differences in the clinical presentation and pathophysiological aspects (1). In 1935, Stein and Leventhal provided the first description of PCOS including hirsutism, amenorrhea, chronic anovulation and infertility; obesity and enlarged cystic ovaries demonstrated after surgery (2). The first modern definition of PCOS came from an expert conference sponsored by the National Institute of Health

(NIH) in 1990. It defined that the major criteria for PCOS, including clinical hyperandrogenism and/or hyperandrogenemia and menstrual dysfunction (oligo-amenorrhea, infertility), provided that all other well-known disorders presenting with the same signs and symptoms have been excluded *a priori* (3). Therefore, this definition identified PCOS as an androgen excess disorder of exclusion, with an ovarian dysfunction at the basis of the disease. In 2003, another expert conference in Rotterdam, sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive

Invited author's profile

Renato Pasquali is full professor of Endocrinology & Metabolism and Director of the division of Endocrinology of the S.Orsola-Malpighi Hospital, Bologna, Italy. He also heads the School of Specialization in Endocrinology and Metabolism, University Alma Mater Studiorum of Bologna, Italy. He is a member of numerous national and international scientific societies and serves on the editorial boards of international journals. His scientific interests relate to (i) the pathophysiology and treatment of the polycystic ovary syndrome; (ii) the endocrinology of obesity (sex hormones, the hypothalamic-pituitary-adrenal axis, and the endocannabinoid system).

Review	R Pasquali and others	Secondary PCOS	175:4	R158
--------	-----------------------	----------------	-------	------

Medicine (ESHRE/ASRM) (4), added specific sonographic findings of polycystic ovaries (defining the polycystic ovarian morphology – PCom) to those previously defined by the NIH conference and recommended that PCOS could be defined when at least two major features were present, whatever their combination, provided that all the other disorders responsible for androgen excess had been excluded. In a relatively short period of time, use of the Rotterdam's criteria has spread everywhere, although these criteria have led to a significant multiplication of the phenotypes, starting from the classic ones up to milder forms, without the presence of a hyperandrogenic state (5). Obviously, this has increased the nosological difficulties in diagnosing PCOS and its multiple phenotypes. For example, some disagreement still remains between gynecologists and endocrinologists on the use of these criteria, the first being more focused on PCom and ovarian dysfunction, whereas the second seem to concentrate more on hyperandrogenism and metabolic issues (6, 7). In fact, there is emerging evidence that metabolic features, including insulin resistance and compensatory hyperinsulinemia, glucose intolerance states and lipid abnormalities are much more associated with the classic phenotype of PCOS and are strictly related to the pattern of androgen excess (8, 9), as previously underlined by the document published in 2009 by the Androgen Excess and PCOS society (AEPCOS) society (5). Finally, in 2012, the Expert Panel following the NIH (USA) (10) suggested that a list of activities aimed at improving the understanding in the diagnosis and management of PCOS is warranted, including the need for specific biological and clinical markers using a phenotype-biological approach and the urgent need to expand the use of precise and accurate techniques to measure circulating androgens. It is expected that scientists and clinicians may be helped by this approach in characterizing classic vs nonclassic forms of PCOS and, eventually, their specific pathophysiological aspects.

Arguments in favor of the definition of secondary PCOS

Several recent studies have shown that extremely accurate and sensitive technologies in the detection and analysis of steroids, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) (8), have significantly improved the definition of the androgenemic blood pattern in women with PCOS (11, 12, 13, 14, 15, 16, 17, 18). These studies have clearly shown that a steroid profile, including not only testosterone blood levels but also androstenedione and the free androgen index (FAI), may

optimize the diagnosis of hyperandrogenemia in women with PCOS, therefore improving our understanding on the heterogeneity of its phenotypes. In fact, increasing evidence supports that androgen excess represents a constitutive biomarker of PCOS. As expected, an androgen profile rather than testosterone alone should be used, as documented by several recent studies (12, 13, 14, 15, 16, 18). A fundamental prerequisite is represented by the fact that if different androgens are needed to fully define hyperandrogenemia in these women, it could be that different mechanisms may be involved in regulating the enzyme activity involved in the androgen production rate or in their metabolic clearance rate (18). In addition, although there is a consensus that increased androgen production in PCOS women is mainly dependent on hyperactivity of the ovarian theca cells, some uncertainty still remains regarding the role of adrenals and extraglandular sites (8), such as the adipose tissue and the liver and possibly the skin cell, as they express the most crucial genes of steroidogenesis and also utilize sex steroid precursors of potent androgens (19, 20). This may have some relevance in explaining the presence of hirsutism in women without evidence for an altered blood androgen profile.

Since PCOS is a clinical entity diagnosed by exclusion, it is essential to acknowledge that all other main disorders responsible for androgen excess need to be excluded. The concept of secondary PCOS stems from the fact that there are diseases or disorders, some of which were included in the exclusion criteria cited above, with a well-defined etiology and pathophysiology, in which a direct responsibility in determining the excessive production of androgens and, possibly, the development of the different PCOS phenotypes is obvious or widely assumed. The presence of androgen excess, whatever the specific androgen increase, needs to be present in the secondary forms of PCOS, irrespective of the original cause. In addition, specific and well-defined causative factors, either endogenous or exogenous, need to be clearly recognized. Finally, the potential complete recovery of the hyperandrogenemic state as well as the remission of the PCOS phenotype should follow the removal of the cause, whatever it may be (a disease, a disorder or a drug). Accordingly, in most of these cases, the secondary PCOS could be defined as comorbidity.

Obesity and PCOS

Pathophysiological insights on the role of obesity in the development and clinical presentation of PCOS

The origin of PCOS is still largely unknown, and genetic and/or environmental origins of PCOS have been

Review	R Pasquali and others	Secondary PCOS	175:4	R159
--------	-----------------------	----------------	-------	------

postulated (1, 8, 9). It has been reported that the PCOS phenotype can be found from early infancy to puberty (21). Available studies do not support the concept that the common PCOS is due to a single-gene defect, although large association studies have suggested that a cluster of genes may increase the individual susceptibility to develop it (22, 23). Animal studies have also suggested that fetal exposure to androgen excess may contribute to development of the PCOS phenotype later in time (24, 25). These theories imply the potential direct role of intrauterine androgen excess in favoring the metabolic derangements, including hypertrophy of the visceral fat and associated insulin resistance coupled with compensatory hyperinsulinemia. Additional events that have been associated with an increased risk of developing PCOS are low birth weight and subsequent catch-up (8, 21).

