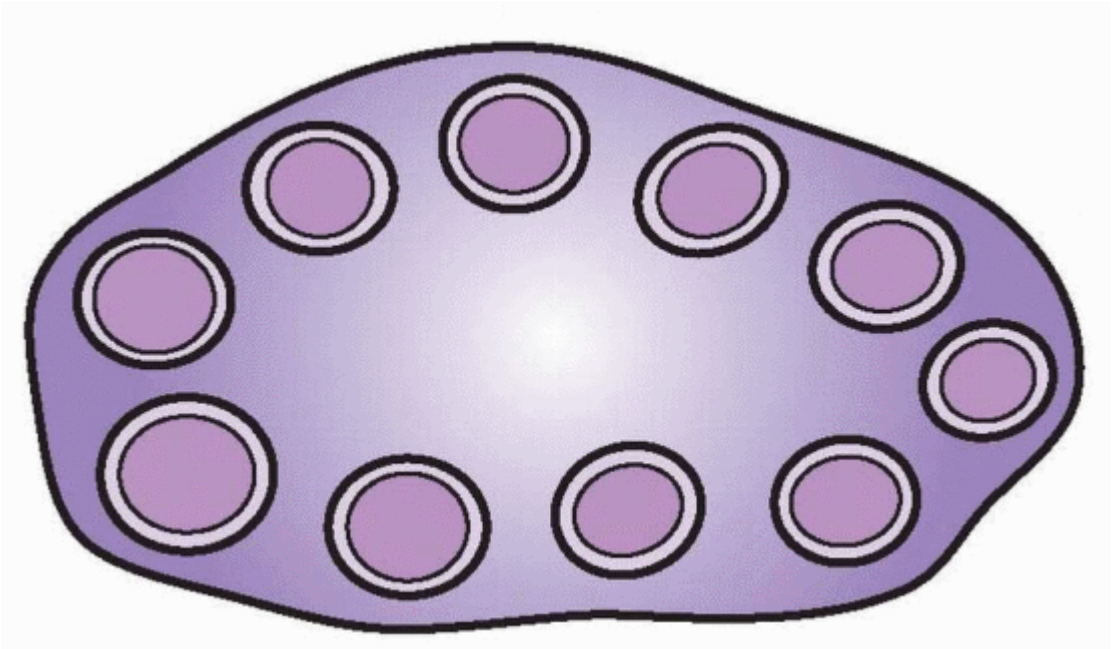
Editors: Fritz, Marc A.; Speroff, Leon
Title: *Clinical Gynecologic Endocrinology and Infertility, 8th Edition*

12

Chronic Anovulation and the Polycystic Ovary Syndrome



Anovulation is very common and has a number of different clinical manfestations, including amenorrhea, dysfunctional uterine bleeding, and hirsutism. The condition also has serious potential consequences, such as infertility and an increased risk for developing endometrial hyperplasia and neoplasia. In many anovulatory women, the pathophysiology involves insulin resistance, which increases the risks for developing diabetes mellitus and cardiovascular disease. In others, overt hypogonadism increases the risk for developing early osteoporosis. All clinicians who care for women must therefore be thoroughly familiar with the evaluation and management of anovulatory women.

Normal ovulatory function requires coordination at all levels of the hypothalamic-pituitarygonadal axis, and anovulation can result from disruption at any level. This chapter considers the variety of mechanisms that can cause anovulation and the clinical consequences of chronic anovulation, focusing on the most common anovulatory disorder, the polycystic ovary syndrome, and its management.

Causes of Anovulation

The complex interaction of neuroendocrine, intraovarian, and endometrial mechanisms that regulate the normal ovulatory menstrual cycle are discussed in detail in other chapters in this text (Chapters 5 and 6). They are summarized briefly here, to provide the foundation for subsequent discussion of the pathophysiology of anovulation.

As the corpus luteum regresses and the menstrual cycle draws to a close in the late luteal phase, serum concentrations of estradiol, progesterone, and inhibin A decline to basal levels, releasing the hypothalamic-pituitary axis from their collective negative

P.496

feedback effects. Accordingly, the frequency of hypothalamic gonadotropin-releasing hormone (GnRH) secretion increases, stimulating an increase in pituitary folliclestimulating hormone (FSH) secretion, which serves to “recruit” a new cohort of small antral follicles or, more accurately, to rescue a group of follicles from otherwise programmed demise via apoptosis. During the early follicular phase, the serum concentration of inhibin B, secreted by the recruited pool of small antral follicles, rises progressively.

During the midfollicular phase, ovarian autocrine and paracrine mechanisms involving activin and insulin-like growth factors enhance FSH-stimulated aromatase activity in granulosa cells to help create and sustain the estrogenic microenvironment required for continued follicular growth and development. Whereas an estrogenic follicular milieu fosters further growth, an androgenic milieu promotes atresia. As the serum inhibin B concentration reaches its peak, estradiol and inhibin A levels, derived from the granulosa cells of growing follicles in the cohort, begin increasing steadily. In response to their combined inhibitory effects, luteinizing hormone (LH) pulse amplitude decreases and pulse frequency increases (presumably reflecting the pattern of hypothalamic GnRH secretion), and serum concentrations of both FSH and LH fall gradually. Declining FSH levels remain sufficient to support continued growth of the selected dominant follicle, which has more granulosa cells and FSH receptors and a more advanced microvasculature, but become inadequate to support further development in smaller follicles in the cohort.

During the late follicular phase, inhibin A and insulin-like growth factors combine to promote LH-stimulated androgen production in theca cells, which provides substrate for aromatization to estrogen in the proliferating mass of granulosa cells within the preovulatory follicle. FSH and estradiol then combine to induce expression of LH receptors on the granulosa cells that will mediate luteinization and ovulation when the follicle reaches full maturity. Ultimately, serum levels of estradiol derived from the preovulatory follicle exceed the threshold concentration required to exert positive feedback effects centrally, acting primarily on the pituitary to induce the midcycle LH surge. The LH surge completes follicular maturation and triggers a cascade of events resulting in extrusion of the oocyte and formation of the corpus luteum. The oocyte completes the first meiotic division and local secretion of plasminogen activator and other cytokines mediates erosion of the follicular wall, allowing the oocyte to emerge with its surrounding investment of cumulus cells. The mural granulosa cells begin to luteinize and produce progesterone.

After ovulation, serum estradiol concentrations fall precipitously, but only transiently, before rising again in parallel with progesterone and inhibin A produced by the corpus luteum. Progesterone transforms the endometrium from a proliferative to a secretory morphology and stimulates a still uncharacterized cascade of biochemical events that renders the endometrium receptive to embryo implantation. As the progesterone level rises to its peak during the midluteal phase, LH pulse frequency decreases again and gonadotropin levels fall progressively to their nadir in the late luteal phase. Unless pregnancy intervenes and rapidly rising levels of human chorionic gonadotropin (hCG) rescue the corpus luteum and stimulate continued high level progesterone secretion, the corpus luteum regresses, estradiol and progesterone levels fall, support for the endometrium is withdrawn, and menses ensue.

Central Defects

Although difficult to demonstrate, hypothalamic dysfunction offers both a logical and likely explanation for ovulatory failure. A normal pituitary response to feedback signals from the follicle requires pulsatile GnRH secretion within a critical range. The onset of

P.497

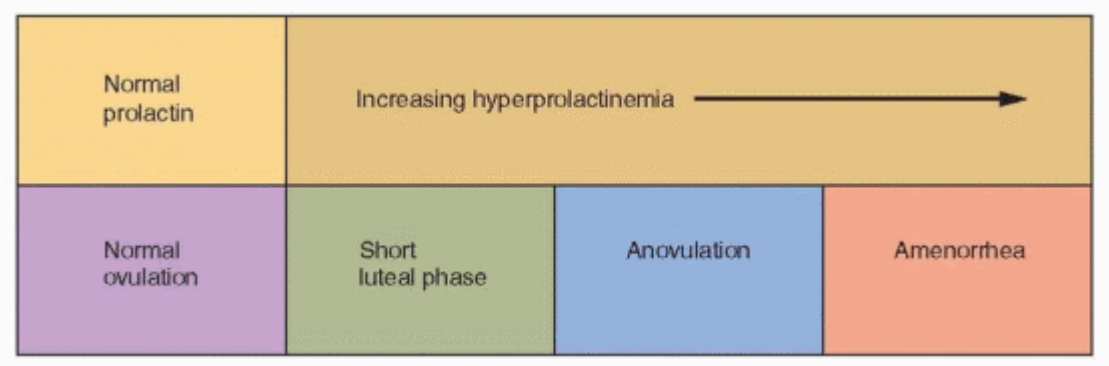
puberty in girls results from decreasing central inhibition of GnRH neuronal activity and increasing pulsatile GnRH secretion, which stimulates a progressive increase in pituitary gonadotropin release and, in turn, ovarian follicular growth and estrogen production (Chapter 10). After menarche, cycle length and menstrual characteristics in adolescent girls typically vary until the hypothalamic-pituitary-ovarian axis matures and the positive feedback relationship between estradiol and gonadotropin secretion and ovulation becomes established. ***Factors that reactivate central inhibitory mechanisms, such as emotional, nutritional (weight loss, eating disorders), or physical stress (excessive exercise), can suppress GnRH neuronal activity, leading to dysfunctional patterns of gonadotropin secretion that fail to promote progressive follicular development, resulting in anovulation.*** Although such patients more commonly present with amenorrhea (Chapter 11), lesser degrees of GnRH neuronal suppression can result in homeostatic levels of pituitary-ovarian function and a euestrogenic chronic anovulatory state.

Pituitary Tumors

Pituitary tumors can cause anovulation by inhibiting gonadotropin secretion. They may compress pituitary gonadotrophs directly, or interrupt delivery of hypothalamic GnRH by compression of the pituitary stalk. Alternatively, they may cause hyperprolactinemia by interfering with the inhibitory actions of hypothalamic dopamine (the putative prolactin inhibitory hormone) on pituitary lactotrophs, resulting in a secondary suppression of pulsatile GnRH secretion.

Hyperprolactinemia

Hyperprolactinemia is another specific example of anovulation resulting from a central defect.[1] The mechanism involves disruption or inhibition of the normal GnRH pulse rhythm, resulting in ineffective or frankly low levels of gonadotropin secretion. It's possible that elevated prolactin levels stimulate a generalized increase in hypothalamic dopaminergic activity, intended to suppress prolactin secretion but also inhibiting GnRH neurons. In any event, increasing prolactin levels can result in a spectrum of ovulatory dysfunction, ranging from a short luteal phase to anovulatory cycles to amenorrhea and hypogonadotropic hypogonadism, depending on the extent to which gonadotropin secretion is disturbed or suppressed. Mild hyperprolactinemia may cause only a short luteal phase, resulting from inadequate preovulatory follicular development.[2,3] Moderate hyperprolactinemia frequently causes oligomenorrhea or amenorrhea, and higher prolactin levels typically result in frank hypogonadism with low estrogen levels.[4,5] ***A breast examination with gentle compression looking for evidence of galactorrhea and measurement of the serum prolactin concentration are important parts of the evaluation of all anovulatory women.***



Abnormal Gonadotropin Secretory Dynamics

Many, but not all anovulatory women with polycystic ovaries exhibit abnormal gonadotropin secretory dynamics. The most common abnormality is an increase in mean serum LH levels, due to an increase in both LH pulse frequency and amplitude.[6,7] Serum concentrations of FSH typically are normal or low. The pattern could result from a decrease in hypothalamic dopamine or opioid inhibition of pulsatile GnRH secretion,[8] or from abnormalities in steroid hormone feedback, including the lack of progesterone (due to anovulation)[9] or increased circulating androgen levels.[10] Other evidence from studies in nonhuman primates and women suggests strongly that prenatal exposure to increased androgen concentrations induced by genetic and/or environmental factors may program the GnRH pulse generator in the female fetus in such a way as to result in increased pituitary LH secretion, causing disordered follicular development and ovarian hyperandrogenism.[11,12] and [13] The increased prevalence of chronic anovulation and polycystic ovaries in women with epilepsy offers another example of how central nervous system dysfunction can disrupt the hypothalamicpituitary-ovarian axis and result in anovulation.[14,15]

Abnormal Feedback Signals

Anovulation can result from abnormal estrogen feedback signals from the periphery, in two ways. Chronically elevated estrogen levels may not permit the increase in FSH secretion required to stimulate or sustain progressive follicular development. Conversely, poor follicular development may not generate or sustain the estradiol level required to induce the ovulatory LH surge.

Chronically Elevated Estrogen Concentrations

The fall in estradiol levels that normally occurs during the late luteal phase (as the corpus luteum regresses) is a prerequisite for the inter-cycle rise in FSH that drives the wave of new follicular development. Sustained high levels of estrogen negative feedback caused by increased production or decreased clearance and metabolism can prevent any significant increase in FSH levels, resulting in a chronic anovulatory state.

Pregnancy is the most common and obvious example of anovulation resulting from sustained high levels of estrogen production. Rare estrogen producing ovarian tumors (e.g., granulosa cell tumors) can have the same effect. Although the adrenals do not normally secrete appreciable amounts of estrogen directly into the circulation, they contribute via their secretion of androgens (androstenedione, dehydroepiandrosterone and its sulfate), which can be converted to estrogen in the periphery. Adipose tissue has significant aromatase activity, which converts androgens to estrogens,[16] thereby providing at least one mechanism for the well-known association between obesity and chronic anovulation (see below).

The clearance and metabolism of estrogen can be impaired in a variety of conditions, such as thyroid or hepatic disease. Both hyperthyroidism and hypothyroidism can cause chronic anovulation by altering the metabolic clearance and peripheral interconversion of steroid hormones.[17,18] and [19] ***Hypothyroidism can be associated with elevated prolactin levels, providing the rationale for measuring serum thyroid-stimulating hormone (TSH), as well as prolactin, in the evaluation of anovulatory and amenorrheic women***. Hepatic disease also disturbs the normal clearance and metabolism of sex steroids.[20]

P.499

Failure of the LH Surge

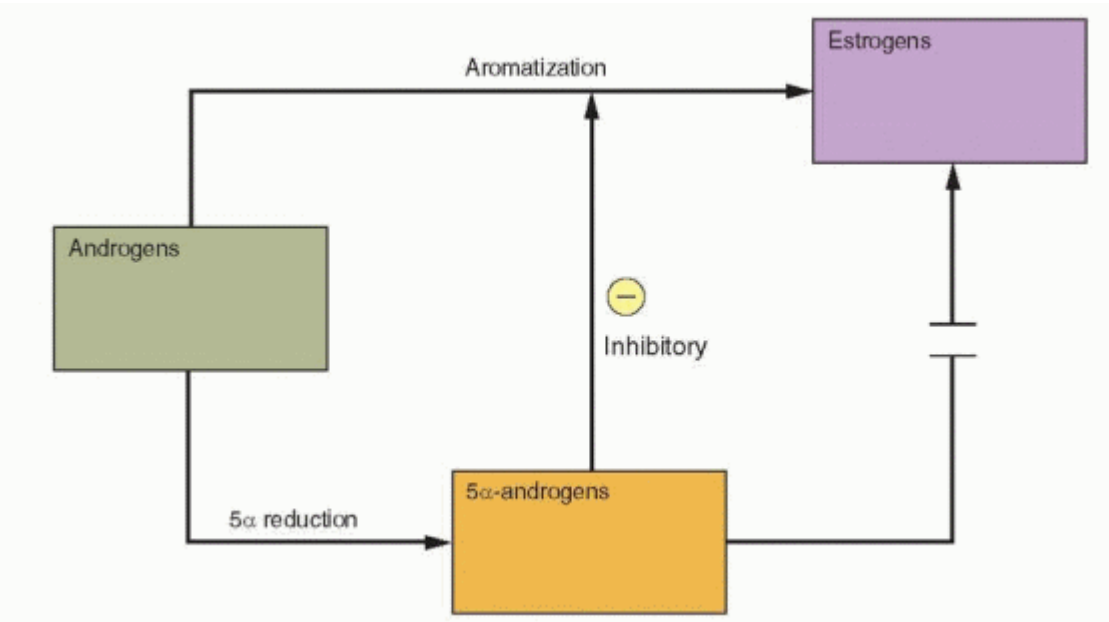
The rising tide of estradiol arising from the preovulatory follicle in the late follicular phase induces the midcycle LH surge that stimulates ovulation. Quite obviously, women with gonadal dysgenesis or ovarian

failure are anovulatory because they have no remaining functional ovarian follicles and no significant estrogen production. More commonly, clinicians encounter patients with normal serum levels of gonadotropins and estradiol who do not ovulate, whose anovulation results from the failure to achieve complete follicular development and to generate and sustain the level of estradiol required to induce the LH surge. Normal women typically also become anovulatory during the years immediately preceding the menopause, probably reflecting intrinsic deficiencies in aging follicles that impair normal follicular maturation.

