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REVIEW

New perspectives on the definition and management of polycystic ovary syndrome

R. Pasquali[1,3] · **A. Gambineri**[2]

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Abstract

Background There is a growing debate on the opportunity of improving the understanding in the diagnosis and management of polycystic ovary syndrome (PCOS).

Objective This review article summarizes recent research related to the definition of polycystic ovary syndrome (PCOS).

Methods Review of the recent literature on the topic.

Results New ideas on the definition of hyperandrogenism, based on new scientific data and clinical perspectives are presented. (i) In fact, recent studies have pointed out the need to improve the concept of androgen excess by using a larger androgen profile, rather than simply measuring the testosterone blood levels. (ii) Due to the poor correlation between androgen blood levels and the degree of hirsutism, it is proposed that the definition of hyperandrogenism should be based on the presence of blood androgen excess and hirsutism, considered separately, because their pathophysiological mechanisms may differ according to the different phenotypes of PCOS. (iii) The potential role of obesity in favoring the development of PCOS during adolescence is also discussed and the concept of "PCOS secondary to obesity" is developed. (iv) Finally, the need for greater appropriateness in the evaluation of possible coexistence is highlighted, in patients with PCOS who have fasting or glucose-stimulated very high insulin levels, or severe insulin-resistant states.

Conclusions Based on what was discussed in this review, we believe that there are margins for modifying some of the current criteria that define the various PCOS phenotypes.

Keywords Polycystic ovary syndrome · Hyperandrogenemia · Hirsutism · Obesity · Severe insulin resistance syndrome

Introduction

Polycystic ovary syndrome (PCOS), the most common endocrinopathy of women in reproductive age, is largely heterogeneous, including both classic and non-classic forms, characterized by major differences in clinical presentation and pathophysiological aspects [1]. For many decades, the definition of this syndrome was clinically based on the well-known work by Stein and Leventhal [2]. In 1990, an expert conference sponsored by the National Institute of Health (NIH) established that the major criteria to diagnose PCOS included clinical hyperandrogenism (determined by the presence of hirsutism) and/or blood total testosterone (TT) excess associated with ovarian dysfunction (OD) (defined by the presence of oligo-amenorrhea and chronic anovulation), provided that all other well-known disorders characterized by androgen excess are excluded a priori [3]. In 2003, the expert conference in Rotterdam added a third criterion, based on the ovarian morphological appearance by ultrasonography (defined as polycystic ovarian morphology—PCOm) [4]. Intriguingly, the Rotterdam panel decided that PCOS could be defined when at least two major features were present, whatever their combination. Therefore, the Rotterdam criteria identified the OD-PCOm phenotype, which was suggested to be a non-hyperandrogenic entity of PCOS. In a short period of time, the Rotterdam criteria

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✉ R. Pasquali
renato.pasquali@unibo.it

[1] University Alma Mater Studiorum of Bologna, Bologna, Italy

[2] Unit of Endocrinology, S. Orsola-Malpighi Hospital, University Alma Mater Studiorum of Bologna, Bologna, Italy

[3] Via Santo Stefano 38, 40125 Bologna, Italy

became very popular, despite the number of possible different phenotypes being greatly expanded, from classic to milder forms, characterized by the absence of a hyperandrogenic state. A few years later, the Androgen Excess and PCOS society (AEPCOS) [5] emphasized the relevance of hyperandrogenism as a primary criterion to define PCOS. On the other hand, most of the studies published over the past 15 years did not take into account the various phenotypes, but only reported the definition of PCOS as a single entity. However, there is scientific evidence showing that metabolic features, chiefly insulin resistance and compensatory hyperinsulinemia, glucose intolerance states and lipid abnormalities are predominantly associated with the classic hyperandrogenic phenotype of PCOS [6], although some studies had a different perspective, partly conditioned by the phenotypic criteria used to diagnose PCOS [7–9]. The fact that PCOS can also be diagnosed in the absence of hyperandrogenemia can obviously lead to different interpretations by medical specialists. This has been confirmed by some studies reporting some disagreement between gynecologists and endocrinologists in Australia [10] and in Europe [11] on the relevance of these criteria, the earliest focusing on the OD-PCOm criterion, while the second paid more attention to the presence of hyperandrogenism. Over time, the need to improve the evaluation of the diagnostic criteria of PCOS as well as the clinical and therapeutic management of each patient became increasingly evident. In 2012, a document from the Expert Panel at the NIH (US) suggested a list of actions aimed at improving the understanding in the diagnosis and management of PCOS, including (1) the need for specific biological and clinical markers using a phenotype–biological approach, (2) the opportunity to expand the use of more precise and accurate techniques to measure circulating androgens, (3) a scientific effort to better define the causes, predictors, and long-term metabolic consequences of PCOS and, finally, (4) how to improve optimal prevention and treatment strategies based on individual needs and the specific phenotypes [12–14].

In this review article, we will try to update recent research related to the aforementioned areas and propose new ideas on how the definition of the different criteria, specifically hyperandrogenism, could be modified according to new scientific data and clinical perspectives and on the potential impact of obesity, particularly during adolescence, and the severe insulin-resistant states in the development of PCOS.

Should the concept of hyperandrogenism in PCOS be revisited?