The impact of overweight or obesity developed during pubertal years on the maturation of the hypothalamic–pituitary–gonadal (HPG) axis and the fertility processes deserves some attention (26). Intriguingly, the early exposure to inappropriate androgens may also be responsible for altered hypothalamic control of gonadotropin secretion and, in turn, of the ovarian function (26). In adolescent girls, excess body weight often precedes or manifests at the same time as the development of irregular menses and is often associated with increased testosterone (total and free) blood levels (27). In these girls, body mass index (BMI) may represent the best predictor of unbound testosterone levels, much more than luteinizing hormone (LH) and insulin levels, and the Tanner stage (27). Large population-based studies have also shown that half of oligomenorrheic girls may have significantly increased LH and androgen levels (28), although this finding still remains controversial, since other studies performed in healthy normal-weight girls with anovulatory regular menses showed that they were characterized by a significantly higher androgen levels in comparison with those with ovulatory menses (29). Intriguingly, longitudinal studies have shown that adolescent serum androgen levels may be preserved into adulthood and are associated with menstrual dysfunction, which suggests a potential risk to develop PCOS (30). A study performed in a large cohort women with PCOS, to investigate the relationship between the prevalence of PCOM and blood androgen levels with the women's body size at birth and the length of gestation, found that the PCOS group characterized by obesity and high testosterone had above-average birth weight and were born from overweight mothers, which suggests that intrauterine factors may in some way predispose to the development of PCOS (31). At variance, a

more recent large study did not support the hypothesis that maternal androgens, within the normal range for pregnancy, directly program PCOS in the offspring (32). In any case, an increase in body weight and fat during early adolescence may disrupt the HPG axis by neuroendocrine mechanisms both by involving suppression of gonadotropins independently of androgen excess and by altering diurnal neuroendocrine rhythms (33). In addition, excess body fat and obesity may also produce other specific negative effects on the sex steroid system. For example, an increased production rate of testosterone from androstenedione, through activation of adipocyte type 5 17β -hydroxysteroid dehydrogenase (possibly mediated in part by insulin), has been found (33). The role of enzyme dysregulation has been confirmed by the fact that its expression in the subcutaneous fat correlated with BMI values and decreased after weight loss (34, 35).

Although insulin excess has been proved to be responsible, at least in part, for ovarian androgen production rate in the theca cells (36), there is consistent evidence that the impact of obesity on androgen balance cannot be totally ascribed to insulin, since insulin resistance and compensatory hyperinsulinemia are present in about 25% of PCOS adolescents and in 50–70% of adults, based on clinical studies (37, 38), although the data obtained from euglycemic clamp studies support much higher rates (39). In any case, it seems that insulin resistance in PCOS is disproportionate to the degree of obesity; it should, however, be considered that it is invariably present in all obese women (39). Interestingly, it has been reported that ovulatory women with PCOS are less insulin-resistant than anovulatory PCOS ones (40). Obesity is known to increase androgen and insulin levels and, consequently, it is suggested that it may increase PCOS prevalence and exacerbate the clinical features of PCOS (41). Therefore, the available data support the concept that obesity *per se* may determine or reveal the PCOS phenotype from adolescence to adulthood in susceptible individuals.

An important aspect that needs to be emphasized is represented by the fact that an expansion of the visceral adipose tissue is believed to play an important role in the pathophysiology of PCOS. Increased visceral adiposity is very common in women with PCOS, is partly independent of the BMI values and may significantly contribute to the expression and severity of the PCOS phenotype (8). There is consistent evidence that in PCOS women, visceral fat may have an aberrant morphology and function, which is partly dependent on androgen excess. The main alterations are represented by the development of hypertrophic adipocytes and their relative inefficiency in responding to

Review	R Pasquali and others	Secondary PCOS	175:4	R160
--------	-----------------------	----------------	-------	------

the sympathetic system (42). These features appear to be different with respect to those observed in the visceral fat cells in the insulin resistance syndrome, which occurs at the level of adrenergic receptors (42). Other studies have shown that an increased catecholamine-induced lipolysis in women with PCOS may be due to unique alterations in the stoichiometric properties of the protein kinase A (PKA)–hormone sensitive lipase (HSL) complex, which could represent early and possibly primary lipolytic defect in these women (43). Interestingly, an alteration of the lipolytic catecholamine resistance can also be present in the subcutaneous fat, and could probably be attributed to a combination of decreased amounts of β 2-adrenergic receptors, other than to the regulatory II b component of both PKA and HSL systems (44). In addition, it has been reported that a reduced adipose tissue vascularization can be present, leading to a consequent hypoxia and, definitely, to the development of a local low-grade inflammation with an increased production of cytokines, chemokines and adipokines that are able to antagonize insulin functions linked with a decreased production of the natural insulin sensitizer adiponectin (45). Therefore, in this way, the development of local and systemic insulin resistance may develop over time. Although the cause of the abnormal structure and function of the adipose tissue in PCOS are still relatively unclear, there is convincing evidence supporting a role of androgen excess. First, androgens stimulate the hypertrophy of adipocytes by affecting the expression of enzymes and proteins involved in lipid and carbohydrate metabolism, in oxidative stress and in the differentiation of pre-adipocytes into mature adipocytes (42). Secondly, androgens regulate lipolysis, resulting in an increased release of free fatty acids (46). These and other still undefined effects of even modest androgen excess in women may explain the association between visceral fat enlargement and PCOS status in both adolescent and adult women. Consequently, it could be suggested that early visceral fat expansion, particularly during childhood and in adolescent age, may play a role in the development of PCOS.

Effect of weight loss on PCOS: lifestyle and bariatric surgery

Whether obesity plays a causative role or only represents a condition amplifying the development of the PCOS, particularly during adolescence, is at present still unclear. Undoubtedly, weight loss *per se* may improve PCOS to varying extents. In fact, a number of interventional studies with lifestyle modification with or without the

association of insulin sensitizers (particularly metformin) or weight lowering drugs uniformly demonstrated a significant improvement in many key features of PCOS, including androgen blood levels (47). Unfortunately, most of these studies are short, rarely exceeding 6 months (47), which represents their major limitation. In addition, a great inter-individual variability in the response to weight loss has been reported, and predictive factors are still largely under-evaluated (48, 49). However, it has been shown that when the objectives are well defined, patient empowerment can be increased, and the extent of weight loss can, therefore, be amplified (50).

A long-term retrospective study performed in a relatively large group of obese PCOS patients treated with a hypocaloric diet (1200–1400 kcal/day) followed by mild caloric restriction and physical activity, with careful reinforcement at the periodical checkups and with a follow-up period of 20.4 ± 12.5 months reported that, in spite of similar weight loss, 15.4% had a persistence of the full PCOS phenotype, 47.7% had a partial improvement (still hirsute, discordant recovery in androgen blood levels) and, finally, 36.9% completely recovered from all features of PCOS (51). Figure 1 depicts the major findings on androgen blood levels, and ovarian morphology in PCOS women who completely recovered from PCOS. Importantly, the study also found that high abdominal fatness (measured by waist circumference) and particularly baseline androstenedione blood levels ($P < 0.001$) predicted the worse outcome. Interestingly, an inverse relationship between androstenedione blood levels at baseline and the improvement periods has also been described in another study carried out in women with PCOS following a 6-month metformin treatment (52). These findings confirm that the response to weight loss in overweight/obese PCOS women is largely heterogeneous. However, they show that a subset of women with the classic PCOS phenotype may achieve full recovery. Unfortunately, very few studies are available on the efficacy of long-term lifestyle intervention, particularly in overweight or obese adolescent girls, in which the major interest is often represented by the improvement of menstruations and excessive androgens, rather than by infertility.

Circulating blood concentrations of androstenedione may be high or relatively normal in women with PCOS, often independent of testosterone values, which may reflect a different origin, production rate or metabolic processes (18). They may also refer to a subset of PCOS women with a primary ovarian functional hyperandrogenism, since overproduction of androstenedione from both the ovaries and the adrenals has been demonstrated (53).

Figure 1

Efficacy of long-term lifestyle intervention and weight loss in overweight-obese women with PCOS included in the “fully recovered subgroup” (see text). The complete disappearance of the criteria defining PCOS is shown.

However, this finding may also disclose a specific subset of women with PCOS, characterized by decreased aromatase activity in the ovarian tissues, as suggested by previous studies (54) and recent genetic findings on the activity of this enzyme in PCOS (55).