Local Ovarian Conditions

A disturbance in one or more of the delicately balanced intra-ovarian regulatory mechanisms that serve to select the dominant follicle and allow it alone to grow and develop in the face of declining levels of FSH may lead to anovulation. Activins, inhibins, and insulin-like growth factors act via local autocrine and paracrine mechanisms to first enhance the action of FSH by increasing the concentration of FSH receptors within the dominant follicle, then combine to induce the appearance of LH receptors required to mediate the actions of LH during the midcycle surge that drives the final stages of follicular maturation and stimulates ovulation. A follicle can thus fail to grow and ovulate due to a failure or interference with any of these local mechanisms (Chapter 6).

The "two-cell, two gonadotropin" concept of ovarian follicular development (Chapters 2 and 6) emphasizes the critical importance of local androgen concentrations. At low levels, androgens serve as substrate for FSH-induced aromatization and estrogen production. At higher concentrations, androgens are converted alternatively to more potent 5α-reduced androgens, which cannot be converted to estrogen and also inhibit aromatase activity and FSH induction of LH receptors on granulosa cells. ***Consequently, abnormally high local androgen concentrations, from any cause, impede follicular maturation, promote atresia, and predispose to a chronic anovulatory state.***



P.500

Obesity

The prevalence of obesity in women with chronic anovulation and polycystic ovaries is high, ranging between 35% and 60%.[21,22,23] and [24] Obesity predisposes to chronic anovulation in at least three distinct ways:

1. Increased peripheral aromatization of androgens, resulting in chronically elevated estrogen concentrations.
2. Decreased levels of hepatic SHBG production, resulting in increased circulating concentrations of free estradiol and testosterone.
3. Insulin resistance, leading to a compensatory increase in insulin levels that stimulates androgen production in the ovarian stroma, resulting in high local androgen concentrations that impair follicular development.

Combined, these effects can be difficult to overcome, but even modest weight loss, which results in decreased circulating insulin and androgen concentrations, frequently restores ovulatory function and normal menstrual cyclicity.[25,26,27] and [28]

Defining the Cause of Anovulation

Whereas the cause of anovulation may be relatively clear in women with ovarian failure, pituitary tumors, eating disorders, hyperprolactinemia, or obesity, frequently it is not possible to isolate the specific mechanism responsible. However, it also is often not necessary. Regardless of its cause, the clinical manifestations and consequences are predictable, easily documented, and generally not difficult to manage. Women with absent or abnormal menstrual function who are otherwise healthy can be categorized as follows:

1. Ovarian failure. Hypergonadotropic hypogonadism, reflecting the inability of the ovary to respond to gonadotropin stimulation, due to follicular depletion (Chapter 11).
2. Central defects. Hypogonadotropic hypogonadism, reflecting hypothalamic or pituitary failure or suppression (Chapter 11).
3. Hypothalamic-pituitary-ovarian dysfunction, resulting in asynchronous gonadotropin and estrogen production, having a wide variety of causes and clinical manifestations that depend on the level of ovarian function, including amenorrhea (Chapter 11), hirsutism (Chapter 13), dysfunctional uterine bleeding (Chapter 15), endometrial hyperplasia and cancer (Chapter 18), and infertility (Chapters 27 and 31).

The polycystic ovary syndrome (PCOS) is the most obvious and common condition associated with chronic anovulation, affecting 4-6% of reproductive age women.[29,30] Several mechanisms contribute to the pathophysiology of anovulation in PCOS, operating at every level of the reproductive system. ***It is inaccurate to state that PCOS is the most common "cause" of anovulation, because PCOS does not cause anovulation; rather, PCOS is the consequence of chronic anovulation, which can result from a wide variety of causes. In that context, the disorder is described more accurately as chronic anovulation with***

polycystic ovaries. Although the term PCOS is now firmly established in our scientific and clinical lexicon, it is important to emphasize that PCOS is not a discrete or specific endocrine disorder having a unique cause or pathophysiology. Instead, the condition is best viewed as a final common pathway in the chronic anovulatory state.

The Polycystic Ovary Syndrome

Multicystic or "sclerocystic" ovaries were recognized as early as the mid-18th century, but associated primarily with pelvic pain or menorrhagia. In the early 20th century, prevailing hypotheses viewed them as resulting from inflammation due to infection, congestion due to pressure or partial torsion that disrupted normal blood flow to the ovary, or from dystrophy due to abnormalities in ovarian nutrition.[31]

In 1935, Irving F. Stein and Michael L. Leventhal first described a symptom complex associated with anovulation.[32] Both gynecologists were born in Chicago, both were graduates of Rush Medical College, and both spent their entire professional careers at the Michael Reese Hospital.[33] Stein and Leventhal described seven patients (four being obese) with amenorrhea, hirsutism, and enlarged, polycystic ovaries. They reported that all seven resumed regular menses and that two became pregnant after bilateral ovarian wedge resection, involving the removal of one-half to three-fourths of each ovary. Stein and Leventhal developed the wedge resection procedure after observing a resumption of menses following ovarian biopsy in several patients with amenorrhea. They speculated that the thickened ovarian capsule prevented follicles from reaching and escaping from the surface of the ovary.

Careful histologic studies of the "Stein-Leventhal ovary" revealed that they had twice the cross-sectional area of normal ovaries, the same number of primordial follicles, double the number of developing and atretic follicles, a 50% thicker and more collagenized tunica, a 5-fold thicker subcortical stroma, and a 4-fold greater number of hilar cell "nests" than normal ovaries. These studies further suggested that "hyperthecosis," characterized by an abundance of such nests and a markedly increased stroma, was likely just a later or more advanced stage of a progressive process.[34]

The pathophysiology responsible for development of polycystic ovaries has puzzled gynecologists and endocrinologists for many years and proven very difficult to define. However, there is an answer that is very simple, logical, and clinically useful. ***The characteristic polycystic ovary develops when a chronic anovulatory state persists for a sufficient length of time***. A cross section of anovulatory women at any one point in time will demonstrate that approximately 75% have multicystic or polycystic ovaries.[24,35] ***Because there are many causes of anovulation, there are many causes of polycystic ovaries***. Any of the causes of anovulation outlined earlier can yield the same or a similar clinical presentation. ***The polycystic ovary results from a functional derangement, not from a specific central or local defect***.

Pathophysiology

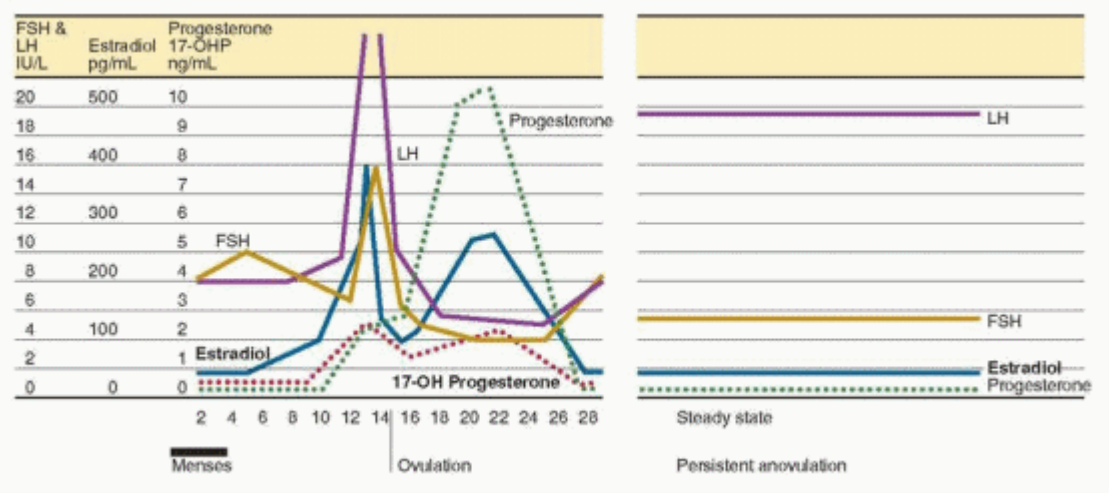
Whereas the morphological characteristics of polycystic ovaries were attributed at first to pathological changes in the ovaries themselves that prevented ovulation,[34] they now are recognized as reflecting the disordered endocrine milieu that results from chronic

anovulation. In contrast to the cyclic pattern of hormone concentrations that occurs during the normal cycle, the endocrine milieu in women with chronic anovulation is characterized by a "steady state" in which gonadotropin and sex steroid concentrations vary relatively little, by comparison.

The average daily production of both androgens and estrogens is increased in women with PCOS, as reflected by elevated serum concentrations of testosterone, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), 17α-hydroxyprogesterone (17-OHP), and estrone. The results of treatment with a long-acting GnRH agonist (aimed at suppressing gonadotropin-dependent ovarian steroid production) indicate that the increases in serum testosterone, androstenedione, and 17-OHP derive from the ovary and are LH-dependent, whereas those in DHEA and DHEA-S derive from the adrenal.[36,37,38] and [39] Serum estrone concentrations are modestly elevated, due to peripheral conversion of increased amounts of androstenedione. In contrast, serum estradiol levels in women with PCOS fluctuate

but generally remain within the range typically observed in the early follicular phase,[40] reflecting continued low-level production from limited follicular development.[41,42]

The endocrine milieu in women with PCOS reflects the chronic anovulatory state, which may result from a wide variety of causes. ***Current perspectives view PCOS as a complex disorder, similar to cardiovascular disease and type 2 diabetes mellitus, wherein numerous genetic variants and environmental factors interact, combine, and contribute to the pathophysiology.***[43] Not surprisingly, attention has focused on identifying genetic variants involving the regulation of gonadotropin secretion and action, insulin secretion and action, weight and energy regulation, and androgen synthesis and action.



Gonadotropin Secretion and Action

Stein and Leventhal suggested that polycystic ovaries were likely to result from abnormal anterior pituitary hormonal stimulation,[32] based on earlier observations that treatment with a urinary extract of anterior pituitary hormones could induce changes similar to those in polycystic ovaries.[44] Subsequent studies employing an LH bioassay (based on the ovarian

response of immature female rats or the prostatic response of hypophesectomized male rats to urinary extracts) demonstrated excessive LH activity in women with PCOS,[45,46,47] and [48] which later was confirmed by studies using a radioimmunoassay.[49]

Compared to normally cycling women, those with PCOS generally exhibit increased serum LH concentrations, low-normal FSH levels, and increased LH:FSH ratios.[7,50,51] ***The increase in serum LH levels results from abnormal LH secretory dynamics, characterized by an increase in LH pulse frequency, and to a lesser extent, also in pulse amplitude.***[6,52,53] ***and*** [54] ***The decrease in FSH levels results from the increase in GnRH pulse frequency, the negative feedback effects of chronically elevated estrone concentrations (derived from peripheral aromatization of increased androstenedione), and normal or modestly increased levels of inhibin B (derived from small follicles).***[55,56]

LH pulse frequency in women with PCOS does not exhibit the normal cyclic variation seen in ovulatory women and is relatively constant, at approximately one pulse per hour. The pattern presumably reflects a similar increase in hypothalamic GnRH pulse frequency, which favors secretion of LH more than FSH.[57,58] and [59] The LH response to an acute exogenous GnRH stimulus also is exaggerated in women with PCOS, but

to a lesser extent in obese than in lean women; accordingly, LH pulse amplitude and serum LH levels generally are somewhat lower in obese than in lean women with PCOS.[7,60] Elevated serum LH concentrations in women with PCOS also exhibit increased bioactivity in bioassay systems *in vitro*, reflecting a difference in glycosylation with a predominance of more basic (alkaline) LH isofoms, which have greater bioactivity.[53,61,62] and [63]

The approximate hourly LH pulse frequency in women with PCOS is within the range of frequencies usually observed across the normal ovulatory cycle, suggesting it results from a failure of the mechanisms that normally slow the GnRH pulse generator rather than from an abnormal acceleration in pulse frequency. The increased pulse frequency might reflect intrinsic hypothalamic dysfunction, the effects of abnormal feedback signals from the periphery, or both.[64]

Since dopamine and opioids normally inhibit hypothalamic GnRH neuronal activity, the higher GnRH pulse frequency observed in women with PCOS could be caused by a decrease in dopaminergic or opioidergic neuronal stimulation. However, experimental evidence from studies involving treatment with medications that stimulate or inhibit these pathways does not support either mechanism. Treatment with a dopamine agonist has no discernible effect on the pattern of gonadotropin secretion in women with PCOS.[65,66] Treatment with a progestin slows LH pulse frequency,[9] just as progesterone does during the normal luteal phase, indicating that the opioid-dependent process that normally mediates the effects of progesterone is operating,[67,68] and [69] and suggesting that any decrease in opioid tone results primarily from the lack of progesterone feedback, due to anovulation.

Infusion of exogenous insulin[70,71] and [72] and treatments that decrease insulin levels (metformin, thiazoladinediones) have no significant effect on the pattern of LH secretion in women with PCOS.[72,73] LH levels also are lower in obese than in lean women with PCOS, even though insulin levels are higher in the obese.[7,60] These observations suggest that hyperinsulinemia has no significant direct effect on LH secretion.

Treatment with exogenous estrone does not increase basal or GnRH-stimulated LH concentrations in women with PCOS,[74] and treatment with an aromatase inhibitor does not decrease LH pulse frequency,[75] indicating that increased circulating levels of estrone may exert negative feedback effects on FSH, but probably do not have any important direct influence on LH secretion in women with PCOS. Whereas the lack of progesterone feedback resulting from anovulation undoubtedly contributes to the higher LH pulse frequency,[9] evidence suggests that the GnRH pulse generator also is less sensitive to the feedback inhibition of sex steroids.

P.504

Treatment with an estrogen-progestin contraceptive or with physiologic doses of exogenous estrogen and progesterone slows LH pulse frequency in women with PCOS, but to a lesser extent than in normal women.[76,77] and [78] However, after pretreatment with flutamide (an androgen receptor antagonist), the effects of estrogen and progesterone on LH pulse frequency in women with PCOS are the same as in normal women,[10] suggesting that increased circulating androgen levels help to sustain the higher LH pulse frequency observed in women with PCOS by decreasing sensitivity to estrogen and progestin feedback.

Androgens also may contribute more directly to the abnormal pattern of gonadotropin secretion in women with PCOS. Evidence from studies in rats, sheep, monkeys, and women indicates that prenatal exposure to increased androgen concentrations may affect GnRH pulse generator programming, predisposing to an increased pulse frequency and LH secretion.[12,13,79,80,81] and [82] At least in rodents, prenatal androgen treatment also decreases basal and estrogen-induced hypothalamic progesterone receptor

concentrations,[81] offering a mechanism to explain how androgens might decrease hypothalamic sensitivity to progesterone feedback. It could be that hyperandrogenemia from any cause, arising during fetal life (maternal hyperandrogenism, classical congenital adrenal hyperplasia), adolescence (premature adrenarche, nonclassical congenital adrenal hyperplasia), or in adulthood (obesity, hyperinsulinemia) induces abnormalities in the feedback control of pulsatile GnRH secretion, resulting in increased LH secretion, which stimulates increased ovarian androgen production, in a self-perpetuating cycle.