Currently, the definition of hyperandrogenism in PCOS is based on the presence of hyperandrogenemia [namely, TT and/or free testosterone (FT)] and/or hirsutism. However, there is evidence that approximately 50–60% of women with PCOS have high blood total TT or FT levels, respectively, and that hirsutism is present in less than three-quarters of these patients [15]. Though it can be accepted that TT and FT are major components of the androgen pool in women, at present it should also be accepted that the restriction of TT measurement alone could represent a simplistic limitation, due to the fact that a large number of weak or more potent androgens are not considered and, particularly in females, most of the available immunoassays for androgen measurement are decidedly unsatisfactory [6, 16–19]. The use of more advanced technologies capable of measuring a variety of steroids could in fact reveal that androgen excess in women with PCOS may not be restricted to the contribution of the ovaries, but also that of adrenal glands, the adipose tissue and, possibly, the skin, in which there is a rich steroidogenesis, with specific mechanisms of regulation of both the synthesis and metabolism of these hormones. For example, although androstenedione (Δ4-A) may be used to define hyperandrogenemia in PCOS women, neither the NIH [3] nor the Rotterdam [4] conference included it in the panel of androgens useful for the diagnostic panel. On the other hand, it is well known that Δ4-A can be equally synthesized in the adrenal cortex and in ovarian theca cells [20]. In the past, increased Δ4-A from theca cells of polycystic ovaries along with higher levels in the follicular fluid of PCOS patients have been detected [21] and, subsequently, it was described that a subset of women with PCOS had an isolated increase in Δ4A [22].

It is well known that the common immunoenzymatic assays used to measure are characterized by poor sensitivity and specificity [11] and this may explain the low relevance given to Δ4-A levels in the evaluation of hyperandrogenemia in women with PCOS. At variance, very recent studies have clearly defined that Δ4-A is often higher than normal in most women with PCOS and that the combination of TT, Δ4-A, and the free androgen index (FAI) better predicts any adverse metabolic risk, chiefly insulin resistance and glucose intolerance states [23, 24]. In addition, these studies [23, 24] confirmed that the combined use of this triad of parameters can accurately define hyperandrogenemia in the majority of PCOS patients, partially independent of the association with hirsutism [23–26]. These studies have also shown that high Δ4-A levels were significantly associated with higher levels of adrenal steroids and, in addition, significantly contributed to worsened glucose tolerance, fasting insulin levels, and the homeostasis model assessment for insulin resistance (HOMA-IR) [23, 24]. By contrast, high FAI values were associated with lower SHBG levels, higher fasting glucose, a worse insulin resistance state and, finally, low HDL-cholesterol [24]. Overall, these data strongly support that the combined use of total TT, Δ4-A, and FAI can accurately define hyperandrogenemia in the majority of PCOS

patients and that their blood concentrations tend to increase with the severity of the phenotype and, ultimately, favor the categorization of the heterogeneous presentation of hyperandrogenemia in PCOS. At variance, a recent study reported that blood levels of free testosterone (FT) alone, measured by equilibrium dialysis, provided that TT is assayed by the gold standard methodology, could be safely used to identify hyperandrogenemic PCOS women and their individual metabolic risk [27].

Additional confirmation on the potential role of other androgens in the pathophysiology of PCOS has recently been provided. In fact, it has been reported that in women with PCOS, particularly during adolescence, a systemic upregulation of 5α-reductase activity, which activates the conversion of TT (and probably Δ4-A) to the most potent androgen, 5α-dihydrotestosterone (DHT), may be detected [28]. Accordingly, a significant link between the TT/DHT ratio values and an adverse metabolic phenotype has been found in PCOS patients [29]. In addition, there are new data supporting an intriguing role of adrenals in determining the hyperandrogenemic state in these patients [30]. In fact, recent studies have highlighted the importance of the 11-oxygenated C19 steroid pathway to androgen metabolism in humans, a pool of hormones that was thought to possess only minimal activity. For example, it has been known for a long period of time that the adrenal is capable of converting D4A to 11β-hydroxyandrostenedione (11OHA4), catalyzed by the 11β-hydroxylase activity of the cytochrome P450 enzyme cytochrome P450 11β-hydroxylase [31]. It has also been shown that, using appropriate analytical techniques, 11OHA4 is a major product of adrenal steroidogenesis [32] and that it can generate two additional steroids such as 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone [33], which bind and activate the androgen receptor. Very recently, a study by 0'Reilly and coworkers [34], performed in a large cohort of women with PCOS, provided very good evidence that, apart from elevated blood levels of TT, D4A and DHEA, serum levels of adrenal 11-oxygenated androgens, such as 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone, and 11-ketotestosterone, were significantly higher than in control subjects, as was the urinary 11-oxygenated androgen metabolite 11β-hydroxyandrosterone, without any difference in their concentrations between normal-weight and obese PCOS. Finally, they found that the blood levels of 11β-hydroxyandrostenedione and 11-ketoandrostenedione correlated significantly with markers of insulin resistance, which potentially implies a cause–effect relationship. Overall, these new data strongly support that 11-oxygenated androgens may play a potentially important role in defining the androgenic status in women with PCOS and that their activity can be supported by the close correlation to markers of metabolic risk. If these findings are confirmed by additional studies, it could be of interest to investigate whether they may disclose different hyperandrogenic patterns according to the different phenotype of PCOS.

To summarize, the new scientific studies strongly support the concept that an androgen profile is much more appropriate to define hyperandrogenemia in women with PCOS and that both the ovaries as well as the adrenals may participate in determining this hyperandrogenic status. Whether this may differ according to the various PCOS phenotypes may represent an exciting challenge for future research.