Studies on the effects of bariatric surgery in PCOS women with severe obesity represent convincing evidence on the potential recovery from the PCOS phenotype, provided adequate weight loss is achieved. A pioneer study from Escobar-Morreale and coworkers (56) found that in 12 women with massive obesity and PCOS followed-up for 12 ± 5 months after bariatric surgery, most of them displayed a complete remission of the PCOS phenotype. A recent meta-analysis (57), including 13 primary studies and involving 2130 female patients, provided additional information on the efficacy of bariatric surgery in obese PCOS women. The authors reported that the preoperative incidence of PCOS was 45.6%, which significantly decreased to 6.8% ($P < 0.001$) at the 12 month follow-up, parallel to the decrease in BMI from 46.3 to 34.2. Focusing on the criteria defining the PCOS phenotypes, the study found that 56.2% of patients reported preoperative menstrual irregularity, which significantly decreased to 7.7% ($P < 0.0001$). The incidence of hirsutism declined from 67.0% at baseline to 38.6% ($P = 0.03$) and that of infertility declined from 18.2 to 4.3% ($P = 0.0009$). Notably, some heterogeneity in the response was found, which was significant for all outcomes, except for infertility. These data

confirm the relevant effectiveness of sustained weight loss on the PCOS phenotype and suggest that a number of patients achieved complete recovery from it.

PCOS secondary to other endocrinopathies and other disorders in hormonal action and metabolism

It is currently accepted that many endocrine disorders with a well-defined etiology may develop clinical and biochemical features fully representative of the classic PCOS. An essential prerequisite is that all these disorders need to be ruled out in order to make the diagnosis of PCOS (2, 3, 4). In fact, these disorders may often present with signs and symptoms of androgen excess, ovarian dysfunction and PCOM. From the conceptual point of view, all patients with the combined presence of these specific features should be defined as having a secondary PCOS phenotype or PCOS-like syndrome. In most cases, this requires a careful clinical evaluation, combined with biochemical hormone measurement, specific functional tests, genetic profile and, finally, imaging studies.

There is a list of these disorders that can mimic PCOS, and, as reported above, they need to be excluded in a woman with a PCOS phenotype. Some of them have been cited above according to all the consensus criteria (3, 4, 5). Notably, all these diseases are characterized by a specific

Review	R Pasquali and others	Secondary PCOS	175:4	R162
--------	-----------------------	----------------	-------	------

etiology and pathophysiological derangements. However, there are other clinical entities characterized by androgen excess and, possibly, the PCOS phenotype. They include conditions due to glucocorticoid resistance or altered cortisol metabolism, acromegaly, severe hypothyroidism, hyperprolactinemia, severe insulin resistance disorders and some cases of type 1 diabetes as well as gestational diabetes (58, 59, 60, 61, 62). In accordance with the reasoning followed in this paper, it is important to point out that almost all these diseases can be treated, although not all can be completely cured, because of their genetic background and/or the incomplete clarification of pathophysiological mechanisms. There is clinical evidence that when these patients are healed or cured, the PCOS-like phenotype tends to completely disappear in most of them, which obviously confirms that PCOS represents a secondary disorder. In the following section, we enter into the merits of the heterogeneous entity represented by severe insulin-resistant states, a chapter in rapid expansion due to the important growth of the knowledge in the pathophysiological and genetic aspects.

Severe insulin-resistant syndrome and PCOS

In 1976, Kahn and coworkers (63) first proposed the severe insulin-resistant states in non-obese patients and classified them as “type A” or “type B,” respectively, based on the absence or presence of anti-insulin receptor antibodies. At around the same time, the term “HAIR-AN” (hyperandrogenism, insulin resistance and acanthosis nigricans) (64) was coined, identifying a specific combination of severe insulin resistance associated with a PCOS-like phenotype, and remained use for several years, although it intrinsically represented a generic description of severe IR in women. In the last decade, the increased understanding of the different forms of insulin resistance allowed a chance for a more detailed new classification of these conditions, now called severe insulin resistance syndrome (SSIR), based on clinical features and related molecular and genetic aspects (65). SSIR can be subcategorized by the type of defect that leads to severe insulin resistance, when noted. These defects include insulin receptor autoantibodies, mutations in the insulin receptor or in the signaling pathway downstream of the insulin receptor and, most frequently, a defect in the function or in the development of the adipose tissue, leading in turn to different patterns of lipodystrophies (65). There are several different lipodystrophic phenotypes, due to the partial or total loss of subcutaneous fat with a different distribution due to

the different etiology (66). Lipodystrophy is often, though not always, accompanied by metabolic derangements, including insulin resistance, diabetes mellitus, hepatic steatosis or steatohepatitis and dyslipidemia. Other manifestations of metabolic derangements can include polycystic ovarian syndrome (PCOS), acanthosis nigricans (due to severe insulin resistance) and eruptive xanthomas (due to severe hypertriglyceridemia) (67, 68). While congenital generalized forms of lipodystrophy are often diagnosed during childhood, some forms of partial lipodystrophies, particularly familial partial lipodystrophy, may have some similarity with the most common metabolic disorders managed by adult endocrinologists, and appear after pubarche. A recent consensus statement aimed at improving the detection of all types of lipodystrophies has recently been published (68). The fact that, in the past, women with the HAIR-AN syndrome were characterized by the classic PCOS phenotype strongly supported a role of insulin excess in determining all features of PCOS, chiefly androgen excess (Fig. 2). In fact, it is well known that insulin excess is able to stimulate theca cell steroidogenesis and reduces hepatic SHBG synthesis, thereby leading to hyperandrogenemia (8). This has been confirmed by a long list of studies showing a significant association between insulin and androgens, particularly testosterone and androstenedione (13, 14, 18). In addition, there is a general consensus that in most women with PCOS, a mild-to-moderate insulin resistance may be responsible for the high prevalence of the metabolic syndrome (39) and that insulin sensitizers, such as metformin, may improve total and free testosterone in these women (1, 8). Notably, it should also be recognized that there has been uncertainty as to whether insulin resistance in PCOS has unique characteristics, since androgen excess *per se* may explain the development of insulin resistance in the insulin-sensitive tissues, such as fat, muscles and liver (46). In any case, the role of severe insulin resistance and very high circulating insulin in determining the PCOS phenotype in patients with SSIR states is confirmed on both clinical and basic research studies (69). One aspect of the association between PCOS and SSIR is that, in the last two decades, it has become increasingly clear that the responsible genetic factors of PCOS linked to SSIR are quite specific, and completely different with respect to the susceptible genes of the common PCOS, as proposed by recent genome-wide association and linkage studies (22, 23). In fact, a combination of linkage analysis and candidate gene screening has led to the identification of a list of genes associated with congenital lipodystrophy, either generalized or partial, that encodes protein essential for normal fat tissue development and/or function in

Figure 2

Mechanisms linking severe insulin-resistant syndrome and PCOS.

humans. Based on what is currently known, it is possible to functionally categorize genes implicated in the pathogenesis of lipodystrophy as being primarily involved in: (i) the transcriptional regulation of adipocyte differentiation (PPARG, LMNA, Zinc metalloproteinase STE24 homolog-ZMPSTE24); (ii) fatty acid uptake by adipocytes (CAV1, PTRF); (iii) triacylglycerol (TAG) synthesis (AGPAT2) or (iv) lipid droplet formation (BSCL2, CIDED, PLIN1) (69). Recently, two new inherited forms of lipodystrophies due to mutation in polymerase delta 1 (*POLD1*) or hormone-sensitive lipase (*LIPE*) gene have been described (70, 71). The expanding genetic background responsible for the development of the different lipodystrophy phenotypes indirectly supports the primary role of exaggerated circulating insulin in the development of the forms of secondary PCOS in these patients.