The primary evidence indicating that excessive LH stimulation plays an important role in the pathophysiology of PCOS comes from studies examining the effects of treatment with GnRH antagonists and long-acting GnRH agonists. In women with PCOS, treatment with a GnRH antagonist induces an acute dose-dependent decrease in both LH and testosterone concentrations,[54] and long-term treatment with an agonist can suppress ovarian androgen production to postmenopausal levels.[83,84] However, normally cycling women with polycystic ovaries exhibit higher androgen and insulin levels and lower SHBG concentrations than women with normal ovarian morphology, even though LH levels and secretory dynamics are not different.[85] ***These observations suggest that excessive LH secretion or stimulation may be an important cause of disordered follicular development and anovulation, but is not the proximate cause of polycystic ovaries or of increased ovarian androgen production in women with PCOS.***

Insulin Secretion and Action

An association between glucose intolerance and hyperandrogenism was first recognized by Archard and Thiers in 1921, in a famous report describing a bearded diabetic woman.[86] Insulin resistance was first described in diabetic patients who required progressively higher doses of insulin to maintain effective glucose control, most often because they developed antibodies to preparations of insulin derived from animal sources.[87] Today, we recognize insulin resistance as a feature of a wide variety of disorders and conditions, ranging from extreme insulin-resistance syndromes (auto-antibodies to the insulin receptor, insulin receptor mutations, lipodystrophic states)[88,89] and [90] to common problems such as type 2 diabetes, obesity, stress, infection, pregnancy, and PCOS. The importance of insulin resistance, hyperinsulinemia, and insulin action in the pathogenesis of PCOS was first suggested by a study conducted in 1980, demonstrating significant correlations between basal levels of plasma insulin, androstenedione, and testosterone, and between insulin and testosterone levels after an oral glucose load.[91]

Insulin resistance is a common feature in obese and, to a lesser extent, lean women with PCOS; the overall prevalence ranges between 50% and 75%.[92,93,94] ***and*** [95] Insulin sensitivity is decreased by an average of 35-40% in women with PCOS, compared to normal

women, similar to what is observed among women with noninsulin dependent diabetes mellitus.[96,97] and [98] ***Up to 35% of women with PCOS exhbit impaired glucose tolerance and 7-10% meet criteria for type 2 diabetes mellitus.***[99,100] Conversely, women with type 2 diabetes are 6-fold more likely than non-diabetic women of similar age and weight to have PCOS.[101]

Insulin resistance is a condition in which endogenous or exogenously administered insulin has less than normal effects on fat, muscle, and the liver.[102] In adipose, insulin resistance results in increased hydrolysis of stored triglycerides and elevated circulating free fatty acid levels. Decreased glucose utilization (primarily in muscle) and increased hepatic gluconeogenesis (which insulin normally inhibits) result in increased blood glucose concentrations and a compensatory hyperinsulinemia (in those with adequate pancreatic reserve). ***Increased circulating insulin levels cause or contribute to***

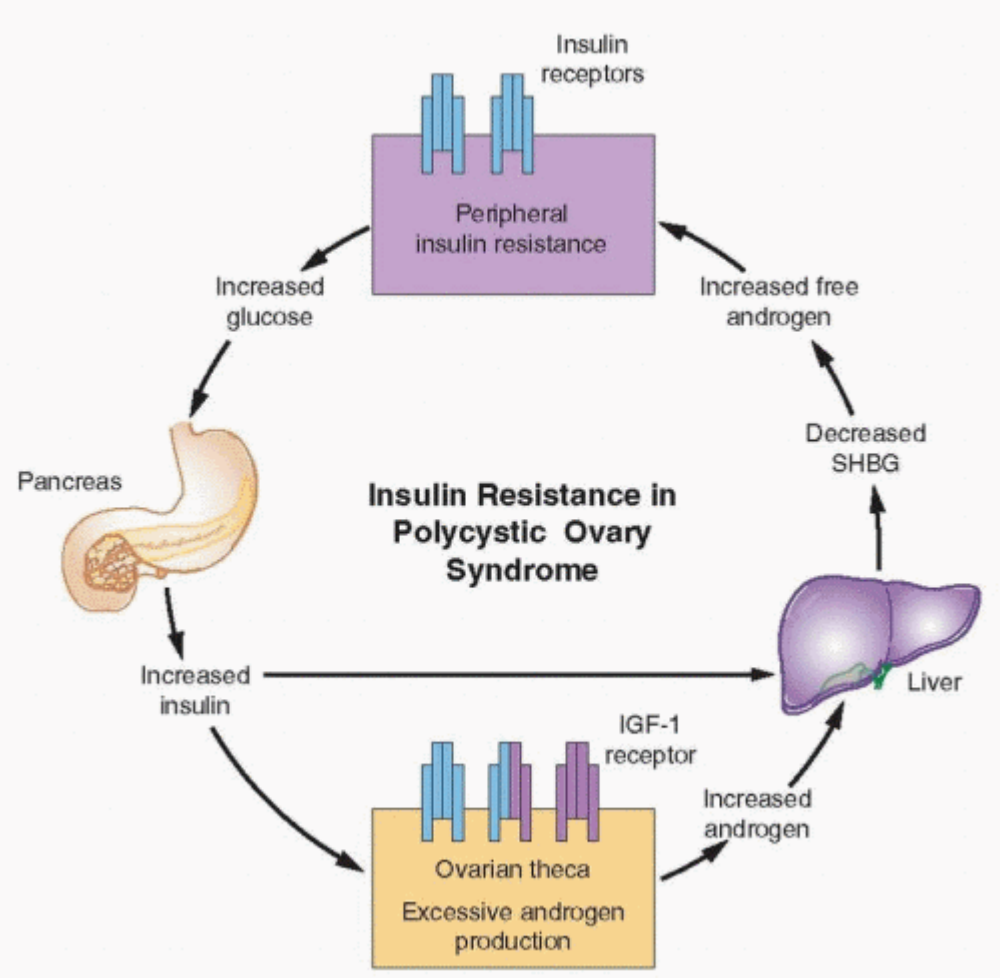
hyperandrogenism in women with PCOS in at least two important ways, by stimulating increased ovarian androgen production, and by inhibiting hepatic SHBG production.

Numerous studies have demonstrated that insulin stimulates androgen production in ovarian theca cells *in vitro*.[103] Theca cells from women with PCOS also exhibit increased sensitivity to insulin, compared to those from normal women. Physiologic levels of insulin can stimulate androgen synthesis in theca cells of women with PCOS, whereas higher insulin concentrations are required in normal theca cells.[104,105] ***Because insulin also potentiates the action of LH,[106] insulin and LH act synergistically to stimulate androgen production.***[104,107]

Clinical investigations in women with PCOS have demonstrated that insulin also stimulates ovarian androgen production *in vivo*. Notably, the cumulative sum insulin response during an oral glucose tolerance test correlates positively with the rise in serum androstenedione and testosterone above baseline concentrations.[108] Moreover, suppression of serum insulin

P.506

levels by treatment with diazoxide or an insulin-sensitizing agent (troglitazone) decreases serum androstenedione and testosterone levels in women with PCOS.[109,110]



High insulin concentrations also inhibit hepatic SHBG production,[111,112] as do high androgen concentrations. ***The combined actions of insulin and androgens lower SHGB concentrations, yielding increased free androgen levels, which aggravate the underlying insulin resistance.***[113] ***Ultimately,***

these conditions foster a self-propagating positive feedback loop that can increase in severity over time.

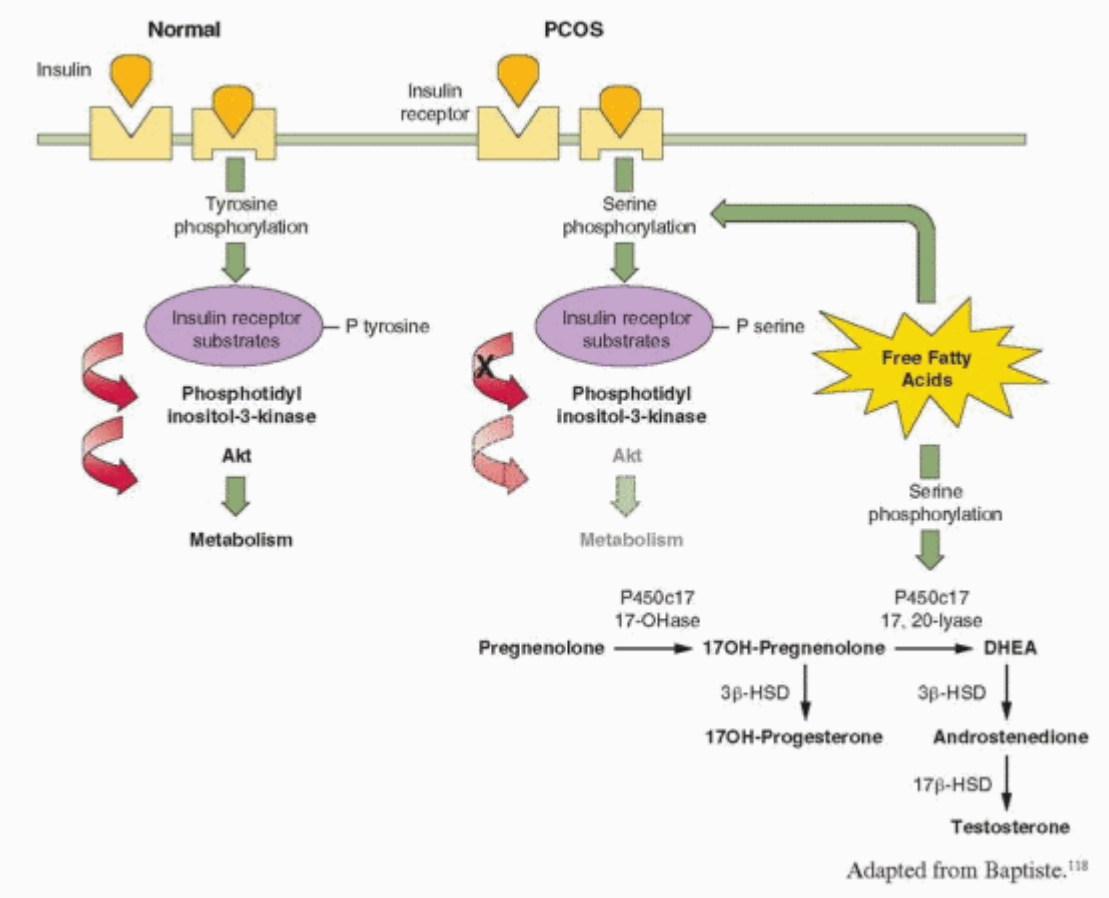
Insulin stimulates ovarian androgen production acting via insulin receptors on theca/ interstitial cells in the ovarian stroma.[105,114] At high concentrations, insulin also binds to IGF-1 receptors (and possibly hybrid receptors) which are structurally similar and use a similar signaling mechanism.[96] However, evidence indicates that insulin acts primarily via its own receptor, by activating a signaling system separate from that involved in glucose transport. Whereas an anti-insulin receptor antibody effectively inhibits insulin-stimulated steroidogenesis in cultured human granulosa cells, an anti-IGF receptor antibody has no effect.[105,115,116] A d-chiro-inositol containing glycan increases theca cell testosterone production *in vitro*, and preincubation with an anti-inositolglycan antibody blocks insulin stimulation, but not that of hCG.[105] These observations suggest inositolphosphoglycan mediators act as second messengers in signal transduction for insulin stimulation of theca cell androgen synthesis and that the mechanism differs from that mediating the actions of LH.[117]

What causes insulin resistance in women with PCOS is not entirely clear. Not surprisingly, given the complexity and polygeneic nature of the disorder, evidence suggests that more than one mechanism may be involved.

The classical actions of insulin are mediated via its receptor and two distinct intracellular pathways. The phosphatidyl-inositol 3-kinase (PI-3K) pathway mediates the metabolic effects of insulin, and the mitogen-activated protein kinase (MAPK) pathway mediates the proliferative actions of insulin. Normally, insulin binding to its receptor induces a conformational change, resulting in tyrosine phosphorylation of the receptor and protein substrates, which bind and serially activate PI-3K and Akt, an effector molecule that plays the major role in signal transduction for glucose regulation and metabolism.[118,119] Akt activation potentiates the translocation of glucose transporter 4 (GLUT4) from intracellular compartments to the plasma membrane, thereby increasing glucose uptake. Other effector molecules mediate insulin inhibition of gluconeogenesis and glycogenolysis,[120,121] stimulation of lipid synthesis, and inhibition of lipid catabolism.[122,123] Luteinized granulosa cells obtained from women with PCOS display both a selective increase in insulin activation of the mitogenic pathway, via MAPK, and resistance in the PI-3K-mediated metabolic pathway of insulin action.[124] ***These and similar observations illustrate how insulin actions can be selectively inhibited and enhanced at the same time, via different signaling pathways,[96,124] explaining how insulin can stimulate hyperandrogenism in women who are "insulin-resistant."***

Studies in cultured skin fibroblasts, muscle, and adipocytes from women with PCOS indicate that insulin resistance results from defects early in the postreceptor signaling pathway.[98,125,126] and [127] The number and affinity of insulin receptors in both obese and lean women with PCOS are not decreased,[128,129] but insulin receptors exhibit a constitutive increase in phosphorylation of serine residues and a decrease in insulin-stimulated phosphorylation of tyrosine residues. Serine phosphorylation of insulin receptor substrates prevents their binding with PI-3K and thereby inhibits insulin signaling. Increased serine phosphorylation can be induced by intracellular metabolites of free fatty acids,[126,130] which are increased in most women with PCOS and have been demonstrated to cause insulin resistance *in vivo*.[131,132] High circulating free fatty acid levels also can increase androgen production in women,[133] by inducing serine phosphorylation of P450c17, which results in increased 17,20 lyase

activity.[134,135] These observations offer a mechanism for insulin resistance that also further helps to explain the link between insulin and hyperandrogenism in women with PCOS.



Although hyperandrogenism can decrease insulin sensitivity, the effect is relatively modest.[113] ***Insulin resistance and hyperinsulinemia are the primary factors; they are the cause, not the result, of hyperandrogenism.*** Treatment with a GnRH agonist can normalize elevated serum androstenedione and testosterone levels in women with PCOS, but has limited or no effect on insulin resistance.[37,136,137] and [138] Similarly, although bilateral ovarian cautery can decrease serum androgen concentrations by nearly 50% in women with PCOS, glucose utilization (insulin sensitivity) remains unchanged.[139]

Accumulating evidence suggests that deficiency or dysfunction in downstream signaling mediated by inositolphosphoglycans also may contribute to insulin resistance in women with PCOS.[140,141,142] and [143] Finally, obesity is a common feature of women with PCOS, representing yet another important mechanism contributing to the development of insulin resistance, as discussed below.

Insulin resistance and hyperinsulinemia are undoubtedly an important part of the pathophysiology of PCOS. However, it is important to emphasize that 25-50% of women with PCOS have no demonstrable insulin resistance. Moreover, among all women with insulin resistance, the prevalence of PCOS is relatively low (approximately 15%).[144] Therefore, insulin resistance and hyperinsulinemia are not the primary cause or pathogenic factor in all women with PCOS.

Weight and Energy Regulation

The risk for developing PCOS rises with increasing obesity,[144,145] and [146] as does the severity of insulin resistance, hyperinsulinemia, and ovulatory dysfunction, and the prevalence of metabolic syndrome, glucose intolerance, risk factors for cardiovascular disease, and sleep apnea.100, 147-150

Obesity, by itself, is associated with insulin resistance and compensatory hyperinsulinemia. Insulin resistance is most highly correlated with intra-abdominal obesity, because visceral fat is more active metabolically than subcutaneous fat, more sensitive to lipolysis, releases more free fatty acids, and produces a number of cytokines involved in insulin resistance, such as tumor necrosis factor-α (TNF-α), interleukin-6, leptin, and resistin.[151] The accumulation of free fatty acids in tissues causes lipotoxicity and insulin resistance, in part via TNF-α, which increases serine phosphorylation and thereby inhibits insulin signaling.[152] Insulin resistance due to obesity also induces leptin resistance and decreases adiponectin levels, thereby decreasing fatty acid oxidation and promoting lipotoxicity.[151,153] Obesity in women with PCOS typically is distributed centrally, with a greater increase in visceral than in subcutaneous fat.[154,155,156] and [157] However, even lean women with PCOS have an increased percentage of body fat, a higher waist-hip ratio, and greater intra-abdominal, peritoneal and visceral fat, compared to normal women matched for body mass index (BMI).