The ovarian dysfunction (oligo-amenorrhea) PCOm phenotype can be associated with androgen excess: hyperandrogenic vs non-hyperandrogenic sub-phenotypes

According to the Rotterdam criteria [4], the combination of ovarian dysfunction (that is oligo-amenorrhea and chronic anovulation) and PCOm represents a specific phenotype of PCOS, characterized by the absence of hyperandrogenism, defined by increased TT blood levels alone and absence of hirsutism. Intriguingly, this phenotype is often difficult to define, particularly in relation to the definition of PCOm, which is largely related to the operator and to the available technique, as clearly reported in some recent studies [35–38]. In addition, as noted above, the definition of hyperandrogenemia may be somewhat restrictive if it is based solely on TT measurement. Therefore, some authors question that the definition (according to the Rotterdam criteria) of the non-hyperandrogenic forms should be PCOS, due to the fact that hyperandrogenism is considered the cornerstone of the syndrome, according to the AEPCOS society [5]. Whether women with the OD-PCOm phenotype may be defined as normoandrogenemic was first investigated by Dewailly and coworkers 10 years ago [39]. They reported that a subset of patients with the OD-PCOm phenotype, without hirsutism and normal TT blood levels according to the reference values, nevertheless had significantly higher TT and D4A (measured by an ELISA assay) than control non-PCOS women, though their blood levels were in the reference normal range. These authors suggested that the absence of overt hyperandrogenemia might simply represent a false-negative finding in the subset of PCOS women with the OD-PCOm phenotype. Accordingly, we recently demonstrated that, using more sensitive LC–MS/MS, more than 50% of patients with the OD-PCOm phenotype displayed a specific pattern of steroid abnormalities, characterized by elevated D4A and/or FAI, but normal TT blood levels [24]. This may imply that a specific hyperandrogenemic profile may characterize most PCOS women with the OD-PCOm

phenotype, although these findings must necessarily be confirmed by further studies.

At present, we suggest that the non-hyperandrogenic phenotype OD-PCOm may represent a PCOS-like mild form, presumably characterized by specific pathophysiologic mechanisms. On the other hand, these findings further support the need to move toward a widespread use of sensitive analytical methodologies in the measurement of androgen blood levels and to expand the investigation to an androgen profile, rather than relying on TT alone.

The definition of hyperandrogenism revisited: hirsutism and hyperandrogenemia may not be synonymous

Hirsutism has been considered a clinical biomarker of hyperandrogenism [3–5]. Although hirsutism is considered to reflect hormone imbalances, a subset of women with PCOS do not manifest any androgen imbalance, based on TT blood levels. Undoubtedly, the major problem is that worldwide the evaluation of hirsutism is performed by visual scoring, which has been shown to be potentially subject to inter-observer variability [40]. The modified Ferriman–Gallwey score (mFG) proposed by Hatch et al. [41] for many years has become the gold standard for the evaluation of hirsutism. Unfortunately, although objective methods for assessing hair growth (such as photographic evaluations, weighing of shaved or plucked hairs, and microscopic measurements) have been proposed, nonetheless they are complex, inconvenient and costly, all aspects that limit their widespread clinical use [42]. Undoubtedly, an important aspect that should be revisited is represented by the cutoff value of the mFG score used for the diagnosis of hirsutism, which was selected by Hatch et al. [41] on the basis that they found that only approximately 4% of the reproductive age female population studied by Ferriman and Gallwey in the UK scored 8 or above for the combined nine body areas they termed 'hormonal' [43]. Finally, there are two potentially relevant aspects that should be considered. First, the cutoff value of the mFG score should ideally be established for the population to which it is applied, according to the variability among ethnicities. Second, it should be considered that the mFG scoring system provides estimation of the total amount of body hair, and not that related to regional distribution on the body, especially the face and trunk, where the presence of hirsutism can have an extremely negative impact on the patient, especially during the adolescent age [44]. Undoubtedly, this is an area of considerable interest that deserves much more attention [45].

It is well known that the growth of sexual hair is mainly dependent on the presence of androgens. The specific differentiation patterns still remain unexplained, suggesting that androgens may have paradoxically different effects on human hair follicles depending on their body site [46]. In reality, hirsutism reflects the interaction between circulating and local (cutaneous) androgen concentrations as well as the sensitivity of the hair follicle to androgens. The hair follicle response to circulating androgens varies considerably within and between individuals, which may explain why some women with blood androgen excess do not manifest clinically relevant hirsutism [45]. In our view, this implies the possibility that hirsutism may represent the clinical manifestation of excess androgen due to heterogeneous and specific alterations of steroidogenesis, some of which may be located predominantly at the level of the skin more than at the level of the ovaries or the adrenal glands. In fact, one must consider that skin cells contain the entire biochemical apparatus necessary for the production of steroids (glucocorticoids, androgens, and estrogens) either from precursors of systemic origin or, alternatively, through the conversion of cholesterol to pregnenolone and its subsequent transformation to biologically active steroids. The cutaneous steroidogenic system can also have systemic effects, which are emphasized by a significant skin contribution to circulating blood androgens [47].

Although hirsutism is a marker of excessive androgen action at the pilosebaceous unit, it has been repeatedly shown that the severity of hirsutism poorly correlates with the severity of androgen excess or does not correlate at all [45, 46, 48, 49] (Fig. 1). This may suggest that hirsutism not only reflects circulating androgen levels, but is also influenced by the peripheral metabolism of androgens and the sensitivity of the skin target elements to androgens [50]. In addition, there is evidence that insulin resistance and associated hyperinsulinemia may contribute to the development of hirsutism [51]. It is possible that the poor or absent correlation between the mFG score and blood androgen levels may depend on the significant variability among direct immunoassays used for serum androgen measurements. On the other hand, we have recently confirmed that a poor or absent correlation between the mFG score and androgen blood levels is detected when the LC–MS/MS technology is applied [24]. Much more research should be performed to establish whether circulating androgen levels may or may not reflect local androgen concentrations at the pilosebaceous unit [52] and whether cutaneous androgen effects also depend on the expression of the androgen receptor in the pilosebaceous unit, which has all the necessary tools to utilize sex steroid precursors for the transformation to more potent sex hormones other than the ability to directly produce active androgens [47, 52]. In addition, there are theoretical possibilities that the heterogeneity of clinical (hirsutism) and biochemical (hyperandrogenemia) presentation in different PCOS phenotypes may result from activation/deactivation of specific enzymatic pathways and that the skin is an