In the most common condition from the clinical point of view, represented by a patient who asks for a medical consultation for features of PCOS, in addition to a thorough medical examination, it is also necessary to evaluate insulin blood levels. In the presence of severe insulin resistance and very high circulating insulin, it is mandatory to search for the presence of a lipodystrophic phenotype, particularly the partial ones. In most cases, it is mandatory to plan adequate clinical and laboratory investigations to make a correct diagnosis. Although no formal criteria for the biochemical diagnosis of SSIR exist, there is a general consensus to consider the following conditions indicating (i) a fasting insulin value above 20.9 µg/mL and/or a peak insulin value on glucose tolerance testing above 209 µg/mL when diabetes is absent and a BMI below 30 kg/m² is present; (ii) or an exogenous insulin requirement of

>3 units/kg per day when diabetes with absolute insulin deficiency is present with a BMI below 30 (65, 68). However, the biochemical diagnosis of SSIR becomes difficult in the presence of partial β-cell compensation and/or in BMI above 30 due to background obesity-related insulin resistance, glucotoxicity impairing islet function and mixtures of endogenous and exogenous insulin in the blood. In this condition, insulin resistance should be interpreted in the light of clinical history and physical signs. A physical sign always present in SSIR is acanthosis nigricans. Other physical signs of SSIR, although not always present, are unusual severe combined dyslipidemia (high triglycerides and low high-density lipoprotein-cholesterol levels) sometimes complicated by unusually severe hepatic steatosis and early-onset hypertension (65, 69).

The prevalence of PCOS secondary to SSIR has not been formally assessed, though referrals to our center in the last 10 years suggest a rate of approximately 1.5% among patients visiting the out-patient clinic for symptoms of PCOS (oligo-amenorrhea, hirsutism, infertility). Among 1200 patients with PCOS included in our database, we have extracted 97 patients with SSIR, defined according to the criteria reported above. Eighteen of these patients (18.6%) had a lipodystrophic phenotype (representing 1.5% of the entire cohort). Ten of these lipodystrophic patients proved to be affected by a genetically determined lipodystrophy, seven by a heterozygous missense mutation in *LMNA* gene, one by a heterozygous missense mutation in *PPARG* gene and two by a heterozygous missense mutation in *PLIN1* gene (72). Notably, all these patients had PCOS, according to the NIH criteria (3). The main complaint by each

Review	R Pasquali and others	Secondary PCOS	175:4	R164
--------	-----------------------	----------------	-------	------

Table 1 Clinical and biochemical characteristics of a group of patients with a genetically determined severe insulin-resistant states associate with a PCOS phenotype.

Genetics	Main complaint	Age (years)	BMI	AN	High Tg	Steatosis	Diabetes	Hypertension	CV events
LMNA									
Pt 1	Hirsutism	21	26	Y	Y	N	N	N	N
Pt 2	Secondary amenorrhea	15	25	Y	Y	Y	N	N	N
Pt 3	Primary amenorrhea	22	23	Y	Y	N	N	N	N
Pt 4	Hirsutism	35	22	Y	Y	N	N	N	N
Pt 5	Hirsutism	62	24	Y	Y	Y	N	Y	Y
Pt 6	Hirsutism	59	26	Y	Y	Y	N	Y	Y
Pt 7	Hirsutism	31	26	Y	Y	Y	N	N	N
PPARG									
Pt 1	Secondary amenorrhea	30	25	Y	Y	Y	Y	Y	N
PLIN1									
Pt 1	Hirsutism	34	32	Y	Y	Y	Y	Y	N
Pt 2	Secondary amenorrhea	21	23	Y	Y	Y	Y	N	N

AN, acanthosis nigricans; N, no; Tg, triglycerides; Y, yes.

patient and their clinical characteristics of are reported in Table 1. These findings emphasize the concept that the secondary PCOS to SSIR states is much more common than expected. The identification of these secondary phenotypes of PCOS is of importance also because these patients often benefit from tailored therapies, including specific dietary management as well as insulin sensitizers, particularly thiazolidinediones (pioglitazone) (69, 72), which, however, requires the presence of residual adipose depots to achieve metabolic benefit. Finally, in patients with lipodystrophy and low levels of serum leptin, administration of recombinant human leptin may be dramatically beneficial in improving glycemic control, dyslipidemia and lipid accumulation in the liver (73).

To conclude, the endocrinologists who deal with PCOS should be aware of the forms of PCOS secondary to SSIR as they are not so uncommon. Although genetics is useful to substantiate the diagnosis, the diagnosis of these forms remains predominantly clinical by the inspection of patients in their underwear with the search for clinical hallmarks, as well as for collateral clinical features.

Drug-induced PCOS

Antiepileptic drugs and the development of PCOS

An association between PCOS and epilepsy was first suggested in 1984 by Herzog and coworkers (74), who reported that 5 out of 20 women with temporal lobe epilepsy presented with PCOS, supporting a pathophysiological link between the two disorders. In 1993, Isojärvi and coworkers (75) provided the first scientific evidence that in women in the reproductive age treated with valproate (VPA) for epilepsy, most of them had cardinal features of

PCOS, such as PCOM and increased testosterone blood levels. The same findings were present, although to a lesser extent, in a subgroup of women treated with carbamazepine (CBZ). This study introduced the new concept that treatment with antiepileptic drugs, rather than epilepsy *per se*, may be responsible for the development of PCOS in these women. Subsequent studies (reviewed in references 76, 77, 78, 79), provided evidence that the prevalence of PCOS in women affected by primary generalized and localization-related epilepsy was higher than expected, in comparison with the data on the prevalence of PCOS itself in the general population (80). In any case, these studies focused on individual components of the PCOS phenotype reporting a high occurrence of menstrual dysfunctions or hyperandrogenemia, as well as PCOM. The same review articles (76, 77, 78, 79) focused on VPA-treated epileptic patients and found a specific association of VPA treatment with all features of PCOS, and particularly with its full phenotype, according the NIH criteria (3). These findings were reinforced in 2011, when Hu and coworkers (81) published a meta-analysis aimed at quantifying the association between VPA therapy and an increased risk of PCOS in women with epilepsy. This meta-analysis included only 11 prospective controlled studies involving 556 women with epilepsy treated with VPA, 593 women treated with other antiepileptic drugs, 120 women with untreated epilepsy and 329 healthy controls. The authors reported that the calculated incidence of PCOS in VPA-treated women was significantly higher than that in nontreated women ($P < 0.05$, OR 3.04, 95% CI: 2.09–4.43), but some difference in the results was detected according to different definitions or diagnostic criteria of PCOS. The raw incidence of PCOS in VPA-treated epileptic women was approximately 1.95-fold with respect to nontreated

Review	R Pasquali and others	Secondary PCOS	175:4	R165
--------	-----------------------	----------------	-------	------

patients. Collectively, the results of the meta-analysis as well as those of cross-sectional studies emphasized the fact that among the whole cohort of VPA-treated women investigated, the prevalence of PCOS was higher, with particular evidence for adolescent girls with respect to adult women (82).