The overall prevalence of obesity, and in women with PCOS, varies among different patient populations;[158] in the United States, approximately 35% of all adult women and 60% of women with PCOS are obese.[159,160] However, the overall prevalence of PCOS among different populations is quite similar (approximately 7%).[30,161,162] and [163] Moreover, the prevalence of PCOS among unselected women varies relatively little with increasing BMI: 8.2% in underweight women (BMI < 18.5), 9.8% in normal-weight women, 9.9% in overweight women (BMI 25.0-30.0), 9.0% in obese women (BMI ≥ 30.0), 12.4% in those with a BMI between 35.0 and 40.0, and 11.5% in morbidly obese women (BMI > 40.0).[146] ***Combined, these observations indicate that obesity relates primarily to genetic and environmental factors and is a common, but not essential, feature of PCOS. Obesity contributes modestly to the risk for developing PCOS and adds to the pathophysiology in already affected women by aggravating the degree of insulin resistance and hyperinsulinemia.***[164,165] It also is possible that PCOS itself may, to some degree, predispose to weight gain and obesity.

The prevalence of menstrual irregularity, dysfunctional bleeding, hirsutism, and infertility is higher in obese than in lean women with PCOS,[165,166] and [167] as is the risk for developing glucose intolerance and diabetes.[100,168] Moreover, obese women have a higher prevalence of miscarriage, gestational diabetes, and pre-eclampsia, regardless whether they also have PCOS.[169]

Androgen Synthesis and Action

Hyperandrogenism is the key feature of PCOS, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals.[170] In women with PCOS, approximately 60% of circulating androstenedione derives directly from the ovaries and the remainder from the adrenals; similarly, 60% of circulating testosterone is secreted directly by the ovaries, with most of the remainder deriving from peripheral conversion of androstenedione.[171]

The primary mechanisms driving increased ovarian androgen production in PCOS include increased LH stimulation resulting from abnormal LH secretory dynamics and

increased LH bioactivity, and hyperinsulinemia due to insulin resistance, which potentiates the action of LH and is worsened by obesity. Other evidence indicates that increased ovarian androgen synthesis in women with PCOS probably also relates to the increased volume of theca cells in an expanded ovarian stroma, and to increased sensitivity to LH stimulation,[172,173] possibly due to over-expression of LH receptor in theca and interstitial (stromal) cells.[174] Androgen production remains elevated in long-term cultures of theca cells from women with PCOS,[175] even after suppression of LH levels by treatment with a

GnRH agonist,[176] suggesting that increased androgen production in women with PCOS, to some extent, also reflects an intrinsic dysregulation of key steroidogenic enzmes, such as 3ß-hydroxysteroid dehydrogenase (3ß-HSD) and 17,20-lyase,[104,172,177,178] and [179] which may have a genetic foundation.[180]

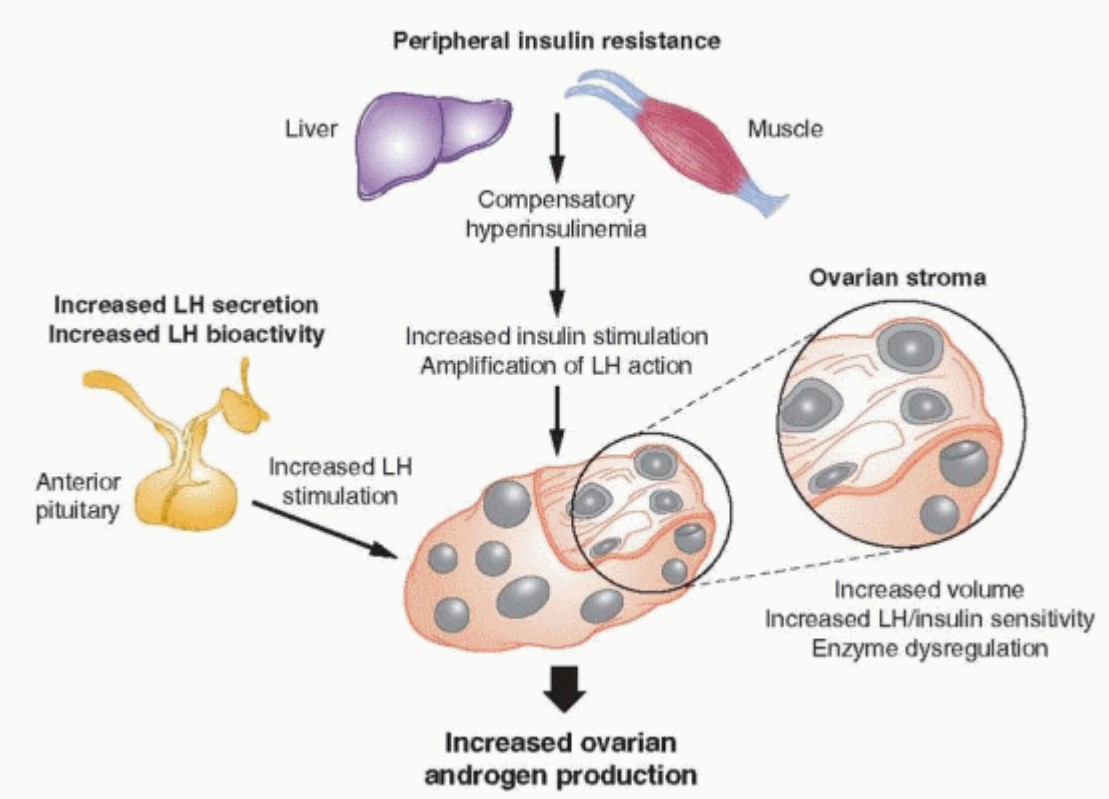
Adrenal androgen production (androstenedione, DHEA, DHEA-S) also is increased in women with PCOS; over half exhibit moderately increased circulating DHEA-S levels.[181] When ovarian synthesis is suppressed by treatment with a long-acting GnRH agonist, adrenal androgen levels remain higher in women with PCOS than in normal women.[92,172,176,182] Adrenal androgens have little or no intrinsic androgenic activity, but contribute to the pathophysiology of PCOS via conversion to testosterone in the periphery.

A number of potential mechanisms for the increase in adrenal androgen production have been investigated, but the explanation remains uncertain. Chronic estrogen stimulation due to anovulation could decrease adrenal 3ß-HSD activity, as it does in the fetal adrenal cortex, but evidence for the mechanism is conflicting.[84,183,184,185] and [186] Increased pituitary ACTH secretion or increased sensitivity to ACTH could provide an explanation, but neither can be demonstrated.[181,187,188] In some, but clearly not all women with PCOS, adrenal androgen excess might result from intrinsic upregulation of P450c17 17,20 lyase activity,[39,135,189,190] and [191] or from hyperinsulinemia.[192,193,194,195] and [196] ***In sum, no one mechanism explains the moderate adrenal androgen excess commonly observed in women with PCOS.***

High local androgen concentrations contribute to the polycystic morphogenesis of the ovaries, via conversion to more potent 5α-reduced androgens, which cannot be aromatized to estrogen and inhibit both aromatase activity and FSH induction of LH receptors on granulosa cells, thereby impeding or preventing progressive follicular development. Granulosa cells obtained from polycystic ovaries are not functionally impaired. They are sensitive to FSH and insulin-like growth factors and produce estrogen,[197,198,199,200] and [201] but cannot generate and maintain the estrogenic follicular milieu required to achieve more advanced stages of development. Consequently, new follicular growth continues but arrests long before full maturation is achieved, resulting in multiple small follicular cysts (typically measuring 2-10 mm in diameter), surrounded by hyperplastic theca cells, which often become luteinized due to increased LH stimulation. Atretic follicles ultimately contribute to an expanding ovarian stroma that increases in volume over time, further increasing the cellular mass producing androgens, in yet another self-propagating cycle that predisposes to chronic anovulation.

The importance of high local ovarian androgen concentrations in the pathophysiology of PCOS is demonstrated by the results of ovarian wedge resection and by observations in women with other conditions associated with hyperandrogenemia. Wedge resection results in a sustained decrease in androgen levels that precedes the return of ovulatory cycles, indicating that high intraovarian androgen concentrations effectively inhibit follicular development and prevent ovulation.[202,203,204] and [205] The success of ovarian wedge resection correlates with the amount of androgen-producing stromal tissue that is removed; even a unilateral oophorectomy can restore menstrual cyclicity and ovulation in anovulatory women with polycystic ovaries.[206] Although laparoscopic procedures such as ovarian “drilling” with an electrosurgical needle or a laser have replaced the classical wedge resection, the results achieved are similar. Polycystic ovaries also have been observed in women with androgen-producing ovarian and adrenal tumors,[207,208] and [209] and in female-to-male transsexuals

treated with exogenous androgens.[210,211] ***These observations again illustrate the important point that polycystic ovaries are not a characteristic feature of a specific endocrine disorder. They result from a functional derangement in follicular development induced or sustained by increased intraovarian androgen levels as a consequence of chronic anovulation, whatever the cause.***



Genetic Considerations

Familial clustering of hyperandrogenism, anvoulation, and polycystic ovaries suggests an underlying genetic basis or cause. At least one group of patients with a heritable X-linked form of PCOS has been described, albeit with a widely varying phenotype.[212] Studies in large families have suggested autosomal-dominant inheritance, with premature balding as the male phenotype.[213,214] Other studies of siblings and parents of women with PCOS have observed a high prevalence of hyperinsulinemia and hypertriglyceridemia, PCOS in females, and premature balding in males.[215,216] Nearly 50% of sisters of women with PCOS have elevated total or bioavailable testosterone concentrations,[217] and approximately 35% of mothers also are affected.[218,219] The first degree relatives of women with PCOS also exhibit other metabolic abnormalities such as dyslipidemia, which may predispose to an increased risk for cardiovascular disease.[220,221,222] and [223] These observations further suggest a genetic predisposition or susceptibility.

Understandably, efforts to identify genes associated with a susceptibility to anovulation and polycystic ovaries have focused on genes relating to the insulin receptor and substrates[224,225] and [226] and the genes encoding the P450 side-chain cleavage (*CYP11*) and P450c17 (*CYP17*) enzymes.[227,228,229,230] and [231] However, it seems likely that PCOS is a polygenic disorder involving the interaction of numerous genomic variants and the influence of environmental factors.[232] Candidate genes include the long list of molecules that participate in any of the metabolic and reproductive pathways affected in the syndrome, emphasizing yet again that PCOS is not a specific endocrine disorder, but a result of chronic anovulation due to a wide variety of causes.

Summary of Key Points

- Polycystic ovary syndrome is not a specific endocrine disorder having a unique cause. Rather, it is a complex disorder wherein numerous genetic variants and environmental factors interact, combine, and contribute to the pathophysiology.
- Polycystic ovaries and the clinical features of polycystic ovary syndrome reflect a functional derangement in follicular development, resulting in chronic anovulation. Because there are many causes of anovulation, there are many causes of polycystic ovaries and the polycystic ovary syndrome.
- Women with polycystic ovary syndrome generally exhibit increased serum LH concentrations, low-normal FSH levels, and increased LH:FSH ratios. The increase in serum LH levels results from abnormal LH secretory dynamics, characterized by increases in LH pulse frequency and amplitude, reflecting the pattern of pulsatile GnRH secretion. The decrease in FSH levels results from the increase in GnRH pulse frequency and from the negative feedback of chronically elevated estrone concentrations (derived from peripheral aromatization of increased androstenedione) and normal or increased levels of inhibin B (derived from small follicles).
- Insulin resistance and compensatory hyperinsulinemia are common features in women with polycystic ovary syndrome and play an important role in the pathophysiology. Up to 35% of women with polycystic ovary syndrome exhibit impaired glucose tolerance and up to 10% meet criteria for type 2 diabetes mellitus.
- Increased LH and insulin stimulation drives ovarian androgen production, and androgens and insulin combine to inhibit hepatic SHBG production, yielding increased free androgen, which aggravates underlying insulin resistance, in a self-propagating positive feedback loop that can increase in severity over time.
- Obesity contributes to the risk for developing polycystic ovary syndrome and adds to the pathophysiology in already affected women by aggravating the degree of insulin resistance and hyperinsulinemia.
- Hyperandrogenism is a major feature of polycystic ovary syndrome, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals. Increased LH and insulin stimulation are the primary mechanisms driving increased ovarian androgen production. Others include an expanded ovarian stoma having increased sensitivity to insulin and LH, and intrinsic dysregulation of key steroidogenic enzymes.
- Polycystic ovary syndrome is a polygenic disorder likely involving the interaction of numerous genomic variants and the influence of environmental factors. Candidate genes include all of the molecules that participate in the affected metabolic and reproductive pathways.

Diagnosis of Polycystic Ovary Syndrome

It is generally accepted that PCOS is not a specific endocrine disease but a syndrome represented by a collection of signs and symptoms, and that no one sign, symptom, or test is diagnostic. Not surprisingly, establishing criteria for diagnosis of PCOS has proven both challenging and controversial.

It has been argued that having a clear and specific definition for PCOS is important because affected women are at increased risk for a variety of problems (infertility, dysfunctional bleeding, endometrial cancer, obesity, type 2 diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disease), because the diagnosis can have health implications for other family

members, and because the need for life-long treatment may affect access to health care insurance in systems like that in the United States.[43,233] ***In our view, the primary advantage to having specific diagnostic criteria for PCOS relates to research, because varying criteria cloud the conclusions and question the generalizability of results from studies involving women with "PCOS." In clinical medicine, simply knowing and understanding the health implications and consequences of chronic anovulation and methods for their effective management are far more important than assigning a specific diagnosis of PCOS.***

The basis for diagnosis of PCOS has changed with time and advances in medicine and related technology. The earliest descriptions of the disorder were based on findings of enlarged ovaries, hirsutism, and menstrual dysfunction.[32] The advent of hormone assays moved the focus to serum gonadotropin and androgen concentrations.[234] More recent advances in ultrasonography and recognition of the importance of insulin resistance in the pathophysiology have turned attention to ovarian morphology[235] and to the metabolic consequences of the disorder.

There have been three separate and distinct efforts to establish or refine the diagnostic criteria for PCOS. The first was a conference sponsored by the National Institute of Child Health and Human Development (NICHD) in 1990, concluding that the major criteria for diagnosis of PCOS (in order of importance) were (1) hyperandrogenism and/or hyperandrogenemia, (2) menstrual dysfunction, and (3) exclusion of other known disorders having a similar clinical presentation.[233] The second was a conference co-sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), convened in Rotterdam, The Netherlands, in 2003, concluding that diagnosis of PCOS should be based on at least two of three major criteria, including (1) oligo/anovulation, (2) clinical or biochemical signs of hyperandrogenism, and (3) polycystic ovaries (as identified by ultrasonography), also excluding other androgen excess disorders.[236,237] The third was a task force appointed by the Androgen Excess and PCOS Society (AE-PCOS) in 2006, concluding that diagnosis of PCOS requires (1) hyperandrogenism (hirsutism and/or hyperandrogenemia), (2) ovarian dysfunction (oligo/anovulation and/or polycystic ovaries), and (3) exclusion of other androgen excess or related disorders.[43]

The original 1990 NICHD diagnostic criteria were based on traditional concepts of PCOS, requiring evidence of *both* hyperandrogenism (hyperandrogenemia and/or hirsutism) and menstrual dysfunction (oligo/amenorrhea). The 2003 ESHRE/ASRM ("Rotterdam") criteria sought to recognize and accommodate a broader spectrum of the disorder, regarding polycystic ovaries as evidence of ovarian dysfunction and including women having *neither* hyperandrogenemia *nor* hirsutism. The 2006 AE-PCOS Society criteria allowed that polycystic ovaries could be considered a sign of ovarian dysfunction, but again emphasized that PCOS is characterized, first and foremost, by hyperandrogenism, including women with *either* oligo/amenorrhea or polycystic ovaries, but excluding those having *neither* hyperandrogenemia *nor* hirsutism.

Ironically, although the purpose of the consensus conferences and task force was to rigorously define PCOS for purposes of research, it can be argued that the differing sets of criteria succeeded only in creating controversy and confusion where clarity was needed most. ***Published clinical trials involving women with PCOS must be carefully reviewed to determine which diagnostic criteria were applied in selecting the study population.***

Hyperandrogenemia

Biochemical evidence of hyperandrogenism is based on the finding of elevated circulating androgen concentrations. Testosterone is the most important androgen produced by the

ovary and the usual basis for diagnosis of hyperandrogenemia. Other androgens that may be elevated in women with PCOS include androstenedione, DHEA, and DHEA-S.