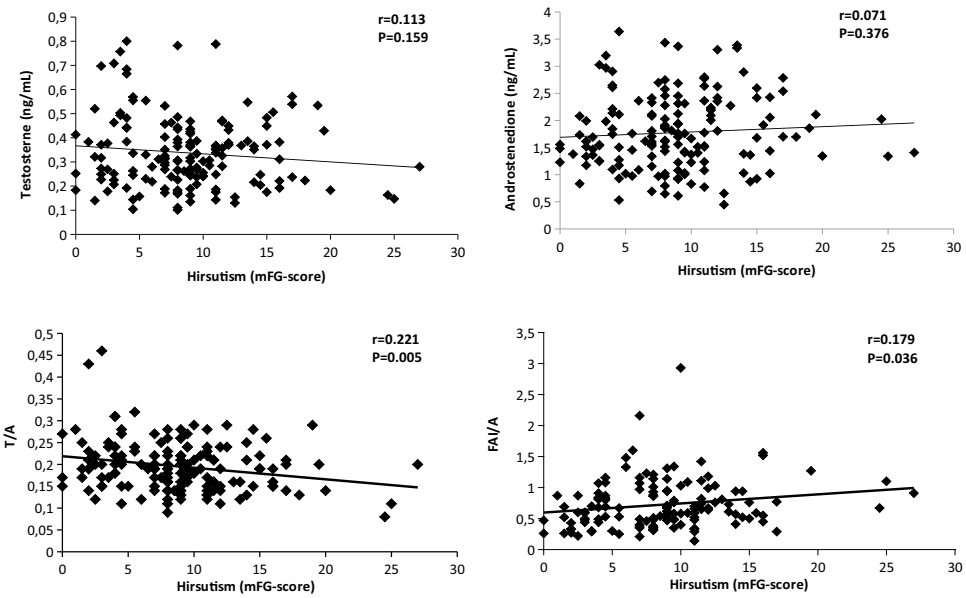

Fig. 1 Relationship between the Ferriman–Gallwey score and androgens (measured by LC–MS/MS) in women with PCOS: the severity of hirsutism poorly correlates with that of androgen excess or does not correlate at all. T/A testosterone/androstenedione, FAI/A free androgen index/androstenedione

additional steroidogenic extraglandular organ responsible for the development of hirsutism. Our preliminary unpublished data seem to support this concept (see after paragraph "Conclusion"). In fact, by calculating the product-to-substrate ratios of several hormones (all measured by LC–MS/MS), we have found that in the classic phenotype of PCOS, characterized by the presence of androgen excess and hirsutism, a specific activation of the 17–20 lyase, the 5a-reductase, and even of the aromatase activity may exist. By contrast, those patients with hirsutism and OD-PCOm, but normal androgen (TT, FAI, D4A) blood levels, seem to be characterized by an increased activity of the 17a-hydroxylase, 17–20 lyase, and sulfotransferase (unpublished data).

Collectively, the data summarized above suggest that hirsutism and hyperandrogenemia should not be used as synonyms; conversely, they should be used separately in defining "hyperandrogenism" in women with PCOS. This would allow a better definition of individual phenotypes of PCOS and, potentially, the planning of different therapeutic approaches.

The impact of obesity on the development of PCOS: does a PCOS secondary to obesity exist?

Obesity has become a major contributor to the global burden of disease and its worldwide incidence is continually increasing, with a large variability among countries and continents [53–55]. This trend is also evident in young individuals, from childhood to adolescence. In fact, there is evidence that, particularly in the USA, the prevalence of high body mass index (BMI) by selected cutoff for youth aged 2–19 years is very high in both boys and girls. In the latter, BMI for age ≥ 85th percentile of the growth charts has been found to be 31.6% in girls aged 2–19 years [56]. In addition, in a retrospective large cohort of individuals participating in the Longitudinal Assessment of Bariatric Surgery-2 assessed before surgery, who were ≥ 80% certain of recalled height and weight at age 18 years (1502 out of 2308), it was found that, compared with those with healthy weight at age 18 years, those with class ≥ 2 obesity at age 18 years had independently increased risk of PCOS by 74% ($P = 0.03$) [57]. These data were in some way expected, since many previous studies had shown that the development of overweight or obesity during adolescence may favor menstrual and ovulatory disorders [58]. Several mechanistic studies have also shown that adolescent girls' alterations in menses and ovulation may persist for many years and that they are frequently associated with high LH levels and mild androgen excess [59]. In fact, excess body weight that develops during puberty may affect the maturation of the hypothalamic–pituitary–gonadal (HPG) axis and, consequently, menses cycles, ovulatory performance and, later in life, fertility processes [60]. This is strictly related to androgen excess, since there is evidence that in adolescent girl's excess body weight often precedes or manifests at the same time as the development of irregular menses and is often associated with increased TT and FT blood levels and that BMI represents the best predictor of altered blood levels of androgens [61]. In an epidemiological study including a large cohort of adolescent students aged 16–19 years, we have confirmed that higher than normal BMI values increased the likelihood of the presence of PCOS (OR 1.42; $P = 0.009$) [62].