Further studies have also found some relationship between other neurological disorders, such as bipolar psychosis, and higher prevalence rates of PCOS (76). The question whether other drugs are involved in the development of the PCOS-like phenotype in susceptible individual requires further attention. One study performed on a population-based controlled cohort reported that oxcarbazepin (OXC), similarly to VPA, was associated with a significant incidence of high testosterone blood levels, and a relatively high prevalence of PCOS (82). A more recent study including a small cohort of patients (83) reported that women treated with OXC had lower serum total and free testosterone blood levels and higher SHBG levels than the control subjects; however, they displayed increased levels of androstenedione and dehydroepiandrosterone sulfate and a relatively high prevalence of PCOS. A recent report (84) drew attention to the fact that second-generation antipsychotics (ziprasidone, quetiapine, aripiprazole, olanzapine and risperidone) are now generally accepted as an iatrogenic cause or aggravator of metabolic illnesses (85) and could be potential contributors to the development of the PCOS phenotype. Given the health implications in reproductive age women, the disproportionality signal between PCOS and atypical antipsychotics warrants further examination.

Pathophysiological aspects: from clinical research to animal models and *in vitro* experiments

There are very few studies on the endocrine correlates of epilepsy *per se* and on the mechanisms of antiepileptic drugs such as VPA. In fact, as reported above, it is well known that epilepsy itself is expected to have negative effects on the reproductive endocrine function because of the complex interaction between the HPG axis and the limbic cortex (76, 77). In addition, it has been shown that specific subtypes of epilepsy involving the medial temporal lobe regions may cause changes in sex hormone secretion (86). A primary involvement is an alteration of γ -aminobutyric acid neurons in the control of noradrenergic inputs to gonadotropin-releasing hormone (GNRH) neurons in regulating the pulsatile gonadotropin secretion and the ovulatory surge in the secretion of GNRH and luteinizing hormone (LH) (75).

This may explain the finding of an increased LH pulse, high levels of LH or an altered LH-to-FSH ratio that has been repeatedly observed in untreated epileptic women with regular menses, which supports the concept that the disease by itself may contribute to neuroendocrine and reproductive abnormalities (86). Further evidence from preclinical studies shows that the induction of temporolimbic seizures in animals was able to cause changes in reproductive hormone levels and also supports the potential combination of both neuroendocrine and ovarian disruption in the regulation of the hypothalamic pituitary axis as well as in the steroidogenetic theca pathways (87, 88). The negative impact of VPA may, therefore, be additional to the intrinsic neuroendocrine alterations of the different pattern of epilepsy, as described above, due to the specific mechanisms by which VPA may disrupt both neuroendocrine circuits and the ovarian theca cell function, leading in turn to the development of PCOS.

The development of a VPA-dependent increase in body weight is a further intriguing aspect that should be considered in defining the mechanisms responsible for the development of PCOS phenotype in these women. Several studies found that epileptic patients may present with insulin resistance and associated hyperinsulinemia (reviewed in references 76, 77, 78, 79). It is well known that in women with the classic PCOS phenotype, insulin excess may be responsible for an increased rate of androgen production by the ovarian theca cells (39). It could be suggested that the negative effects of VPA on body weight and insulin sensitivity may disclose, together with the aforementioned abnormalities in neuroendocrine circuits and an increase in the ovarian androgen production rate, the development of PCOS in susceptible individuals. Much more research is desirable in this area.

Few animal studies have been performed to investigate the potential effectiveness of VPA in determining the PCOS phenotype. Two studies were performed in rats (89) and rhesus monkeys (90), and failed to mimic the VPA-induced PCOS observed in women. It cannot be excluded that these animal models may be not so appropriate in modeling VPA-induced signs and symptoms associated with PCOS. Intriguingly, it could also be that the negative effects of VPA are not merely dependent on the drug by itself, but rather on the combined effect of the disease (epilepsy) and VPA administration. By contrast, very interesting data emerged from *in vitro* studies. In their first study on the topic, Nelson-DeGrave and coworkers (91) demonstrated that in long-term cultures of theca cells isolated from

follicles of normal cycling women and of those treated for 72 h with VPA (30–3000 micromoles), an increase in forskolin-stimulated steroidogenesis could be observed. In particular, the most pronounced effect of VPA on androgen biosynthesis was observed in the dose range of 300–3000 microns, which intriguingly represents the therapeutic levels in the treatment of epilepsy and bipolar disorder. This is the first biochemical evidence to support a direct link between VPA treatment and increased ovarian androgen biosynthesis. Further studies by the same research group (92) focused on determining whether VPA induces a PCOS-like genomic phenotype. They compared the gene expression profiles of untreated normal, VPA-treated normal and PCOS theca cells. They found that VPA- and PCOS-induced changes in gene expression-enhanced Akt/PKB signal transduction in human theca cells were similar, which indicates that common changes in gene expression in PCOS and VPA-treated normal theca cells may occur, therefore suggesting a mechanistic link for the development of PCOS features, including increased steroid synthesis and arrested follicle development in women receiving chronic VPA therapy.

Conclusions and future perspectives

This review summarizes the available evidence for the development of the concept that a secondary PCOS exists and that it may represent a comorbidity of other disorders, with specific pathophysiological mechanism that may differ with respect to those believed to favor the development of the classic PCOS phenotype. We believe that a specific area for much more research is that characterized by the association between obesity and PCOS. This association is largely variable across the world, although it is quite clear that the association between these two disorders is much less important in Europe with respect to North America. We have introduced the concept that early onset of obesity during childhood and adolescence may predispose to the development of PCOS, also because it is intrinsically associated with the development of androgen excess that may differ with respect to those involved for the classic PCOS phenotype. There are also studies supporting the concept that PCOS can completely recover after sustained weight loss. We believe that this may represent a new perspective from the clinical point of view requiring much more scientific interest and research activity. Another area of great interest is the potential responsibility of SSRI and PCOS. There is increasing evidence that genetic factors

may be responsible for the association between SSRI and other endocrine and metabolic issues, including PCOS. Clinicians should be much more involved in this expanding area. Finally, the frequent association between drugs used in the treatment of epilepsy (and other psychiatric disorders) and PCOS phenotype opens new perspectives on the role of neuroendocrine factors responsible for androgen excess as well as their role in ovarian steroidogenesis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Author contribution statement

The authors equally contributed in writing the manuscript.

References

- 1 Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK & Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4565–4592. (doi:10.1210/jc.2013-2350)
- 2 Stein IF & Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. *American Journal of Obstetrics and Gynecology* 1935 **29** 181. (doi:10.1016/S0002-9378(15)30642-6)
- 3 Zawadzki JK & Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In *Polycystic Ovary Syndrome*, pp 377–384. Eds A Dunaif, JR Givens, FP Haseltine & GR Merriam. Boston, MA, USA: Blackwell Scientific Publications, 1992.
- 4 The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* 2004 **19** 41–47.
- 5 Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE *et al.* The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertility and Sterility* 2009 **91** 456–488. (doi:10.1016/j.fertnstert.2008.06.035)
- 6 Cussons AJ, Stuckey BG, Walsh JP, Burke V & Norman RJ. Polycystic ovarian syndrome: marked differences between endocrinologists and gynaecologists in diagnosis and management. *Clinical Endocrinology* 2005 **62** 289–295. (doi:10.1111/cen.2005.62.issue-3)
- 7 Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R *et al.* European survey of diagnosis and management of the polycystic ovary syndrome: results of the ESE PCOS Special Interest Group's Questionnaire. *European Journal of Endocrinology* 2014 **171** 489–498. (doi:10.1530/EJE-14-0252)
- 8 Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R *et al.* The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *European Journal of Endocrinology* 2014 **171** P1–P29. (doi:10.1530/EJE-14-0253)