Testosterone levels are elevated in most, but not all, women with PCOS. The free testosterone level is more sensitive for diagnosis of hyperandrogenic disorders,[43] but measurements of free testosterone have several limitations. Direct radioimmunoassays (RIA) for free testosterone are highly inaccurate,[238,239] and [240] particularly in the lower range and in women with decreased SHBG levels.[241] More sophisticated and accurate methods (equilibrium dialysis, gas or liquid chromatography-mass spectrometry) are technically complex, costly, and not widely available.[242] Moreover, testosterone is converted in androgen-sensitive tissues to dihydrotestosterone (DHT), which has a longer duration of action than testosterone, so serum total testosterone concentrations do not necessarily reflect androgen bioactivity.

For purposes of clinical research, the free testosterone concentration can be calculated, using equations derived from the laws of mass action, the serum concentrations of total testosterone, SHBG, and albumin, and the association constants for the interactions of testosterone with SHBG and albumin.[239,243] Calculated values generally correlate well with those determined by equilibrium dialysis,[238,239] although accuracy varies with the specific assays used to measure total testosterone and SHBG. ***For clinical purposes, measurement or calculation of the free testosterone level, or even measurement of the serum total testosterone concentration, usually is unnecessary***.[244] ***In most cases, hirsutism provides ample evidence of hyperandrogenism, and if not severe, sudden in onset, rapidly progressive, or associated with symptoms or signs of virilization, there is little reason for concern about an androgen-producing tumor (*****Chapter 13*****)***.

Measurement of the serum androstenedione concentration could yield evidence of hyperandrogenemia, but limited data suggest that levels are elevated in less than 20% of women with PCOS.[29] Measurement of serum DHEA also has little or no diagnostic value because levels are relatively low, exhibit a diurnal pattern and high between-subject variability, and are sensitive to stress.[43,245]

The serum DHEA-S concentration is the traditional marker for adrenal androgen excess,[246,247] and [248] because it derives almost exclusively from the adrenal,[249,250] and [251] and concentrations are relatively high and remain stable across the day and cycle.[252,253] Overall, the serum DHEA-S concentration is moderately elevated in over half of women with PCOS.[181] Some have an isolated increase in serum DHEA-S, suggesting a deficiency in 3β-HSD, but no genetic mutation in the enzyme has been found.[254,255] ***Although the AE-PCOS Society regards an elevated serum DHEA-S level as sufficient evidence of hyperandrogenism to support the diagnosis of PCOS,***[43] ***the test has very limited or no clinical value in our view***. First, the test lacks both sensitivity and specificity for identifying women with adrenal causes of hyperandrogenism.[256] Second, DHEA-S, like DHEA and androstenedione, has little or no intrinsic androgenic activity and requires conversion to testosterone to exert androgenic effects. Third, the DHEA-S concentration can be grossly elevated (≥700 μg/dL) in women with rare androgen-secreting tumors, but in almost all such patients, the serum testosterone level also is greatly elevated,[257] due to peripheral conversion of high circulating DHEA-S levels, or because the tumor also secretes testosterone.

Clinical Hyperandrogenism

Clinical evidence of hyperandrogenism includes hirsutism, acne, and androgenic alopecia, all of which relate to the effect of androgens on the pilosebaceous unit. Because the sensitivity of the pilosebaceous

unit varies significantly among individuals, the correlation between these clinical features and biochemical measures of hyperandrogenism is relatively poor.[258,259]

P.514

Hirsutism is the growth of terminal hairs on the face or body in a male pattern. ***Hirsutism is the most obvious clinical indicator of androgen excess and is an important feature of PCOS***. Whereas hirsutism affects 65-75% of White, Black and Southeast Asian women,[43,260] its prevalence is lower in racial or ethnic groups having relatively little body hair.[260,261] and [262] The modified Ferriman-Gallwey score is the most common method for grading the extent of hirsutism, assigning a score from 0-4 in each of 9 androgen-sensitive areas, as illustrated and described in Chapter 13.[263,264] The threshold value that defines hirsutism is not firmly established, but generally has ranged between 6 and 8.[29,260,264] ***The modified Ferriman-Gallwey score is the accepted standard for assessing the severity of hirsutism in clinical investigations. However, in clinical practice, the easiest and most practical way is to determine the method and frequency of hair removal (e.g., shaving, plucking, waxing), which also provides a clinically relevant measure for assessing the response to treatment.***

Acne can be another manifestation of hyperandrogenism. Like hirsutism, its prevalence among women with PCOS varies with ethnicity. The prevalence of acne is 12-14% among White women with PCOS,[159,161,262] higher in Asian Indians and women of Mediterranean descent (approximately 25%),[262,265] and lower among Pacific Islanders.[261] However, it is unclear whether acne is any more prevalent among women with PCOS than in the general population. Approximately 20% of women under age 20, 15% of those ages 20 to 30, and 10% of women ages 30 to 40 complain of acne.[266,267,268,269] and [270] The extent to which PCOS may increase risk for developing acne, if at all, is therefore uncertain.

Androgenic alopecia, describing scalp hair loss in women, also can result from hyperandrogenism and is a recognized, but uncommon, feature of PCOS;[23,159,271,272] and [273] less than 5% of women with PCOS complain of hair loss. Typically, the hair loss is limited to the crown and does not involve the frontal hair line.[272,274] Androgenic alopecia may be more common than is recognized, because 25% or more of scalp hair must be lost before thinning becomes apparent.[159,274]

Ovulatory and Menstrual Dysfunction

Normal cyclic menses result from normal ovulatory function. The normal inter-menstrual interval ranges between 24 and 35 days and menses that occur less or more often are an indication of ovulatory dysfunction. Cyclic menses occurring at normal intervals strongly suggest, but cannot be regarded as proof of ovulation.

The majority of women with PCOS, approximately 60-85%, exhibit gross menstrual dysfunction.[43,158,161] The most common abnormalities are oligomenorrhea and amenorrhea. Polymenorrhea (regular cycles occurring at intervals less than 25 days) is very uncommon, observed in less than 2% of untreated women with PCOS.[159] Classically, menstrual dysfunction in women with PCOS has a premenarcheal onset, but many report regular cycles for varying intervals preceding the onset of oligo/amenorrhea.

In general, anovulatory women seldom have regular menses.[275] However, regular cycles are somewhat more common in anovulatory hyperandrogenic women.[43,276] In studies of menstrual function in women with hyperandrogenism, approximately 15-40% are eumenorrheic, despite evidence of oligo-anovulation.[277,278,279] and [280] The prevalence of eumenorrhea among women with PCOS is significantly increased if the Rotterdam diagnostic criteria are applied, because hirsute eumenorrheic women with

polycystic ovaries are included. The absence of any recognizable pattern of premenstrual molimina suggests anovulation in eumenorrheic women.

P.515

Polycystic Ovaries

PCOS takes its name from the enlarged polycystic ovaries so commonly observed in women with hyperandrogenic chronic anovulation.[32] Observations of mild hyperandrogenemia and insulin resistance in some asymptomatic women with polycystic ovaries provided the rationale for including polycystic ovaries among the Rotterdam diagnostic criteria for PCOS, as a sign of ovarian dysfunction.[85,281,282,283] and [284]

Polycystic ovaries typically exhibit increased size and stromal volume and an increased number of small follicles. The Rotterdam criteria consider only the total number of follicles, requiring 12 or more measuring 2-9 mm in diameter (mean of both ovaries).[236,237,285,286] and [287] Others have defined polycystic ovaries on the basis of volume (>7.0-7.5 mL) and architecture.[288,289] The prevalence of polycystic ovaries is quite high among women with androgen excess (>80%).[235,277,290,291,292,293,294] and [295] ***However, from 8% to 25% of normal women, and even 14% of women using oral contraceptives, also meet the ultrasonographic criteria for polycystic ovaries.***[281,] ***and*** [296,297,298] ***and*** [299] [300] Moreover, polycystic ovaries are commonly observed during normal pubertal development, and even in women with hypothalamic amenorrhea and hyperprolactinemia.[301,302]

The 2003 Rotterdam diagnostic criteria expanded the population of women that might be assigned a diagnosis of PCOS by approximately 50%, compared to the criteria earlier recommended by the NICHD, due entirely to the inclusion of polycystic ovaries.[303] The change in criteria ignited considerable controversy, primarily because polycystic ovaries are so commonly observed in normal women and in other conditions. Moreover, the finding, by itself, has little clinical significance. ***Otherwise normal women with polycystic ovaries generally have regular menstrual cycles, exhibit normal serum gonadotropin and ovarian steroid hormone levels, and are not infertile.***[85,297,304,305] ***and*** [306]

Again, the important point is that PCOS is a functional disorder in which polycystic ovaries result from chronic anovulation. Although present in most women with chronic hyperandrogenic anovulation, polycystic ovaries do not establish and are not required for diagnosis of PCOS.[163,182,296]

Other Features of the Polycystic Ovary Syndrome

PCOS has other common features besides hyperandrogenism and ovulatory dysfunction that are not included in any diagnostic criteria, including abnormal patterns of gonadotropin secretion, insulin resistance, and related metabolic abnormalities, such as dyslipidemia.

Abnormal Gonadotropin Secretion

Abnormal patterns of gonadotropin secretion have long been recognized as a common characteristic of women with PCOS. As discussed earlier in the section of this chapter devoted to the pathophysiology of the disorder, increased serum LH concentrations, lownormal FSH levels, and increased LH:FSH ratios are typical, but more so in lean than in obese women with PCOS. In the past, an increased LH:FSH ratio (e.g., >2:1) has been regarded as a marker of PCOS, but the ratio varies with the assays used to measure gonadotropin concentrations, and the prevalence of obesity is high among women with PCOS.[7,60,] [307,308] and [309] ***Consequently, gonadotropin levels or ratios are not a reliable diagnostic criterion; they neither make, nor exclude, the diagnosis.***

P.516

Insulin Resistance

Insulin resistance and hyperinsulinemia are common but not universal features of women with PCOS, no matter what method is used to assess insulin sensitivity.[92,94,310] ***The overall prevalence of insulin resistance among women with PCOS is between 50% and 75%, and greater in obese than in lean women with PCOS***.

Most women with PCOS and insulin resistance are young and have ample pancreatic ß-cell reserve. Consequently, they are able to generate a compensatory hyperinsulinemia, allowing them to maintain normal glucose homeostasis, at least in the fasting state.[100] Although most therefore display an exaggerated insulin response to a glucose challenge, some also exhibit evidence of ß-cell dysfunction,[311,312] and [313] particularly those having a family history of type 2 diabetes mellitus.[314]

Whereas there is no debate that insulin resistance and hyperinsulinemia play an important role in the pathophysiology of PCOS, or that the prevalence of unrecognized diabetes is sufficiently high to warrant testing to exclude the diagnosis in women with PCOS, the practical importance of detecting insulin resistance and what tests, if any, should be performed for that purpose remain highly controversial.

The gold standard method for measuring insulin sensitivity, to which all other methods are compared, is the ***hyperinsulinemic euglycemic clamp***.[315] The technique involves a fixedrate intravenous infusion of insulin and a simultaneous intravenous glucose infusion, varying the rate as needed to establish a steady state plasma glucose level within the normal fasting range. The glucose infusion rate at steady state estimates the rate of glucose uptake in tissues at the defined plasma insulin concentration and is inversely proportional to the degree of insulin resistance; the lower the glucose infusion rate at steady state, the greater the degree of insulin resistance. Insulin sensitivity is defined as the ratio of the glucose disposal rate to the steady state insulin concentration (glucose disposal rate [mmol/kG] × min per mU/L × 100). The clamp technique and other methods involving intravenous infusions of glucose and/or insulin (e.g., the frequently sampled intravenous glucose tolerance test, the insulin sensitivity test, the insulin tolerance test, and the continuous infusion of glucose with model assessment) have been used extensively in clinical investigations of glucose and insulin dynamics. However, they have no real practical clinical application because they are time-consuming, invasive, costly, and require experienced personnel.

The complexities of clamp techniques and other methods requiring intravenous infusions and multiple blood samplings spurred efforts to find an uncomplicated and inexpensive quantitative method for evaluating insulin sensitivity. A number of fasting state (homeostatic) measures have been described, all based on the fasting glucose and insulin concentrations and using straightforward calculations.[316] One common weakness that all such methods have is that they assume a linear relationship between glucose and insulin that is, in fact, parabolic.

The ***fasting serum insulin concentration*** is easy to obtain and requires no calculations;[317] in euglycemic White women with PCOS, values greater than 20-30 mU/mL suggest insulin resistance. The ***fasting glucose/insulin ratio*** has been used widely as an index of insulin sensitivity in women with PCOS; a ratio less than 4.5 has reasonable sensitivity and speci-ficity for insulin resistance.[94] The ***homeostatic model assessment of insulin resistance (HOMA-IR)*** is another measure of insulin sensitivity commonly used in larger epidemiologic studies. The HOMA-IR is calculated by dividing the product of the fasting glucose (mg/dL) and insulin (mU/mL) concentrations by a constant: [glucose (mg/dL)][insulin (mU/mL)]/405, or [glucose (mmol/L)][insulin (mU/mL)]/22.5.[318,319] The HOMA-IR value correlates relatively well with results from clamp studies,[320,321] and unlike the fasting insulin concentration and the glucose/insulin ratio, compensates for fasting hyperglycemia;

values greater than 3.2-3.9 generally indicate insulin resistance.[43,95,322] The ***quantitative insulin sensitivity check index (QUICKI)*** is yet another method for assessing insulin sensitivity in clinical investigations. Like the HOMA-IR, QUICKI can be applied in both euglycemic and hyperglycemic patients.[323] The QUICKI value is the inverse of the sum of the fasting glucose and insulin concentrations, expressed logarithmically: (1/[log(Glucose)+log(Insulin)]); values greater than 0.33 indicate insulin resistance.[43,324] Still other methods use a weighted combination of the fasting insulin and triglyceride concentrations and BMI to estimate insulin sensitivity.[315] All of the calculated indices have limitations, primarily the lack of a standardized insulin assay. ***As the sheer number of different measures of insulin resistance demonstrates, there is no uniformly accepted test for measuring insulin resistance in a clinical setting. Consequently, routine screening for insulin resistance is not recommended.***

The standard ***oral glucose tolerance test*** (OGTT) is the mainstay of methods for diagnosis of impaired glucose tolerance and diabetes mellitus and also can be used to assess insulin sensitivity, when indicated (discussed below). Although techniques vary, all involve measures of plasma glucose and insulin at intervals over 2 to 4 hours after a 75-g or 100-g oral glucose load. ***A baseline 2-hour OGTT is recommended for all women with PCOS, as up to 35% exhibit impaired glucose tolerance and up to 10% have diabetes mellitus.***[99,237,325,326]

Screening for glucose intolerance also is recommended for girls with premature adrenarche or menstrual irregularity that persists for more than 2 years after menarche because hyperinsulinemia often is the cause and they are at high risk for developing diabetes and severe hyperandrogenism.[327,328,329] and [330] In this population, specific screening for insulin resistance also is warranted because evidence indicates that early intervention in those affected can prevent progressive debilitating disease.[329,331,332] Specific screening for insulin resistance also is recommended for women with markely elevated serum androgen levels (≥150 ng/dL), to differentiate the severe insulin resistance syndromes (discussed below) from androgen-secreting tumors.

Interpretation	*2-hour Glucose*	*2-hour Insulin*[43]
Normal	<140 mg/dL	
Impaired glucose tolerance	140-199 mg/dL	
Diabetes mellitus	≥200 mg/dL	
Normal		<80-100 mU/mL
Insulin resistance		>80-100 mU/mL
Severe insulin resistance		>300 µU/mL

Dyslipidemia

Dyslipidemia is perhaps the most common metabolic abnormality observed in women with PCOS. Applying the National Cholesterol Education Program guidelines, nearly 70% have at least one borderline or elevated lipid level,[333] although many women with PCOS have entirely normal lipid profiles.[334,335,336] and [337] Insulin resistance and hyperinsulinemia are associated with decreased high-density lipoprotein (HDL) cholesterol and elevated triglyceride levels, and numerous studies have observed such abnormalities in

women with PCOS.[334,338,339] Some also have observed elevated low-density lipoprotein (LDL) concentrations,[333,335,336] and [337] which are not usually associated with insulin-resistant states and may result from hyperandrogenism or reflect a genetic or dietary influence.[223,340,341]

P.518

Obesity

Obesity is a common feature of PCOS. The prevalence of obesity is approximately 50% overall,[159] but varies significantly with country of origin. The prevalence is highest in the United States, probably reflecting the higher overall prevalence of obesity;[161] in other countries, women with PCOS generally are leaner.[294,342,343] and [344]

The risk for PCOS increases with obesity.[145,345] Although the effect appears relatively modest,[146] it is clear that obesity adds to the pathophysiology of PCOS in affected or predisposed women by aggravating the degree of insulin resistance and hyperinsulinemia.[164, 165] As discussed earlier in the section of this chapter devoted to the pathophysiology of PCOS, high insulin levels stimulate ovarian androgen production and suppresses hepatic SHBG production, thereby increasing bioavailable androgen levels. In turn, high androgen concentrations and chronically elevated estrogen levels (derived from aromatization of androgens in adipose), help to induce or perpetuate an abnormal pattern of gonadotropin secretion (increased LH, low FSH) by increasing LH pulse frequency and amplitude and inhibiting FSH secretion.