Several studies have reported that obesity per se may be associated with elevated androgen production rates in adult

women, particularly those with the abdominal–visceral phenotype [63, 64]. Similar findings have also been found in a study in adolescent girls. In fact, it has been shown that peripubertal obesity is associated with hyperandrogenemia (TT and/or FAI) and hyperinsulinemia, being especially marked shortly before and during early puberty and, intriguingly, the best factor responsible for androgen alterations was BMI [61]. Another study reported that morning LH and fasting insulin blood levels were significant predictors of FT in obese girls, even after adjusting for potential confounders, suggesting that abnormal LH secretion and hyperinsulinemia may be responsible for the development of hyperandrogenemia in a subset of peripubertal girls with obesity [65]. A more recent study [66] also reported that overweight pubertal girls were characterized by elevated non-adrenal FT blood levels after dexamethasone suppression and by exaggerated adrenal androgens in response to cosyntropin stimulation, compared to their normal-weight counterparts. This further supports the concept that, in the presence of excess body weight, a mixed adrenal and ovarian oversecretion of androgens in mid-to-late puberty may be a factor favoring the development of a PCOS-like phenotype. 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 of developing PCOS, particularly in the presence of high BMI [67]. Moreover, it has been repeatedly reported that adiposity in early childhood appears to be linked to advanced puberty in girls [68]. This can be partly due to a premature central activation of the HPG axis and central initiation of puberty [69]. In addition, it has been shown that the neuroendocrine disruption can be associated with alterations in the sex hormone balance, including ample substrate aromatization in the fat tissue, which obviously contributes to early estrogenization in girls and increased adrenal androgen production. Insulin excess by itself could be responsible for these abnormalities, since insulin is able to stimulate adrenal steroidogenesis [70–73] and to increase insulin growth factor-1 (IGF-1) bioavailability via reduction in IGF binding proteins [71]. In addition, the potential role of leptin excess at the hypothalamic level has also been suggested [59]. Another factor potentially involved may be represented by an 11b-hydroxysteroid dehydrogenase activation in the expanded adipose tissue compartment, which in turn may lead to enhanced deactivation of cortisol and subsequent increase in adrenocorticotropin hormone (ACTH) drive, thereby favoring an increase in the adrenal androgen production [74]. Interestingly, a German study reported that, compared to a normal-weight group, obese pubertal girls had significantly higher TT, D4A, DHEA, cortisol, 11-deoxycortisol, and cortisone associated with lower SHBG levels and, above all, that after a sustained weight loss achieved over 1 year, these alterations all tended to almost normalize [75]. Therefore, peripubertal obesity seems to be associated with hyperandrogenemia, although it is still unclear why all girls with increased adiposity do not have androgen excess. The evidence for an important role of adipose tissue, particularly the visceral depots, in generating both (particularly, D4A, DHT, and DHEA) and estrogens and in regulating their metabolic pathways metabolism, has been well known for a long time [74]. This has been shown in obese males [76], but it is likely that the same may occur in obese females. An adequate investigation of factors responsible for androgen and glucocorticoid overproduction in obese pubertal girls might help to identify the subjects potentially susceptible to developing the PCOS phenotype later in time. In any case, the expansion of the visceral fat may play an important role in the pathophysiology of PCOS, thus suggesting that obese girls may be at high risk for the development of PCOS [59, 77]. This concept totally agrees with what was hypothesized by Burt Solorzano and McCartney [59], who emphasized that obesity-related hyperinsulinemia could produce hyperandrogenemia during the pubertal transition in susceptible individuals, possibly by interfering with the normal negative feedback mechanisms at the hypothalamic level, therefore enhancing both gonadotropin releasing hormone (GnRH) pulsatility and LH secretion. In addition, it could be that some pubertal girls may have an inherent defect in ovarian and adrenal steroidogenesis, resulting in turn in a tendency to excessive androgen production, possibly exacerbated by obesity-related hyperinsulinemia [78]. On the other hand, there is considerable evidence that androgens as such are able to promote insulin resistance not only in the adipose tissue, but also in the muscles [79, 80]. In fact, androgens may interfere with insulin signaling by amplifying phosphorylation of mTOR, ribosomal S6-kinase (S6K), and consequently increasing Ser636/639 phosphorylation of IRS-1. Moreover, in cultured subcutaneous adipocytes, testosterone selectively induces metabolic insulin resistance via the androgen receptor (AR) activation, not involving PI3K, but the impaired phosphorylation of the downstream mediator PKCζ. In addition, in the muscles, androgen excess favors an increase in the number of less sensitive type IIb cells and the inhibition of muscle glycogen synthase [79, 80]. Unfortunately, very few data are available in adolescent pubertal girls, with and without obesity, presenting with androgen excess, and available studies are almost all cross-sectional and often lack a proper assessment of any predictive factors. In addition, there are no studies on the potential impact of genetic factors, for example the fat mass and obesity-associated (FTO) gene. Up to now, numerous case–control studies have reported the associations between fat mass, the FTO gene rs9939609 A/T polymorphism, and PCOS. Notably, a recent meta-analysis including five studies involving 5010 PCOS patients and 5300 controls suggested that rs9939609 A/T polymorphism of FTO gene is associated with PCOS

risk, and that the A allele is a risk factor for PCOS susceptibility simultaneously [81].

Another important issue is that an aberrant morphology and function (hypertrophy and a relative inefficiency in responding to the sympathetic system) of the visceral adipose tissue, which is partly dependent on androgen excess, characterizes women with PCOS [82]. These aspects have been extensively discussed in previous papers to which the reader can refer [63, 83, 84]. The expansion of adipose tissue is therefore potentially responsive to the increase in circulating androgens, suggesting the possibility that it may favor the development of a PCOS phenotype during adolescence.