- 9 Dunaif A & Fauser BC. Renaming PCOS – a two state solution. *Journal of Clinical Endocrinology & Metabolism* 2013 **98** 4325–4328.
- 10 Evidence-based Methodology Workshop on PCOS (Final Report National Institute of Health. Evidence-based Methodology Workshop on Polycystic Ovary Syndrome, 3–5 December 2012. Executive summary at: <http://prevention.nih.gov/workshops/2012/pcos/resource-es.aspx>.
- 11 Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brzyski RG, Christman GM, Trussell JC, Krawetz SA, Snyder PJ *et al.* Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. *Journal of Clinical Endocrinology & Metabolism* 2010 **95** 5305–5313. (doi:10.1210/jc.2010-1123)
- 12 Stener-Victorin E, Holm G, Labrie F, Nilsson L, Janson PO & Ohlsson C. Are there any sensitive and specific sex steroid markers for polycystic ovary syndrome? *Journal of Clinical Endocrinology & Metabolism* 2010 **95** 810–819. (doi:10.1210/jc.2009-1908)
- 13 O'Reilly MW, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, Stewart PM, Tomlinson JW & Arlt W. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. *Journal of Clinical Endocrinology & Metabolism* 2014 **99** 1027–1036. (doi:10.1210/jc.2013-3399)
- 14 Lerchbaum E, Schwetz V, Rabe T, Giuliani A & Obermayer-Pietsch B. Hyperandrogenemia in polycystic ovary syndrome: exploration of the role of free testosterone and androstenedione in metabolic phenotype. *PLoS ONE* 2014 **9** e108263. (doi:10.1371/journal.pone.0108263)
- 15 Keefe CC, Goldman MM, Zhang K, Clarke N, Reitz RE & Welt CK. Simultaneous measurement of thirteen steroid hormones in women with polycystic ovary syndrome and control women using liquid chromatography-tandem mass spectrometry. *PLoS ONE* 2014 **9** e93805. (doi:10.1371/journal.pone.0093805)
- 16 Münzker J, Hofer D, Trummer C, Ulbing M, Harger A, Pieber T, Owen L, Keevil B, Brabant G, Lerchbaum E *et al.* Testosterone to dihydrotestosterone ratio as a new biomarker for an adverse metabolic phenotype in the polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 2015 **100** 653–660. (doi:10.1210/jc.2014-2523)
- 17 Fanelli F, Gambineri A, Belluomo I, Repaci A, Di Lallo VD, Di Dalmazi G, Mezzullo M, Prontera O, Cuomo G, Zanotti L *et al.* Androgen profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in healthy normal-weight ovulatory and anovulatory late adolescent and young women. *Journal of Clinical Endocrinology & Metabolism* 2013 **98** 3058–3067.
- 18 Pasquali R, Zanotti L, Fanelli F, Mezzullo M, Fazzini A, Morselli Labate AM, Repaci A, Ribichini D & Gambineri A. Defining hyperandrogenism in women 1 with Polycystic Ovary Syndrome: a challenging perspective. *Journal of Clinical Endocrinology & Metabolism* 2016 **101** 2013–2022. (doi:10.1210/jc.2015-4009)
- 19 Chen WC, Thiboutot DM & Zouboulis CC. Cutaneous androgen metabolism: basic research and clinical perspectives. *Journal of Investigative Dermatology* 2002 **119** 992–1007. (doi:10.1046/j.1523-1747.2002.00613.x)
- 20 Slominski A, Zbytek B, Nikolakis G, Manna PR, Skobowiat C, Zmijewski M, Li W, Janjetovic Z, Postlethwaite A, Zouboulis CC *et al.* Steroidogenesis in the skin: implications for local immune functions. *Journal of Steroid Biochemistry and Molecular Biology* 2013 **137** 107–123. (doi:10.1016/j.jsbmb.2013.02.006)
- 21 Welt CK & Carmina E. Clinical review: lifecycle of polycystic ovary syndrome (PCOS): from in utero to menopause. *Journal of Clinical Endocrinology & Metabolism* 2013 **98** 4629–4638. (doi:10.1210/jc.2013-2375)
- 22 Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J *et al.* Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nature Genetics* 2011 **43** 55–59. (doi:10.1038/ng.732)
- 23 Brower MA, Jones MR, Rotter JI, Krauss RM, Legro RS, Azziz R & Goodarzi MO. Further investigation in Europeans of susceptibility variants for polycystic ovary syndrome discovered in genome-wide association studies of Chinese individuals. *Journal of Clinical Endocrinology & Metabolism* 2015 **100** E182–E186. (doi:10.1210/jc.2014-2689)
- 24 Abbott DH, Nicol LE, Levine JE, Xu N, Goodarzi MO & Dumesic DA. Nonhuman primate models of polycystic ovary syndrome. *Molecular and Cellular Endocrinology* 2013 **37** 21–28. (doi:10.1016/j.mce.2013.01.013)
- 25 Dumesic DA, Goodarzi MO, Chazenbalk GD & Abbott DH. Intrauterine environment and polycystic ovary syndrome. *Seminars in Reproductive Medicine* 2014 **32** 159–165. (doi:10.1055/s-0034-1371087)
- 26 Rosenfield RL. Adolescent anovulation: maturational mechanisms and implications. *Journal of Clinical Endocrinology & Metabolism* 2013 **98** 3572–3583. (doi:10.1210/jc.2013-1770)
- 27 McCartney CR, Blank SK, Prendergast KA, Chhabra S, Eagleson CA, Helm KD, Yoo R, Chang RJ, Foster CM, Caprio S *et al.* Obesity and sex steroid changes across puberty: evidence for marked hyperandrogenemia in pre- and early pubertal obese girls. *Journal of Clinical Endocrinology & Metabolism* 2007 **92** 430–436.
- 28 van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasig RA, Koppenaal C & Schoemaker J. Predictive value of menstrual cycle pattern, body mass index, hormone levels and polycystic ovaries at age 15 years for oligo-amenorrhoea at age 18 years. *Human Reproduction* 2004 **19** 383–389. (doi:10.1093/humrep/deh079)
- 29 Fanelli F, Gambineri A, Belluomo I, Repaci A, Diana Di Lallo VD, Di Dalmazi G, Mezzullo M, Prontera O, Cuomo G, Zanotti L *et al.* Androgen profiling by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in healthy normal weight ovulatory and anovulatory late adolescent and young women. *Journal of Clinical Endocrinology & Metabolism* 2013 **98** 3058–3067. (doi:10.1210/jc.2013-1381)
- 30 Apter D & Vihko R. Endocrine determinants of fertility: serum androgen concentrations during follow-up of adolescents into the third decade of life. *Journal of Clinical Endocrinology & Metabolism* 1990 **71** 970–974.
- 31 Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI & Fraser RB. Fetal growth, length of gestation, and polycystic ovaries in adult life. *Lancet* 1997 **350** 1131–1135.
- 32 Hickey M, Sloboda DM, Atkinson HC, Doherty DA, Franks S, Norman RJ, Newnham JP & Hart R. The relationship between maternal and umbilical cord androgen levels and polycystic ovary syndrome in adolescence: a prospective cohort study. *Journal of Clinical Endocrinology & Metabolism* 2010 **94** 3714–3720. (doi:10.1210/jc.2009-0544)
- 33 Rosenfield RL, Mortensen M, Wroblewski K, Littlejohn E & Ehrmann DA. Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. *Human Reproduction* 2011 **26** 3138–3146. (doi:10.1093/humrep/der291)
- 34 Coviello AD, Legro RS & Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. *Journal of Clinical Endocrinology & Metabolism* 2006 **91** 492–497.
- 35 Rossi B, Sukalich S, Droz J, Griffin A, Cook S, Blumkin A, Guzick DS & Hoeger KM. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 2008 **93** 4780–4786. (doi:10.1210/jc.2008-1198)
- 36 Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N & Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as