Exclusion of Other Androgen Excess Disorders

PCOS is a diagnosis of exclusion, after considering and eliminating other causes of chronic anovulation (primarily thyroid disorders and hyperprolactinemia) and androgen excess. Together, congenital adrenal hyperplasia, androgen-secreting tumors, severe insulin resistance syndromes, Cushing syndrome, and idiopathic hirsutism account for about 10-30% of hyperandrogenism in women.[159,274,279,346] ***Whereas all should be considered and excluded, few actually warrant specific testing.***

Thyroid Disorders

Thyroid disorders are associated with menstrual dysfunction and also can have serious adverse impact on pregnancy outcomes and child development.[347,348,349,350,351] and [352] ***The overall high prevalence of thyroid dysfunction in women warrants specific testing to exclude the diagnosis (serum thyroid-stimulating hormone, TSH) in all anovulatory women, including those with hyperandrogenism, but not for diagnosis of PCOS.***

Hyperprolactinemia

Hyperprolactinemia is highly associated with menstrual dysfunction and is one of the most common causes of secondary amenorrhea. The many causes of hyperprolactinemia are considered at length elsewhere in this text (Chapters 11 and 16). Hyperprolactinemia is associated with increased adrenal androgen production *in vivo* and *in vitro*,[353,354] but its prevalence among women who present with hyperandrogenism is quite low, and generally less than 3%.[159,274,342,346,355,356,357] and [358] ***The high prevalence of hyperprolactinemia among women with menstrual dysfunction justifies specific testing to exclude the diagnosis in all anovulatory women, but not for diagnosis of PCOS.***

P.519

Nonclassical Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is caused by adrenal steroidogenic enzyme defects that result in excessive adrenal androgen production. The most common cause is 21-hydroxylase deficiency; other enzyme defects (11ß-hydroxylase, 3ß-hydroxysteroid dehydrogenase) are relatively rare. In all, the pathophysiology stems from decreased cortisol production, which stimulates a compensatory increase in pituitary ACTH secretion, causing adrenal hyperplasia; increased levels of steroid hormones proximal to the enzyme block seek an alternative metabolic pathway, resulting in increased production of androgens. The disorder is inherited in an autosomal recessive fashion and is discussed in detail in Chapters 9, 10, and 13.

Females with classical CAH (both salt-wasting and simple virilizing forms) typically present at birth with ambiguous genitalia,[359,360] and [361] and thus would rarely be confused with PCOS, but those with the nonclassical or "late-onset" form of CAH present later, during childhood or early adolescence with precocious puberty, or as young adults with signs of hyperandrogenism, very much like those with PCOS.[362] ***Whereas it is logical to recommend that nonclassical CAH be excluded specifically in all women with hyperandrogenism,[43] we believe that specific testing can be safely reserved for those having an early onset of hirsutism (pre-or perimenarcheal, including girls with premature adrenarche), women with a family history of the disorder, and those in high-risk ethnic groups (Hispanic, Mediterranean, Slavic, Ashkenazi Jewish, or Yupic Eskimo heritage).*** The yield from routine screening is very low, because the disorder is uncommon.[158,159,363] The prevalence of nonclassical CAH among American White and Hispanic women with hyperandrogenism is between 1 and 4%.[364] In other countries, the reported prevalence has ranged from as low as 0.3% among Northern Italians to as high as 6-10% among women from Israel, India, and Jordan.[365,366] Moreover, a diagnosis of nonclassical CAH generally will not change the best choice of treatment, because glucocorticoids are less effective than estrogen-progestin contraceptives and/or antiandrogens for the treatment of chronic anovulation and hirsutism in women with nonclassical CAH.[367,368] Whereas it is important to identify women at risk for conceiving a child with the more severe classical form of the disorder, the risk among women with hyperandrogenism is limited to those who carry one classic mutation and a variant allele associated with mild enzyme deficiency (compound heterozygotes), also having a male partner who carries an occult classic mutation. Data from neonatal screening programs for detection of classical CAH indicate that the overall prevalence of classical CAH is approximately 1 in 15,000 live births and varies with ethnicity, ranging from 1 in 28,000 Chinese[369] and between 1 in 5,000 and 1 in 23,000 Caucasians,[370,371] to as high as 1 in 280 Yupic Eskimos.[372] In the United States, the prevalence of classical CAH is 1 in 15,500 White and 1 in 42,000 African Americans.[373]

Regardless whether universal or selective screening for nonclassical CAH is performed, a follicular phase morning serum 17-OHP concentration less than 200 ng/dL excludes, and a level greater than 800 ng/dL all but establishes the diagnosis.[374,375] and [376] Concentrations between the two threshold values suggest the possibility, which can be confirmed by performing an ACTH stimulation test, obtaining blood samples before and 60 minutes after administering cosyntropin (synthetic ACTH 1-24; 0.25 mg intramuscularly, or intravenously); in most women with nonclassical CAH, the 17-OHP concentration will rise above 1,500 ng/dL.[363,365,377]

Androgen-Secreting Ovarian and Adrenal Tumors

Androgen-secreting ovarian and adrenal tumors are rare. The prevalence of ovarian androgen-producing tumors is between 1 in 300 and 1 in 1,000 among women with hyperandorgenism.[159,346,357,378] Androgen-secreting adrenal tumors are even less common.[356]

P.520

In addition, androgen-secreting tumors almost always are accompanied by severe or rapidly progressive hirsutism or symptoms or signs of virilization (deepening of the voice, temporal or male pattern balding, breast atrophy, increased muscle mass, and clitoromegaly). ***The possibility of a tumor is excluded primarily by the clinical history and physical examination. Very few women will require specific evaluation to exclude the diagnosis.***

The recommended evaluation for women suspected of having an androgen-secreting tumor is discussed at length in Chapter 13 and briefly summarized here. A ***serum total testosterone concentration*** greater than 150 ng/dL identifies almost all women with a potential androgen-producing tumor. However, a tumor still should be suspected and excluded in women with rapidly progressive hirsutism or signs or symptoms of virilization, even when the serum testosterone concentration is below the threshold value. ***Transvaginal ultrasonography*** will identify almost all solid ovarian mass lesions, although very small tumors located in the hilar region can escape detection. ***Adrenal computed tomography (CT)*** is extremely sensitive for detecting rare androgen-secreting adrenal tumors, most of which are malignant. ***Selective ovarian venous catheterization*** can be considered for the rare patient having no demonstrable ovarian or adrenal mass lesion, but should be reserved only for those in whom a tumor is strongly suspected.

Severe Insulin Resistance Syndromes

Severe insulin resistance is a specific characteristic of a variety of uncommon clinical disorders. The type A insulin resistance syndrome results from defects in the insulin receptor and affects primarily lean women. The type B syndrome is an autoimmune disorder affecting the insulin receptor. The type C syndrome is a variant of type A and is characterized by marked acanthosis nigricans, hyperandrogenism, obesity, and the absence of insulin receptor defects, and also is known as the hyperandrogenic-insulin resistant-acanthosis nigricans (HAIR-AN) syndrome. Other rare disorders involving severe insulin resistance include leprechaunism, the Rabson-Mendenhall Syndrome, and a variety of lipodystropic syndromes.[43,379]

Although the type C syndrome might reasonably be viewed as a severe form or phenotype of PCOS, the more profound insulin resistance and related metabolic abnormalities in the syndrome distinguishes the two.[380,381] Ovarian hyperthecosis, characterized by distinct clusters of luteinzed theca cells scattered throughout the ovarian stoma and associated with severe hyperandrogenism,[382,383] frequently is observed in women with severe insulin resistance syndromes. Skin tags and acanthosis nigricans (a gray-brown, velvety, sometimes verrucous, discoloration of the skin, usually involving the neck, groin, axillae, and the area beneath the breasts) are other common features of the severe insulin resistance syndromes. The mechanism responsible for their development is uncertain.

Although specific diagnostic criteria for the severe insulin resistance syndromes have not been established, the diagnosis can be substantiated by findings of markedly elevated levels of insulin, typically greater than 80 mU/mL fasting, or greater than 300 mU/mL 2 hours after an oral glucose load.[380,381] As might be expected, most patients will have normal glucose levels in the early stages of the disorder, but are at high risk to develop β-cell failure, diabetes, and dsylipidemia. Accordingly, they require careful long-term follow-up and treatment.

Cushing Syndrome

Cushing syndrome results from excess adrenal cortisol secretion and can be ACTH-dependent (pituitary and ectopic ACTH-secreting tumors) or ACTH-independent (adrenal adenomas,

P.521

exogenous glucocorticoid treatment). ***The disorder has features commonly observed in women with***

PCOS, including menstrual dysfunction, hyperandrogenism, and central obesity. However, the prevalence of Cushing syndrome in women presenting with hyperandrogenism is extremely low, well below 1%.[158,159,274,346,384] ***Consequently, routine screening is not justified and should be limited to the very few patients who also have distinct signs and symptoms of hypercortisolism.*** These include hypertension, severe fatigue and muscle weakness, atrophy of the skin and subcutaneous tissue (easy bruising and purple striae on the abdomen and flanks), hyperpigmentation (caused by excess secretion of α-melanocyte-stimulating hormone, as a byproduct of ACTH synthesis from pro-opiomelanocortin, the common precursor molecule) in areas most exposed to light (the face, neck, and back of the hands) or chronic mild trauma, friction, or pressure (the elbows, knees, knuckles, and shoulders), diabetes, and cognitive impairment.

Methods of screening for Cushing syndrome, specific tests for those who screen positive, and evaluation to differentiate among the causes of Cushing syndrome are discussed in detail in Chapter 13. ***The overnight dexamethasone suppression test is the best single screening test because of its simplicity and ability to discriminate.*** The test is performed by administering 1.0 mg of dexamethasone between 11:00 P.M. and midnight and measuring the serum cortisol at 8:00 A.M. the next morning; values less than 1.8 mg/dL are normal.[385]

Idiopathic Hirsutism

Idiopathic hirsutism is defined classically as hirsutism accompanied by normal ovulatory and menstrual function, in the absence of hyperandrogenemia. Using that definition, the prevalence of idiopathic hirsutism among hirsute women is approximately 5-7%.[158,159,279,280,386] If the 2003 Rotterdam criteria for diagnosis of PCOS are used, the definition would also include the absence of polycystic ovaries, further decreasing the prevalence of idiopathic hirsutism.

By definition, diagnosis of idiopathic hirsutism requires measurement of serum androgen levels, which otherwise is not necessary for those with mild hirsutism (Chapter 13). It is generally assumed that idiopathic hirsutism results from increased peripheral 5ß-reductase activity, which amplifies the action of normal circulating testosterone concentrations via increased intracellular conversion to the more potent androgen, dihydrotestosterone (DHT). Given that many hirsute eumenorrheic women will be found oligo-ovulatory on closer scrutiny, a test of ovulation (e.g., serum progesterone during the putative luteal phase) further helps to differentiate women with PCOS from those with idiopathic hirsutism.

Exclusion of Androgen Excess Disorders other than PCOS

Diagnosis	*Method of Exclusion*
Nonclassical CAH	Follicular phase morning serum 170HP <2ng/mL (Early onset hirsutism, family history of CAH, high- risk ethnicity)
Androgen-secreting tumor	Primarily by clinical history and physical examination; serum testosterone
Servere insulin resistance syndrome	Primarily by clinical history and physical examination; 2-hour OGTT (glucose, insulin levels)

Cushing syndrome	Primarily by clinical history and physical examination; overnight dexamethasone suppression test
Idiopathic hirsutism	Menstrual history, serum progesterone (putative luteal phase), serum testosterone

P.522

Summary of Key Points

- Polycystic ovary syndrome is not a specific endocrine disease but a syndrome represented by a collection of signs and symptoms, and no one sign, symptom, or test is diagnostic.
- Diagnosis of polycystic ovary syndrome is based primarily on the clinical history and physical examination. The major clinical features of polycystic ovary syndrome are hyperandrogenism and menstrual dysfunction.
- Although present in most women with chronic hyperandrogenic anovulation, polycystic ovaries do not establish and are not required for diagnosis of polycystic ovary syndrome.
- Gonadotropin levels or ratios are not a reliable criterion for diagnosis of poly-cystic ovary syndrome.
- Knowing and understanding the health implications and consequences of chronic anovulation and methods for their effective management are far more important than assigning a specific diagnosis of PCOS.
- Evaluation of women with suspected polycystic ovary syndrome should include:

1. Serum thyroid-stimulating hormone (TSH)
2. Serum prolactin
3. 2-hour oral glucose tolerance test
4. Fasting lipid profile
5. Endometrial sampling (in women whose history indicates potential long-term exposure to unopposed estrogen stimulation)
6. Serum testosterone (in women with moderate or severe hirsutism)
7. Morning follicular phase serum 17-hydroxyprogesterone (in women with a pre- or perimenarcheal onset of hirsutism, a family history of congenital adrenal hyperplasia, or high-risk ethnicity)
8. Overnight dexamethasone suppression test (in women with signs or symptoms of hypercortisolism)

Clinical Management

The management of women with PCOS should seek to correct or prevent both its immediate and longer-term clinical consequences, which may include all of the following:

- Menstrual abnormalities.

- Increased risk for developing endometrial hyperplasia and neoplasia.
- Hyperandrogenism (hirsutism, acne, alopecia).
- Infertility.
- Increased risk for developing type 2 diabetes.
- Increased risk for developing cardiovascular disease.

P.523

In many cases, lifestyle changes will be an important part of the clinical management, requiring careful education, counseling, encouragement, and follow-up. For patients having no immediate desire to attempt pregnancy, estrogen-progestin contraceptives provide effective management for menstrual dysfunction and protect against the risk for development of endometrial hyperplasia and cancer. Estrogen-progestin contraceptives and antiandrogens help to prevent or decrease hyperandrogenism. Those seeking to conceive are candidates for ovulation induction. Women with impaired glucose intolerance at risk for developing type 2 diabetes or having features of the metabolic syndrome, indicating a high risk for developing cardiovascular disease, may warrant treatment with insulin sensitizing agents or other medications aimed specifically at reducing those risks. The important point to emphasize is that women with chronic anovulation require comprehensive clinical management that addresses their immediate needs, but also considers their longer-term health and incorporates appropriate risk reduction strategies.

Lifestyle Changes

The strong association between obesity, hyperandrogenism, impaired glucose tolerance, menstrual abnormalities, and infertility emphasizes the importance of addressing lifestyle issues in women with PCOS, focusing on nutrition and exercise. At least 50% of women with PCOS are obese. ***It is important to stress that even a small reduction in weight (2-5%) can result in significant improvements in metabolic and reproductive function***.[387,388,389,390,391] ***and*** [392] The loss of abdominal fat may be the best predictor of the effects of weight loss.

Weight reduction is the first best treatment for obese women.[393] Weight loss increases SHBG concentrations, thereby reducing free androgen levels and decreasing androgen stimulation of the hair and skin. Weight loss also improves ovulatory function, thereby increasing conception rates and also possibly decreasing the risk for miscarriage. ***A significant overall decrease in caloric intake is more important than the specific composition of the diet; there is no compelling evidence to indicate that a low carbohydrate diet is better than a low fat diet***.[394,395] ***and*** [396] ***Although treatment with metformin can facilitate weight loss***,[390,397,398] ***and*** [399] ***primarily by suppressing appetite***,[400] ***the overall effect is modest and inconsistent***.[401,402,403,404,405,406,407,408,409] ***and*** [410] ***Consequently, metformin should not be used primarily for the purpose of weight reduction***.