As mentioned earlier, the criteria for determining the definition of PCOS in adolescent girls are not yet well defined, which implies that the diagnosis of PCOS may be underestimated or, conversely, overestimated. Recent studies have demonstrated that this problem is still open, implying that there are various phenotypes consistent with a possible diagnosis of PCOS and, finally, how important the impact of obesity may be [85], since considerable evidence suggests that PCOS has diverse causes, arising as a complex trait with contributions from both heritable and environmental factors that affect ovarian and adrenal steroidogenesis [86, 87]. The identification of clear diagnostic criteria, especially during adolescence, is a matter of extreme urgency. The presence of hyperandrogenemia in adolescent girls with excess body fat represents an alarm bell that needs proper evaluation, possibly using functional tests, even in the absence of clinical hyperandrogenic manifestations such as hirsutism and acne. Finally, investigation of potentially heritable biochemical traits in the family, also including metabolic issues, may in some way help to characterize the extent of the risk of developing PCOS in adolescent girls [88–90].

Recently, the Pediatric Endocrine Society defined appropriate criteria for the diagnosis of PCOS in adolescence, which included (1) an otherwise unexplained combination of abnormal uterine bleeding pattern, abnormal for age and persistent for 1–2 years, and (2) evidence of hyperandrogenism, by increased TT levels and/or moderate–severe hirsutism and/or moderate–severe inflammatory acne vulgaris [87]. Whether this will improve the diagnosis of PCOS in adolescents remains to be seen, since the heterogeneity of the clinical and biochemical criteria to define PCOS during adolescence can make any clinical framing difficult. Recently, Rosenfield RL focused attention on the spectrum of ovarian androgenic dysfunction that ranges from forms of subclinical hyperandrogenism associated with some normal variants of PCOS to severe ovarian hyperandrogenism in classic PCOS [91]. On the other hand, although most cases lacked evidence of steroid secretory abnormalities, most of them were obese, supporting the concept that obesity per se may account for the development of their atypical PCOS phenotype [60]. Due to the large prevalence of obesity in adolescent girls, this hypothesis requires much more detailed research.

Very convincing data on the potential causative role of obesity in the development of PCOS can be represented by the effects of weight loss achieved by lifestyle intervention, insulin sensitizers (metformin), or antiobesity drugs [92, 93]. Unfortunately, most of these studies are relatively short, rarely exceeding 6 months. Notably, a great inter-individual variability in the response to weight loss has been reported, and predictive factors are still largely underevaluated [94, 95], although it has been shown that when patient empowerment has been increased, the extent of weight loss may be significantly higher [95]. In a previous study, in which this strategy was applied, 37% of patients (24 out of 65) completely recovered from all features of PCOS, although the extent of weight lost was similar to those in whom the phenotype of PCOS was unchanged (15%, out 10 of 65) or in those who had only a marginal improvement (36.9%, 31 out of 65) [96] (Fig. 2). Intriguingly, both abdominal adiposity (measured by waist circumference) and particularly D4A blood levels predicted the outcome. Studies on the effects of bariatric surgery in PCOS women with severe obesity reported much more convincing data on the benefits of sustained weight loss. A recent meta-analysis [97], including 13 primary studies and involving more than 2000 female patients, provided additional information on the efficacy of bariatric surgery in severely obese PCOS women. The most astounding findings were that after 1 year and sustained weight loss (BMI decreased from 46.3 to 34.2), the prevalence of women with PCOS decreased from 45.6% preoperatively to 6.8% ($P < 0.001$) at the 12-month follow-up. Interestingly, among the criteria used for the definition of PCOS at baseline, the study found that menstrual irregularities decreased 56.2–7.7% ($P < 0.0001$), the incidence of hirsutism declined from 67.0 to 38.6% ($P = 0.03$), and infertility declined from 18.2 to 4.3% ($P = 0.0009$).

Taken together, these findings strongly support the concept that that obesity may play a relevant role in the development of the PCOS phenotype in susceptible individuals, a concept that is entirely compatible with the development of a secondary PCOS in obese women and particularly in adolescents (Fig. 3). This hypothesis merits in-depth investigation by targeted clinical and prospective studies, to identify the biological factors responsible for individual variability.

Severe insulin-resistant states and the development of different PCOS phenotypes

Several decades ago, the term "HAIR-AN" (Hyperandrogenism, Insulin Resistance and Acanthosis Nigricans) [98] was coined, identifying a specific combination of severe insulin