Review	R Pasquali and others	Secondary PCOS	175:4	R168
--------	-----------------------	----------------	-------	------

- the signal transduction system. *Journal of Clinical Endocrinology & Metabolism* 1998 **83** 2001–2005. (doi:10.1210/jcem.83.6.4886)
- 37 Lewy VD, Danadian K, Witchel SF & Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. *Journal of Pediatrics* 2001 **138** 38–44. (doi:10.1067/mpd.2001.109603)
 - 38 Apter D, Büttow T, Laughlin GA & Yen SS. Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. *Journal of Clinical Endocrinology & Metabolism* 1995 **80** 2966–2973.
 - 39 Diamanti-Kandarakis E & Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocrine Reviews* 2012 **33** 981–1030. (doi:10.1210/er.2011-1034)
 - 40 Robinson S, Kiddy D, Gelding SV, Willis D, Niththyananthan R, Bush A, Johnston DG & Franks S. The relationship of insulin insensitivity to menstrual pattern in women with hyperandrogenism and polycystic ovaries. *Clinical Endocrinology* 1993 **39** 351–355. (doi:10.1111/j.1365-2265.1993.tb02376.x)
 - 41 Teede HJ, Joham AE, Paul E, Moran LJ, Loxton D, Jolley D & Lombard C. Longitudinal weight gain in women identified with polycystic ovary syndrome: results of an observational study in young women. *Obesity* 2013 **211** 526–532. (doi:10.1002/oby.20213)
 - 42 Villa J & Pratley R. Adipose tissue dysfunction in polycystic ovary syndrome. *Current Diabetes Reports* 2011 **11** 179–184. (doi:10.1007/s11892-011-0189-8)
 - 43 Ek I, Arner P, Rydén M, Holm C, Thörne A, Hoffstedt J & Wahrenberg H. A unique defect in the regulation of visceral fat cell lipolysis in the polycystic ovary syndrome as an early link to insulin resistance. *Diabetes* 2002 **51** 484–492. (doi:10.2337/diabetes.51.2.484)
 - 44 Faulds G, Rydén M, Ek I, Wahrenberg H & Arner P. Mechanisms behind lipolytic catecholamine resistance of subcutaneous fat cells in the polycystic ovarian syndrome. *Journal of Clinical Endocrinology & Metabolism* 2003 **88** 2269–2273.
 - 45 Repaci A, Gambineri A & Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. *Molecular and Cellular Endocrinology* 2011 **15** 30–41. (doi:10.1016/j.mce.2010.08.002)
 - 46 Corbould A. Effects of androgens on insulin action in women: is androgen excess a component of female metabolic syndrome? *Diabetes/Metabolism Research and Reviews* 2008 **24** 520–532. (doi:10.1002/dmrr.v24:7)
 - 47 Moran LJ, Pasquali R, Teede HJ, Hoeger KM & Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. *Fertility and Sterility* 2009 **921** 966–982. (doi:10.1016/j.fertnstert.2008.09.018)
 - 48 Moran LJ, Hutchison SK, Norman RJ & Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. *Cochrane Database of Systematic Review* 2011 **7** CD007506. (doi:10.1002/14651858.CD007506.pub3)
 - 49 Nicholson F, Rolland C, Broom J & Love J. Effectiveness of long-term (twelve months) nonsurgical weight loss interventions for obese women with polycystic ovary syndrome: a systematic review. *International Journal of Women's Health* 2010 **2** 393–399. (doi:10.2147/IJWH)
 - 50 Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A & Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Human Reproduction* 2003 **18** 1928–1932.
 - 51 Pasquali R, Gambineri A, Cavazza C, Ibarra Gasparini D, Ciampaglia W, Cognigni GE & Pagotto U. Heterogeneity in the responsiveness to long-term lifestyle intervention and predictability in obese women with polycystic ovary syndrome. *European Journal of Endocrinology* 2011 **164** 53–60. (doi:10.1530/EJE-10-0692)
 - 52 Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolini E & Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebocontrolled 6-month trial, followed by open, long-term clinical evaluation. *Journal of Clinical Endocrinology & Metabolism* 2000 **85** 139–146.
 - 53 Pasquali R, Patton L, Pocognoli P, Cognigni GE & Gambineri A. 17-Hydroxyprogesterone responses to gonadotropin-releasing hormone disclose distinct phenotypes of functional ovarian hyperandrogenism and polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 2007 **92** 4208–4217.
 - 54 Loughlin T, Cunningham SK, Culliton M, Smyth PP, Meagher DJ & McKenna TJ. Altered androstenedione and estrone dynamics associated with abnormal hormonal profiles in amenorrheic subjects with weight loss or obesity. *Fertility and Sterility* 1985 **43** 720–725. (doi:10.1016/S0015-0282(16)48554-7)
 - 55 Xita N, Georgiou I, Lazaros L, Psafaki V, Kolios G & Tsatsoulis A. The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype. *European Journal of Endocrinology* 2008 **158** 861–865. (doi:10.1530/EJE-07-0905)
 - 56 Escobar-Morreale HF, Botella-Carretero JI, Alvarez-Blasco F, Sancho J & San Millán JL. The polycystic ovary syndrome associated with morbid obesity may resolve after weight loss induced by bariatric surgery. *Journal of Clinical Endocrinology & Metabolism* 2005 **90** 6364–6369.
 - 57 Skubleny D, Switzer NJ, Gill RS, Dykstra M, Shi X, Sagle MA, de Gara C, Birch DW & Karmali S. The impact of bariatric surgery on Polycystic Ovary Syndrome: a systematic review and meta-analysis. *Obesity Surgery* 2016 **26** 169–176. (doi:10.1007/s11695-015-1902-5)
 - 58 Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C & Jacobs HS. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. *Human Reproduction* 1995 **10** 2107–2111.
 - 59 Kaltsas GA, Isidori AM, Besser GM & Grossman AB. Secondary forms of polycystic ovary syndrome. *Trends in Endocrinology and Metabolism* 2004 **15** 204–210. (doi:10.1016/j.tem.2004.05.009)
 - 60 Kelestimur F, Unluhizarci K, Baybuga H, Atmaca H, Bayram F & Sahin Y. Prevalence of polycystic ovarian changes and polycystic ovary syndrome in premenopausal women with treated type 2 diabetes mellitus. *Fertility and Sterility* 2006 **86** 405–410. (doi:10.1016/j.fertnstert.2006.01.019)
 - 61 Bizzarri C, Benevento D, Ravà L, Patera IP, Schiaffini R, Ciampalini P, Giannone G & Cappa M. Ovarian hyperandrogenism in adolescents and young women with type I diabetes is primarily related to birth weight and body mass index. *Fertility and Sterility* 2011 **96** 1497–1502. (doi:10.1016/j.fertnstert.2011.09.023)
 - 62 Alexandraki KI & Kaltsas GA. Endocrinopathies and other disorders inducing a polycystic ovary syndrome phenotype. *Frontiers in Hormone Research* 2013 **40** 142–157. (doi:10.1159/000341846)
 - 63 Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM & Roth J. The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *New England Journal of Medicine* 1976 **294** 739–745. (doi:10.1056/NEJM197604012941401)
 - 64 Barbieri RL & Ryan KJ. Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. *American Journal of Obstetric and Gynecology* 1983 **147** 90–101. (doi:10.1016/0002-9378(83)90091-1)
 - 65 Semple RK, Savage DB, Cochran EK, Gorden P & O'Rahilly S. Genetic syndromes of severe insulin resistance. *Endocrine Reviews* 2011 **32** 498–514. (doi:10.1210/er.2010-0020)
 - 66 Chan JL & Oral EA. Clinical classification and treatment of congenital and acquired lipodystrophy. *Endocrine Practice* 2010 **16** 310–323. (doi:10.4158/EP09154.RA)
 - 67 Garg A. Lipodystrophies: genetic and acquired body fat disorders. *Journal of Clinical Endocrinology & Metabolism* 2011 **96** 3313–3325. (doi:10.1210/jc.2011-1159)
 - 68 Handelsman Y, Oral EA, Bloomgarden ZT, Brown RJ, Chan JL, Einhorn D, Garber AJ, Garg A, Garvey WT, Grunberger G *et al.* The clinical approach to the detection of lipodystrophy – an AACE consensus statement. *Endocrine Practice* 2013 **19** 107–116. (doi:10.4158/endorp.19.1.v767575m65p5m9r06)