The benefits of exercise for improving diabetes and cardiovascular health have been demonstrated in the general population. Incorporation of moderate activity into daily activities appears as effective for reducing the risk of developing diabetes and cardiovascular disease as that achieved with vigorous physical activity, is more likely to be sustained, and is essential for maintaining weight loss over time.[411]

Menstrual Abnormalities and Risk for Developing Endometrial Cancer

Oligomenorrhea is the most common presentation of women with chronic anovulation, although many present with amenorrhea or dysfunctional uterine bleeding, and some even have regular menses. The

typical patient presents with irregular or infrequent menses or with amenorrhea, making any formal assessment of ovulatory function (e.g., basal body temperature, serum progesterone measurement) unnecessary. The overall number of menstrual cycles is less important than preventing abnormal bleeding and the other potential consequences of chronic anovulation. The evaluation and treatment of amenorrhea are discussed in depth in Chapter 11. Dysfunctional uterine bleeding is the focus of Chapter 15.

P.524

Chronic anovulation, obesity, and hyperinsulinemia all are associated with risk for developing endometrial cancer.[412,413,414,415] and [416] Presumably, the mechanism relates to constant, unrelenting estrogen stimulation of the endometrium, predisposing to abnormal patterns of growth. Endometrial hyperplasia, and even endometrial cancer can be encountered in young anovulatory women.[417,418] and [419] Overall, the risk for developing endometrial cancer may be increased by as much as 3-fold. Consequently, for those with long-standing anovulation, endometrial sampling to exclude endometrial hyperplasia is a prudent precaution. ***The decision on whether to perform an endometrial biopsy should not be based on the patient's age, but on the duration of potential exposure to unopposed estrogen stimulation.*** Whereas a grossly increased endometrial thickness (greater than 12 mm) clearly suggests the possibility of endometrial hyperplasia,[420] a normal thickness does not exclude the diagnosis.[421,422]

Estrogen-progestin contraceptives are the most common treatment for the menstrual abnormalities associated with chronic anovulation because they induce regular cyclic menses and attenuate endometrial growth, thereby preventing dysfunctional uterine bleeding and also eliminating the risk for developing endometrial hyperplasia and neoplasia. In those who refuse or have a contraindication to the use of estrogen-progestin contraceptives, the same can be achieved with cyclic or continuous treatment with progestins alone. However, progestin treatment forfeits some of the other important actions of estrogen-progestin contraceptives that help in the treatment of hyperandrogenism, as discussed below. Metformin is another alternative that can restore ovulatory menses in many women with PCOS. However, results vary widely and may require up to 6 months of treatment before they are known.[423,424,425,426,427,428,429] and [430]

Hirsutism

True virilization is rare, but nearly 70% of anovulatory women complain of cosmetically disturbing hirsutism, the severity relating primarily to the level of hyperandrogenemia, but also to the genetic sensitivity of the individual's hair follicles to androgens. Hirsutism is more common in obese anovulatory women, because free androgen levels increase with BMI, due to insulin resistance, hyperinsulinemia, and the combined inhibitory effects of insulin and androgens on hepatic SHBG production. Skin and hair disorders can be both physically and psychologically very damaging. The spectrum of treatments for hirsutism is discussed in Chapter 13 and summarized here.

Mild focal hirsutism can be managed effectively with cosmetic measures (shaving, plucking, waxing, depilatories), but most who present with a complaint of hirsutism are already using one or more such methods and will require treatment. Medical management options include primarily estrogen-progestin contraceptives and antiandrogens (e.g., spironolactone).

Estrogen-progestin contraceptives are an effective treatment for hirsutism primarily because they suppress LH-dependent ovarian androgen production and stimulate hepatic SHBG production.[431,432,433,434,435] and [436] Some have questioned the wisdom and safety of estrogen-progestin contraceptives in women with PCOS, primarily because they have been associated with modest decreases in insulin sensitivity in some studies.[437,438,439,440] and [441] However, the overall weight of available evidence supports their safety in women with PCOS, with and without insulin resistance.[407,442,443,444,445,446,447,448,449,450,451] and [452]

Antiandrogens are effective for the treatment of hirsutism, but generally should be used in combination with an estrogen-progestin contraceptive or another highly reliable method (e.g, an intrauterine device) because of their potential to adversely affect sexual development in a male fetus if the patient were to conceive unexpectedly. Options include spironolactone (50-100 mg twice daily),[453,454] cyproterone acetate (12.5-100 mg daily, or in combination oral contraceptives containing the progestin),[455] and flutamide (62.5 mg daily).[456]

P.525

Although insulin sensitizing agents (metformin, thiazolidinediones) decrease circulating insulin and androgen levels in women with PCOS,[110,457,458,459,460,461,462] and [463] a systematic review including 9 placebo-controlled trials concluded that they have no important benefits for the treatment of hirsutism,[464] and guidelines issued by the Endocrine Society suggest against their use for the treatment of hirsutism.[244]

Infertility

Chronic anovulation is one of the most common causes of infertility. In women with PCOS, other factors relating to oocyte quality or endometrial and implantation abnormalities also might contribute.[465] Infertile anovulatory women who want to conceive are candidates for ovulation induction. Methods for ovulation induction are the subject of Chapter 31 and are outlined briefly here.

The first drug of choice is clomiphene citrate, which is typically administered in an empiric incremental fashion to identify the lowest effective dosage (50-150 mg daily × 5 days, beginning on cycle day 3-5). The cumulative pregnancy rate with clomiphene treatment is approximately 50% after 3 induced ovulatory cycles, and approaches 75% within 6-9 cycles of treatment.[466] The risk for multiple gestation is approximately 5-8%. Approximately 20% of patients prove refractory to clomiphene treatment, most of those having severe hyperandrogenism or obesity.[467]

Treatment with insulin sensitizing agents (metformin, thizolidinediones, D-chiro-inositol) can increase ovulation rates in some women with PCOS.[463,468,469] Metformin has been used widely for that purpose, but there is no practical way to predict reliably those who will respond. Preliminary evidence suggests that a response to metformin may be less likely in women having a polymorphism of a gene encoding a hepatic serine-threonine kinase (*STK11*).[470] Fasting insulin concentrations and glucose:insulin ratios do not predict response to metformin,[471] and overall, metformin appears most effective in patients who also respond to clomiphene.[469,472]

A 2003 meta-analysis of studies involving treatment with metformin in women with PCOS concluded that its efficacy for improving ovulatory function compared favorably with that of clomiphene.[471] However, subsequent randomized multicenter trials comparing the two drugs, alone and in combination, have found clomiphene clearly superior to metformin and observed that combined treatment offers no significant additional benefit.[473,474] and [475] In the largest trial, clomiphene yielded a significantly higher live birth rate than metformin (22.5% vs. 7.2%), and the results of combined treatment were not significantly better (26.8%).[474] In a few small studies involving clomiphene-resistant anovulatory women with PCOS, combined treatment has increased ovulation and pregnancy rates over those achieved with clomiphene alone.[476,477,478] and [479] A 2008 meta-analysis including 17 randomized trials concluded that combined treatment with metformin and clomiphene achieves higher ovulation and pregnancy rates than treatment with clomiphene alone.[469] Although there is no convincing evidence that combined treatment with metformin and clomiphene can increase live birth rates over those achieved with clomiphene alone,[480] the

attempt seems justified for women having few alternatives besides ovarian drilling or treatment with exogenous gonadotropins. Limited evidence indicates that combined treatment with metformin and roziglitazone,[481] or with clomiphene and rosiglitazone,[482] is no more effective than metformin alone. ***In summary, clomiphene should be the first choice of therapy for ovulation induction in women with PCOS, and in those who prove resistant, combined treatment with metformin and clomiphene deserves consideration before proceeding to ovarian drilling or treatment with gonadotropins.***

Although there is no evidence that metformin treatment during pregnancy is associated with any increased risk for major fetal malformations,[483] the safety of its use during pregnancy

P.526

is not yet established. Some have advocated metformin treatment to reduce the increased risk for miscarriage in women with PCOS, which might relate to an underlying metabolic disorder.[150,484,485] and [486] However, no difference in the miscarriage rates of women who did or did not receive metformin treatment have been observed in large randomized trials.[473,474] and [475] Metformin treatment during pregnancy also has been advocated to reduce the risk for developing gestational diabetes and other pregnancy complications in women with PCOS.[487] In diabetic women, treatment with metformin during pregnancy has been associated with an increased prevalence of pre-eclampsia and increased perinatal mortality in some studies,[488] but not in others.[489] Currently, routine metformin treatment during pregnancy is not recommended for women with PCOS.[472]

Induction of ovulation with exogenous gonadotropins is highly effective, but requires careful monitoring to avoid the intrinsic risks of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). Many women are highly sensitive to low doses of medication and exhibit a relatively narrow therapeutic range.[490,491,492,493,494] and [495] Although whether metformin treatment can improve outcomes for women with PCOS in gonadotropin-stimulated[485,496,497] or in vitro fertilization (IVF) cycles[403,498,499] remains unclear, evidence indicates the risk for OHSS may be decreased.[500]

Laparoscopic ovarian drilling with laser or diathermy also can be effective for restoring ovulatory function in women with PCOS, but has risk for causing postoperative adnexal adhesions and decreased ovarian reserve.[501] There is no evidence that metformin treatment improves outcomes achieved with ovarian drilling.[500]

Metabolic Abnormalities and Associated Health Risks

Women with chronic anovulation commonly exhibit insulin resistance and other risk factors for the development of type 2 diabetes and cardiovascular disease. These observations have focused a great deal of attention on the importance of incorporating risk reduction strategies into the clinical management of women with PCOS.

Insulin resistance results in compensatory hyperinsulinemia, which predisposes to a progressive decline in pancreatic ß-cell reserve, leading to glucose intolerance, and ultimately, type 2 diabetes mellitus. In women with PCOS, pancreatic ß-cell dysfunction can be demonstrated even before glucose intolerance becomes apparent, and the rate of progression from glucose intolerance to diabetes is increased;[99,502,503] up to 10% of women with PCOS develop diabetes by the age of 40.[99,100] Obesity adds to the risk, by aggravating the underlying insulin resistance. Overall, the risk for developing impaired glucose tolerance or type 2 diabetes is increased 3- to 7-fold in women with PCOS, compared to women of comparable age without PCOS.[99,100,503]

Although direct evidence for an increased incidence of cardiovascular disease in women with PCOS is lacking, the prevalence of known risk factors is substantially increased.[504] Insulin resistance and hyperinsulinemia are associated with chronic low-grade inflammation, as reflected by elevations in C-reactive protein, interleukin-6, leukocyte count, and other inflammatory markers.[505,506,507,508,509,510,511,512] and [513] Hyperinsulinemia also is associated with hypertension and increased production of plasminogen activator inhibitor type-1 (PAI-1), the principal inhibitor of tissue plasminogen activator (tPA) and urokinase, thereby inhibiting fibrinolysis.[514,515] Elevated androgen levels predispose to increased LDL-cholesterol and aggravate underlying insulin resistance. Consequently, many women with PCOS have some degree of dyslipidemia, such as decreased HDL-cholesterol and increased total and LDL-cholesterol and triglycerides.[338,516] Many also have central obesity, and some even meet criteria for the diagnosis of the metabolic syndrome, predicting a high risk for developing cardiovascular disease.[517,518,519] and [520]

P.527

The ***metabolic syndrome***, originally known as syndrome X,[521] represents a constellation of closely related cardiovascular risk factors, and several studies have observed an increased prevalence of metabolic syndrome in women with PCOS.[517,522] A number of different defi-nitions for the metabolic syndrome have been proposed, varying in emphasis on abnormalities in glucose metabolism (insulin resistance, hyperinsulinemia, glucose intolerance, diabetes mellitus), central obesity, and cardiovascular risk factors (hypertension, increased triglycerides, decreased HDL cholesterol).[523,524,525] and [526] Although all of the definitions yield comparable estimates of the overall prevalence of the metabolic syndrome, they identify different populations in different ethnic groups.[527] For example, the risk for type 2 diabetes increases at much lower levels of body fat in Asians than in Europids (White people of European origin).[528] The definition proposed by the International Diabetes Federation (IDF) in 2005 attempted to reconcile the differences in definitions and to produce a consensus definition that would be useful for identifying those at risk for developing cardiovascular disease in all populations, and also allow comparative long-term studies.[526] The IDF definition views central obesity (as defined by waist circumference) as an essential component of the metabolic syndrome, because of the strength of the evidence linking waist circumference with cardiovascular disease and the other components of the syndrome, and the strong likelihood that central obesity is an early step in the pathophysiologic cascade leading to full expression of the metabolic syndrome.[526] ***In general, the diagnosis of metabolic syndrome requires three of the following five clinical characteristics:***[529]

- **Increased waist circumference (population specific, >88 cm in the United States)**
- **Increased blood pressure (≥130 mm Hg systolic; ≥85 mm Hg diastolic)**
- **Increased triglycerides (≥150 mg/dL)**
- **Decreased HDL-cholesterol (<50 mg/dL)**
- **Increased fasting glucose (≥100 mg/dL) or previously established diabetes mellitus**

Our recognition of the central role of insulin resistance in the pathophysiology of PCOS and our knowledge of its potential longer-term health consequences have focused a great deal of attention on the benefits of insulin sensitizing mediations and other drugs aimed at reducing the risks for developing diabetes and cardiovascular disease.

Metformin is a biguanide oral insulin-sensitizing agent and currently is the most widely used drug in the world for the treatment of type 2 diabetes mellitus. Metformin decreases hepatic glucose production,

decreases intestinal glucose uptake, increases peripheral insulin sensitivity, and also inhibits lipolysis, resulting in decreased circulating concentrations of free fatty acids, which further helps to reduce hepatic gluconeogenesis.[472,530,531] Metformin's mechanism of action is not entirely clear, but involves activation of the adenosine monophosphate-activated protein kinase pathway in the liver and skeletal muscle.[532,533,534,535] and [536]

Metformin is available in both a regular and a sustained release form that may be associated with fewer gastrointestinal side effects (nausea, vomiting, diarrhea, constipation, bloating, flatulence, heartburn, indigestion, unpleasant metallic taste). To improve tolerance and decrease side effects, it is generally recommended that metformin treatment begin with a low dose (250-500 mg daily), increasing gradually over an interval of 4-6 weeks until the desired dose is attained. The drug also can interfere with intestinal absorption of vitamin B12, so patients should be alerted to symptoms of vitamin B12 deficiency, which include numbness, paresthesia, macroglossia, memory loss, behavioral changes, and pernicious anemia. Lactic acidosis is a rare complication of metformin treatment, but for that reason, the drug should not be administered to those with renal insufficiency, liver disease, or alcohol abuse.[537]

A large number of trials have observed beneficial effects of metformin in women with PCOS; in most, the dose has ranged between 1,500 and 2,000 mg daily. ***In general, metformin treatment increases insulin sensitivity,***[402,410,481,538,539,540] ***and*** [541] ***decreases weight and BMI,***[402,406,410,542] ***and decreases blood pressure and LDL-cholesterol.***[401] A meta-analysis

P.528

of 31 trials concluded that metformin increases insulin sensitivity up to 20%, decreases weight and BMI by 3-5%, decreases fasting glucose by about 5%, and increases HDL-cholesterol and decreases triglycerides by approximately 10% in patients at increased risk for developing diabetes.[543] Insulin resistance improves during metformin treatment, no matter how severe, and in lean and overweight women with PCOS as well as in those who are obese.[410,538,539,541] Weight loss enhances the effects of metformin.[410] Metformin appears to decrease levels of C-reactive protein and soluble vascular cellular adhesion molecules (sVCAM), which reflect the low level of chronic inflammation associated with insulin resistance.[506,544,545] Indirect evidence suggests metformin also may improve vascular endothelial function and coronary flow rate in women with PCOS.[546,547,548] and [549]