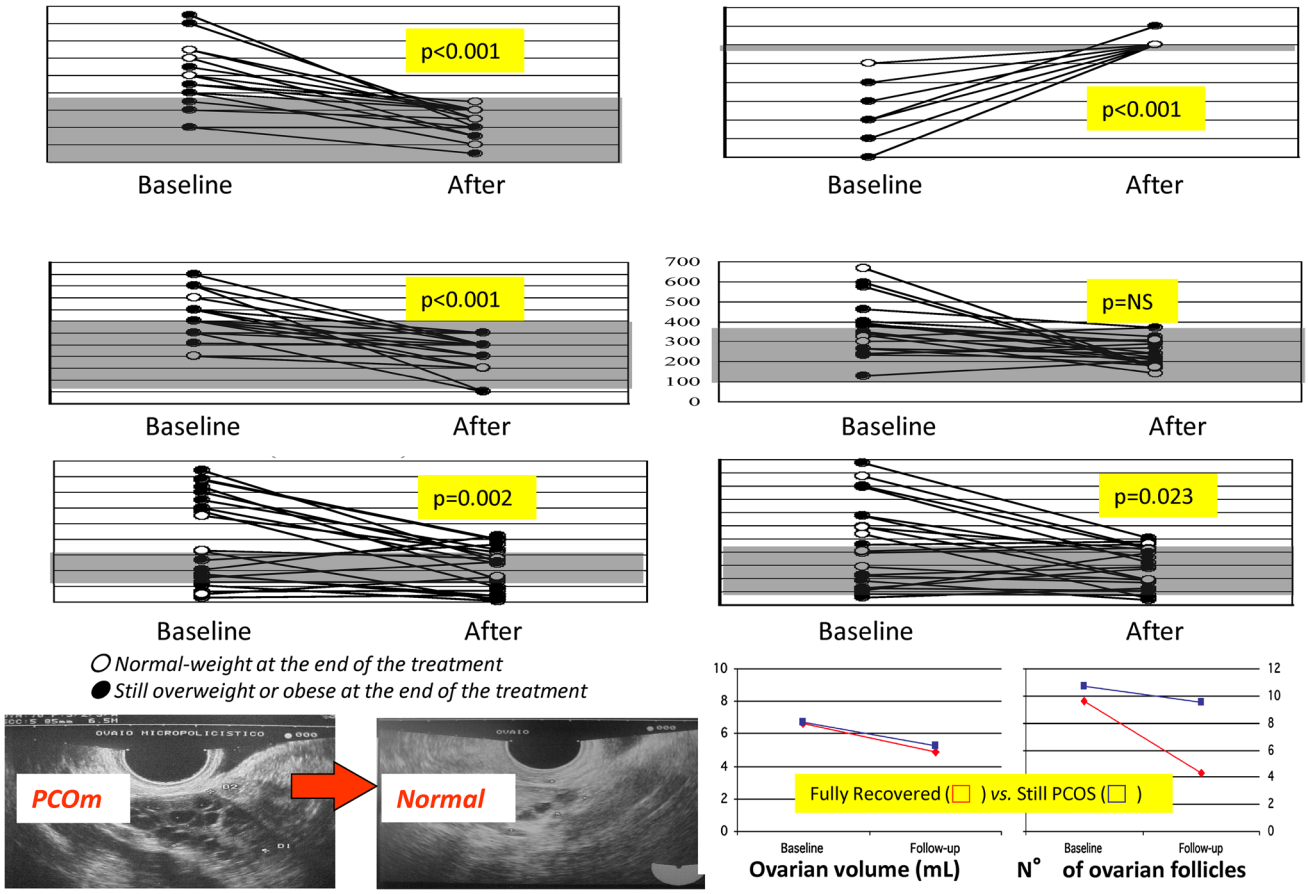

Fig. 2 Changes in androgens, gonadotropins, PCOm, the number of ovarian follicles, and ovarian volume in the subset of patients who completely recovered from the PCOS phenotype are shown. In the "fully recovered" patients with PCOS, blood androgen and gonadotropin blood levels, the F–G score, menses, ovarian morphology [including the number of ovarian follicles and ovarian volume (at ultrasound examination)] were totally within the normal range

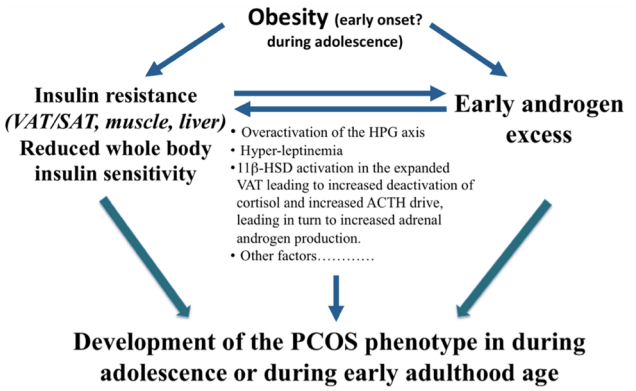

Fig. 3 The concept of "PCOS secondary to obesity": potential pathophysiological mechanisms

resistance associated with a PCOS-like phenotype. In the last decade, the increased understanding of the different forms of insulin resistance favored the development of 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 [99]. These disorders are frequently associated with lipodystrophies and often with a list of metabolic dysfunctions [100]. In a recent review article, we discussed the clinical relevance of the association between SSIR and PCOS to which the reader can refer for more in-depth knowledge [84].

Whereas congenital generalized forms of lipodystrophy are often diagnosed during childhood, some forms of partial lipodystrophies may have a strong similarity to the most common metabolic disorders managed by adult endocrinologists. In most studies performed on women with PCOS, it is assumed that, according to the various consensus [3, 4], the so-called secondary forms of PCOS should be excluded to properly diagnose PCOS. Conversely, very few studies include adequate information on the possible presence, in patients with a PCOS phenotype, of an SSIR. This is an intriguing problem as, by analyzing the data reported in these studies, it is relatively common to find patients with markedly elevated insulin levels both at fasting and after

an oral glucose loading test (OGTT). Moreover, it is very rare that such studies evaluated the possible presence of partial lipodystrophy and, through appropriate investigations (including genetic ones), the diagnosis of SSIR. According to current PCOS diagnostic criteria [3–5], these patients should be excluded or at least described separately.

Preliminary data by our research group on the prevalence of the SSIR state in a large cohort of 1200 adolescents and adult women with PCOS, all included in our database, strongly support the concept that this condition could be relatively more common than expected [84]. Intriguingly, we found that 1.5% of these patients had PCOS, defined according to the NIH criteria associated with very high fasting and/or glucose-stimulated insulin levels (Fig. 4). In most of these patients, we collected frozen blood samples available for possible genetic assessment, in collaboration with Semple MK (at the University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Cambridge, United Kingdom). In a small subset of these patients, we were able to identify a genetically determined lipodystrophy, associated with a genetic background (missense mutations in the following genes: LMNA, PPARG, PLIN1, or CGL (Hypo Leptin) (Fig. 4). Based on what is currently known, it is possible to functionally categorize additional genes implicated in the pathogenesis of lipodystrophy and severe insulin resistance associated with the PCOS phenotypes as being primarily involved in the transcriptional regulation of adipocyte differentiation, fatty acid uptake by adipocytes, triacylglycerol synthesis or lipid droplet formation, and polymerase delta 1 or hormone sensitive lipase [101]. In many of these patients, we are awaiting a genetic evaluation, despite the fact that the identification of any responsible genes is still far from being clarified [101].