- 69 Semple RK. EJE PRIZE 2015: how does insulin resistance arise, and how does it cause disease? Human genetic lessons. *European Journal of Endocrinology* 2016 **174** R209–R223. (doi:10.1530/EJE-15-1131)
- 70 Weedon MN, Ellard S, Prindle MJ, Caswell R, Allen HL, Oram R, Godbole K, Yajnik CS, Sbraccia P, Novelli G *et al.* An in-frame deletion at the polymerase active site of POLD1 causes a multisystem disorder with lipodystrophy. *Nature Genetics* 2013 **45** 947–950. (doi:10.1038/ng.2670)
- 71 Farhan SM, Robinson JF, McIntyre AD, Marrosu MG, Ticca AF, Loddio S, Carboni N, Brancati F & Hegele RA. A novel LIPE nonsense mutation found using exome sequencing in siblings with late-onset familial partial lipodystrophy. *Canadian Journal of Cardiology* 2014 **30** 1649–1654. (doi:10.1016/j.cjca.2014.09.007)
- 72 Gambineri A, Semple RK, Forlani G, Genghini S, Grassi I, Hyden CS, Pagotto U, O'Rahilly S & Pasquali R. Monogenic polycystic ovary syndrome due to a mutation in the lamin A/C gene is sensitive to thiazolidinediones but not to metformin. *European Journal of Endocrinology* 2008 **159** 347–353. (doi:10.1530/EJE-08-0272)
- 73 Tchang BG, Shukla AP & Aronne LJ. Metreleptin and generalized lipodystrophy and evolving therapeutic perspectives. *Expert Opinion on Biological Therapy* 2015 **15** 1061–1075. (doi:10.1517/14712598.2015.1052789)
- 74 Herzog AG, Seibel MM, Schomer D, Vaitukaitis J & Geschwind N. Temporal lobe epilepsy: an extrahypothalamic pathogenesis for polycystic ovarian syndrome? *Neurology* 1984 **34** 1389–1393. (doi:10.1212/WNL.34.10.1389)
- 75 Isojärvi JI, Laatikainen TJ, Pakarinen AJ, Juntunen KT & Myllylä VV. Polycystic ovaries and hyperandrogenism in women taking valproate for epilepsy. *New England Journal of Medicine* 1993 **329** 1383–1388.
- 76 Verrotti A, D'Egidio C, Mohn A, Coppola G, Parisi P & Chiarelli F. Antiepileptic drugs, sex hormones and PCOS. *Epilepsia* 2011 **52** 199–211. (doi:10.1111/j.1528-1167.2011.03310.x)
- 77 Bilo L & Meo R. Polycystic ovary syndrome in women using valproate: a review. *Gynecological Endocrinology* 2008 **24** 562–570. (doi:10.1080/09513590802288259)
- 78 de Vries L, Karasik A, Landau Z, Phillip M, Kiviti S & Goldberg-Stern H. Endocrine effects of valproate in adolescent girls with epilepsy. *Epilepsia* 2007 **48** 470–477. (doi:10.1111/epi.2007.48.issue-3)
- 79 Bilo L & Meo R. Epilepsy and polycystic ovary syndrome: where is the link? *Neurological Science* 2006 **26** 221–230. (doi:10.1007/s10072-006-0675-y)
- 80 Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED & Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *Journal of Clinical Endocrinology & Metabolism* 1999 **84** 4006–4011.
- 81 Hu X, Wang J, Dong W, Fang Q, Hu L & Liu C. A meta-analysis of polycystic ovary syndrome in women taking valproate for epilepsy. *Epilepsy Research* 2011 **97** 73–82. (doi:10.1016/j.epilepsyres.2011.07.006)
- 82 Mikkonen K, Vainionpää LK, Pakarinen AJ, Knip M, Järvelä IY, Tapanainen JS & Isojärvi JI. Long-term reproductive endocrine health in young women with epilepsy during puberty. *Neurology* 2004 **62** 445–450. (doi:10.1212/01.WNL.0000106942.35533.62)
- 83 Löfgren E, Tapanainen JS, Koivunen R, Pakarinen A & Isojärvi JI. Effects of carbamazepine and oxcarbazepine on the reproductive endocrine function in women with epilepsy. *Epilepsia* 2006 **47** 1441–1446.
- 84 Yum SK, Kim T & Hwang MY. Polycystic ovaries is a disproportionate signal in pharmacovigilance data mining of second generation antipsychotics. *Schizophrenia Research* 2014 **158** 275–276. (doi:10.1016/j.schres.2014.06.003)
- 85 Meyer JM & Nasrallah HA Eds. *Medical Illness and Schizophrenia*, 2nd edn. Washington, DC, USA: American Psychiatric Publishing, 2009.
- 86 Herzog AG & Friedman MN. Menstrual cycle interval and ovulation in women with localization-related epilepsy. *Neurology* 2001 **57** 2133–2135.
- 87 Scharfman HE, Malthankar-Phatak GH, Friedman D, Pearce P, McCloskey DP, Harden CL & MacLusky NJ. A rat model of epilepsy in women: a tool to study physiological interactions between endocrine systems and seizures. *Endocrinology* 2009 **150** 4437–4442.
- 88 Edwards HE, Burnham WM, Ng MM, Asa S & MacLusky NJ. Limbic seizures alter reproductive function in the female rat. *Epilepsia* 1999 **40** 1370–1377.
- 89 Lagace DC & Nachtigal MW. Valproic acid fails to induce polycystic ovary syndrome in female rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 2003 **27** 587–594.
- 90 Ferin M, Morrell M, Xiao E, Kochan L, Qian F, Wright T & Sauer M. Endocrine and metabolic responses to long-term monotherapy with the antiepileptic drug valproate in the normally cycling rhesus monkey. *Journal of Clinical Endocrinology & Metabolism* 2003 **88** 2908–2915.
- 91 Nelson-DeGrave VL, Wickenheisser JK, Cockrell JE, Wood JR, Legro RS, Strauss JF 3rd & McAllister JM. Valproate potentiates androgen biosynthesis in human ovarian theca cells. *Endocrinology* 2004 **145** 799–808. (doi:10.1210/en.2003-0940)
- 92 Wood JR, Nelson-Degrave VL, Jansen E, McAllister JM, Mosselman S & Strauss JF 3rd. Valproate-induced alterations in human theca cell gene expression: clues to the association between valproate use and metabolic side effects. *Physiological Genomics* 2005 **20** 233–243. (doi:10.1152/physiolgenomics.00193.2004)

Received 27 April 2016

Accepted 11 May 2016