This fascinating area regarding the interaction between severe insulin resistance and PCOS requires much more attention to avoid misdiagnosis. In the presence of very high fasting or glucose-stimulated insulin levels, any patient with a clear picture of PCOS should be suspected of having an SSIR state, possibly related to a genetic background. Accordingly, these patients should be considered separately, when a clinical study is planned.

Conclusions

This review article intends to introduce some aspects related to the framing of the different PCOS phenotypes, consistent with the concept that PCOS is in fact a "syndrome". We believe that this may represent a new perspective from the clinical point of view requiring much more scientific interest

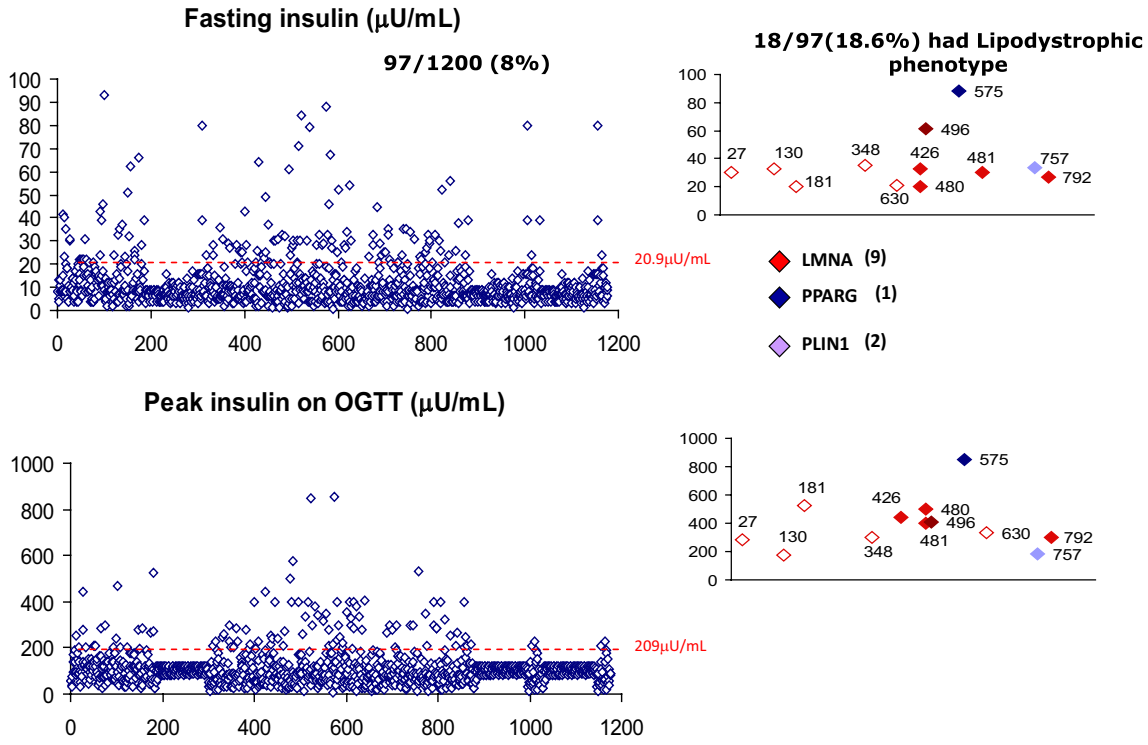

Fig. 4 Fasting and glucose-stimulated insulin blood levels in 1200 patients with PCOS included in the database: 97 patients with SSIR were extracted. Eighteen of these patients (18.6%) had a variable lipodystrophic phenotype (representing 1.5% of the entire cohort) and a specific gene was identified in a subset of them (different colors identify different genes)

and research activity. We support the concept that the different phenotypes of PCO may suggest potentially different pathophysiological mechanisms. In addition, more recent studies, based on much more sensitive and specific methods in the evaluation of blood androgens (such as LC–MS/MS), provide strong evidence for the appropriateness of using a hormonal profile rather than the sole determination of TT. Interestingly, recent data also confirm that a broader androgen profile may highlight a hyperandrogenic condition in many PCOS patients with the OD-PCOM phenotype, currently considered as a non-hyperandrogenic clinical entity.

It is of great interest that recent data available in the literature support the notion that the origin of excess androgen may not only be in the ovaries, but also the adrenals and, presumably, in the skin. Since the relation between circulating androgens and the presence of hirsutism, as well as its severity, is modest or absent, it could be hypothesized that the term "hyperandrogenism", currently defined as "excess of testosterone" and/or "presence of hirsutism", might be more properly defined by distinguishing hirsutism from hyperandrogenemia. An intriguing aspect is the possible responsibility of obesity, especially if its onset is in infancy or puberty, in favoring the development of PCOS in susceptible individuals. If proved, this could imply the possibility of defining a condition of PCOS secondary to obesity. This perspective could undoubtedly represent a new intriguing challenge in relation to the physiopathology of the obesity–PCOS interaction and, obviously, for individual prevention or therapeutic options. In addition, we would like to point out that a better and more appropriate evaluation of elevated fasting or glucose-stimulated insulin levels may disclose the presence of moderate forms of SSIR in young and adult patients with PCOS. Further studies are required on this important issue.

Compliance with ethical standards

Conflict of interest The authors indicate no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent No informed consent.

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