Thiazolidinediones are another type of insulin-sensitizing agent that has been used to improve insulin resistance in women with PCOS. They include rosiglitazone, pioglitazone, and, formerly, troglitazone (withdrawn from the market due to concerns about liver toxicity). Thiazolidinediones are synthetic agonists for the peroxisome proliferator-activated receptor gamma (PPARg), which serves as a nuclear transcription factor in the regulation of genes involved in carbohydrate, lipid, and protein metabolism (free fatty acids and eicosanoids are the natural receptor ligands). In trials involving women with PCOS, treatment with troglitazone improved insulin sensitivity and glucose tolerance in a dosedependent manner.[110,458,468] Similar observations have emerged from studies examining the effects of rosiglitazone and pioglitazone in women with PCOS.[481,482,550,551,552] and [553] However, overall experience with thiazolidinediones is quite limited and they have been associated with cardiac complications. Metformin improves insulin sensitivity as much or more than thiazolidinediones and currently remains the preferred insulin-sensitizing agent for women with PCOS.472, 481, 539, 540

Although the benefits of estrogen-progestin contraceptives in the treatment of women with PCOS are undisputed, they generally do not correct any of the metabolic abnormalities commonly observed in women with PCOS.[407,554,555,556,557] and [558] Although preparations containing drospirenone may have some limited impact,[456] other evidence suggests that even estrogenprogestin contraceptives containing antiandrogenic progestins may aggravate an underlying chronic inflammatory state.[506,508,557] Not

surprisingly, combination therapies aimed at more comprehensive treatment are now emerging, including estrogen-progestin contraceptives and metformin, and low doses of metformin (850 mg daily) and an antiandrogen (flu-tamide, 62.5 mg daily), with or without an estrogen-progestin contraceptive.[456] In women receiving an estrogen-progestin contraceptive, the addition of metformin improves insulin resistance and further reduces hyperandrogenism.[409,444,559] The combination of low doses of metformin (850 mg daily) and an antiandrogen (flutamide, 62.5 mg daily) improves body composition (loss of fat and gain of lean mass) and lipid levels and increases levels of adiponectin, a anti-inflammatory protein secreted from adipose that modulates glucose regulation and fatty acid metabolism.[332] Combined treatment with an estrogen-progestin contraceptive and an antiandrogen has similar effects that are further enhanced if metformin also is added to the treatment regimen.[508] ***Overall, these observations demonstrate that the spectrum of metabolic abnormalities that accompanies PCOS can be improved significantly by treatment with low doses of metformin and antiandrogen in adolescents, and by their addition to estrogen-progestin contraceptives in young women.***[456] At least in theory, alternative or adjunctive treatment with metformin and antiandrogens is attractive because it may improve or reverse "upstream" abnormalities and help to prevent their "downstream" consequences. ***Experience with these combination treatment regimens is still limited, but is growing steadily, suggesting they may soon find their way into clinical practice.***

Dyslipidemia is common in women with PCOS, with many having decreased HDL-cholesterol or increased total and LDL-cholesterol or triglycerides.[516] Because metformin treatment does not have any important or consistent impact on lipid levels,[408,541] interest has turned to the potential benefits of treatment with statins. In the first clinical trial involving

P.529

women with PCOS, lipid profiles improved more in women randomized to treatment with an oral estrogen-progestin contraceptive and simvastatin (20 mg daily) than in those receiving only the contraceptive.[560] In those receiving simvastatin, markers of systemic inflammation and endothelial function also improved, and serum testosterone levels decreased to a significantly greater extent.[560,561] In a placebo-controlled trial, treatment with atorvastatin resulted in a significant decrease in serum testosterone, C-reactive protein, and insulin resistance, and improved lipid profiles.[562] In a trial comparing the effects of simvastatin and metformin, the two drugs decreased testosterone and improved markers of systemic inflammation and endothelial function to a similar extent, but lipid profiles and insulin sensitivity improved only in those receiving simvastatin; results of combined treatment were not different from those of treatment with simvastatin alone.[563]

Statins exert their effects primarily by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting step in the mevalonate pathway leading to cholesterol synthesis.[564] The effects of statins on testosterone concentrations may relate to decreased availability of products of the mevalonate pathway (including cholesterol), to inhibition of the mitogen-activated protein kinase (MAPK) pathway that mediates the proliferative actions of insulin, or to other mechanisms regulating ovarian steroidogenesis.[563,565] ***Statins may offer a promising new approach to the treatment of women with PCOS at risk for developing diabetes and cardiovascular disease. However, it is important to emphasize that statins may be teratogenic and are contraindicated in pregnancy.***[566]

Indications for Treatment with Metformin

The best overall approach to the treatment of chronic anovulation and PCOS currently is somewhat controversial. For decades, estrogen-progestin contraceptives have been standard therapy for women who are not immediately interested in seeking pregnancy, for good reasons. However, the central role of

insulin resistance in PCOS and limited evidence suggesting that estrogen-progestin contraceptives may aggravate insulin resistance have raised concerns that such treatment might increase long-term risks for diabetes and heart disease in women already predisposed.[555]

There is no question that the majority of both lean and obese women with PCOS are insulin resistant,[22,567,568] and [569] and that the prevalence of impaired glucose tolerance and diabetes is increased in women with PCOS.[99,100,502] In the lean patient, insulin resistance is intrinsic, but poorly understood,[97,126,570] and the obese patient carries an additional metabolic burden.[571] There also is no debate that insulin resistance and PCOS are associated with increased risk for developing hypertension,[157,572,573] dyslipidemia,[335,520,574,575] and with a number of other surrogate markers and risk factors for heart disease.[333,458,505,521,525,576,577] and [578] Understandably, some view PCOS as an early sign, or even as a component, of the metabolic syndrome in women.[555]

Some studies have observed that estrogen-progestin contraceptives decrease insulin sensitivity.[404,440,442,579] Overall, the evidence suggests that the effects of estrogen-progestin contraceptives on insulin resistance and glucose tolerance vary with the dose of ethinyl estradiol, with the dose and type of progestin, and with phenotype, and in general, are not clinically important.[407,409,580] No large studies have examined the risk for developing type 2 diabetes in women with PCOS specifically. Studies in healthy women have observed a modest increase in the relative risk for past and current users of estrogen-progestin contraceptives, compared with never users, although the differences were not significant.[581,582]

Estrogen-progestin contraceptives may decrease insulin sensitivity or glucose tolerance, to some extent, in some women, but concerns that the risk may be substantially higher in women with PCOS and underlying insulin resistance are unsubstantiated.

P.530

Similarly, no large studies have examined the effect of estrogen-progestin contraceptives on the risk for developing cardiovascular disease in women with PCOS specifically. Alterations in vascular and endothelial function have been described and increased death rates from cardiovascular disease have been observed in women with history of menstrual irregularity.[583] Case-control studies have observed that estrogen-progestin contraceptives are associated with an increased risk for myocardial infarction, but events are rare and risk is almost entirely limited to women with hypertension and those who smoke.[584,585,586,587] and [588] ***Concerns that use of estrogen-progestin contraceptives might pose greater risk for women with PCOS are understandable, but there is no convincing evidence that they do.***

Treatment with metformin might indeed decrease the risk for developing diabetes and heart disease in women with PCOS, but evidence for those benefits is indirect, inferred primarily from studies in patients with impaired glucose tolerance,[393] and from studies examining surrogate markers and risk factors for heart disease; conclusive evidence is lacking. The two most common and classical features of PCOS are anovulation and hyperandrogenism, and metformin has little impact on either. Metformin improves menstrual cyclicity and ovulatory function in some women with PCOS, but not in most, and standard treatments for anovulatory infertility are clearly more effective. Consequently, for the large majority of women with PCOS, metformin treatment alone will not suffice; treatment with estrogen-progestin contraceptives and antiandrogens, or with clomiphene citrate, will be required. ***The most clinically relevant question is who can benefit most from metformin treatment.***

The most logical candidates for treatment with metformin (aimed at preventing or slowing progression to type 2 diabetes and at reducing longer-term risks for cardiovascular disease) are women with impaired glucose tolerance or diabetes, those with obvious evidence of severe insulin resistance (acanthosis

nigricans),[589] and women having other features of the metabolic syndrome, such as central obesity, hypertension, and dyslipidemia. ***All women with PCOS should therefore be screened with an oral glucose tolerance test at the time of presentation, and every 2 years thereafter, and those with impaired glucose tolerance warrant annual screening.***[326] ***Evaluation also should include blood pressure, waist circumference, and a lipid profile, to help identify those with features of the metabolic syndrome.*** There is good evidence from the Diabetes Prevention Trial that metformin treatment can decrease the risk for progression to diabetes in those with impaired glucose intolerance, by approximately 30% (although better results were observed in those receiving intensive lifestyle interventions).[393] In a retrospective study of 50 women with PCOS treated with metformin (including 11 with impaired glucose tolerance at baseline), impaired glucose tolerance persisted in 5/11 (45%), and reverted to normal in the remainder (6/11, 55%), over an average of 43 months of follow-up.[590]

Anovulatory adolescent girls are another group that warrants periodic screening for glucose intolerance, and specific screening for insulin resistance, particularly if they are obese or had low birthweight.[591,592] Both characteristics are associated with premature adrenarche and the development of PCOS during adolescence, and evidence indicates that hyperinsulinemia is a key pathogenic factor.[327,328,329] and [330] Although menstrual irregularity is common for a time after menarche, those in whom it persists for more than 2 years merit greater scrutiny. There is substantial evidence that early treatment with metformin can decrease hyperinsulinemia and hyperandrogenism, and restore ovulatory menstrual function in girls with demonstrable insulin resistance, at least in those who are not obese.[329,331] Addition of a low dose of antiandrogen has additional beneficial effects on body composition and lipid levels.[332] ***These observations are compelling and indicate that metformin treatment, alone or in combination with antiandrogens, can have enormous impact and benefits for this important population.***

Although most women with PCOS have insulin resistance, at least 25% does not, no matter what method is used to assess insulin sensitivity.[92,94,310] ***Routine screening for insulin resistance is not recommended, primarily because there currently is no validated test***

P.531

for measuring insulin resistance in a clinical setting. The most accurate methods have no clinical application because of their complexity, and calculated indices are limited by the lack of a standardized insulin assay and any data demonstrating that markers of insulin resistance predict response to treatment. Routine treatment with metformin is difficult to justify for women with PCOS who do not have abnormal glucose tolerance, acanthosis nigricans, or the features of metabolic syndrome. However, continued surveillance and periodic screening is warranted, and recommended.

Conclusion

We clearly are in a new era in our understanding and management of women with PCOS. In the past, we treated the specific problems of infertility, dysfunctional uterine bleeding, and hirsutism effectively. We now have the opportunity, indeed the obligation, to offer interventions that can help prevent or reverse some of the metabolic consequences of the disorder that have an important impact on overall health and on the quality and quantity of life.

All references are available online at:
http://www.clinicalgynendoandinfertility.com

References

1. Schlechte J, Sherman B, Halmi N, VanGilder J, Chapler F, Dolan K, Granner D, Duello T, Harris C, Prolactin-secreting pituitary tumors in amenorrheic women: a comprehensive study, *Endocr Rev* 1:295, 1980. http://www.ncbi.nlm.nih.gov/pubmed/7014211

2. Seppala M, Ranta T, Hirvonen E, Hyperprolactinaemia and luteal insufficiency, *Lancet* 1:229, 1976. http://www.ncbi.nlm.nih.gov/pubmed/55535

3. Corenblum B, Pairaudeau N, Shewchuk AB, Prolactin hypersecretion and short luteal phase defects, *Obstet Gynecol* 47:486, 1976. http://www.ncbi.nlm.nih.gov/pubmed/943740

4. Biller BM, Baum HB, Rosenthal DI, Saxe VC, Charpie PM, Klibanski A, Progressive trabecular osteopenia in women with hyperprolactinemic amenorrhea, *J Clin Endocrinol Metab* 75:692, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1517356

5. Schlechte J, Walkner L, Kathol M, A longitudinal analysis of premenopausal bone loss in healthy women and women with hyperprolactinemia, *J Clin Endocrinol Metab* 75:698, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1517357

6. Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF, Jr., Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization, *J Clin Endocrinol Metab* 66:165, 1988. http://www.ncbi.nlm.nih.gov/pubmed/2961784

7. Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE, Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 82:2248, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9215302

8. Barnes RB, Lobo RA, Central opioid activity in polycystic ovary syndrome with and without dopaminergic modulation, *J Clin Endocrinol Metab* 61:779, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3928677

9. Berga SL, Yen SS, Opioidergic regulation of LH pulsatility in women with polycystic ovary syndrome, *Clin Endocrinol (Oxf)* 30:177, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2532984

10. Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS, Marshall JC, Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone, *J Clin Endocrinol Metab* 85:4047, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11095431

11. Barnes RB, Rosenfield RL, Ehrmann DA, Cara JF, Cuttler L, Levitsky LL, Rosenthal IM, Ovarian hyperandrogynism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women, *J Clin Endocrinol Metab* 79:1328, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7962325

12. Zhou R, Bird IM, Dumesic DA, Abbott DH, Adrenal hyperan-drogenism is induced by fetal androgen excess in a rhesus monkey model of polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:6630, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16174719

13. Xita N, Tsatsoulis A, Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies, *J Clin Endocrinol Metab* 91:1660, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16522691

14. Nappi C, Meo R, Di Carlo C, Estraneo A, Bilo L, Reduced fertility and neuroendocrine dysfunction in women with epilepsy, *Gynecol Endocrinol* 8:133, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7942081

15. Bilo L, Meo R, Epilepsy and polycystic ovary syndrome: where is the link?, *Neurol Sci* 27:221, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16998724

16. Siiteri PK, MacDonald PC, Role of extraglandular estrogen in human endocrinology, in *Handbook of Physiology, Section 7, Endocrinology*, S.R. Geyer, E.B. Astwood, and R.O. Greep, Editors. 1973, American Physiology Society: Washington, DC. p. 615.

17. Ridgway EC, Maloof F, Longcope C, Androgen and oestrogen dynamics in hyperthyroidism, *J Endocrinol* 95:105, 1982. http://www.ncbi.nlm.nih.gov/pubmed/7130886

18. Longcope C, Abend S, Braverman LE, Emerson CH, Androstenedione and estrone dynamics in hypothyroid women, *J Clin Endocrinol Metab* 70:903, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2318947

19. Tahboub R, Arafah BM, Sex steroids and the thyroid, *Best Pract Res Clin Endocrinol Metab* 23:769, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19942152

20. Narasaka T, Moriya T, Endoh M, Suzuki T, Shizawa S, Mizokami Y, Matsuoka T, Sasano H, 17Beta-hydroxysteroid dehydrogenase type 2 and dehydroepiandrosterone sulfotransferase in the human liver, *Endocr J* 47:697, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11228044

21. Goldzieher JW, Axelrod LR, Clinical and biochemical features of polycystic ovarian disease, *Fertil Steril* 14:631, 1963. http://www.ncbi.nlm.nih.gov/pubmed/14077325

22. Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A, Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance and/or hyperinsulinemia, *J Clin Endocrinol Metab* 65:499, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3305551

23. Conway GS, Honour JW, Jacobs HS, Heterogeneity of the polycystic ovary syndrome: clinical endocrine and ultrasound features in 556 patients, *Clin Endocrinol* 30:459, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2688996

24. Franks S, Polycystic ovary syndrome, *New Engl J Med* 333: 853, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7651477

25. Bates GW, Whitworth NS, Effect of body weight reduction on plasma androgens in obese, infertile women, *Fertil Steril* 38: 406, 1982. http://www.ncbi.nlm.nih.gov/pubmed/6811336

26. Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R, Balestra V, Melchionda N, Barbara L, Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss, *J Clin Endocrinol Metab* 68:173, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2642485

27. Guzick DS, Wing R, Smith D, Berga SL, Winters SJ, Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women, *Fertil Steril* 61:598, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8150098

28. Huber-Buchholz MM, Carey DG, Norman RJ, Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone, *J Clin Endocrinol Metab* 84:1470, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10199797

29. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R, Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study, *J Clin Endocrinol Metab* 83:3078, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9745406

30. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF, A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain, *J Clin Endocrinol Metab* 85:2434, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10902790

31. Fogue E, Massabuau G, L'ovaire a petits kystes., *Rev Gynecol Chirurg Abdom* 14:97, 1910.

32. Stein IF, Leventhal ML, Amenorrhea associated with bilateral polycystic ovaries, *Am J Obstet Gynecol* 29:181, 1935.

33. Speert H, *Obstetrics & Gynecologic Milestones Illustrated*. 1996, New York: The Parthenon Publishing Group.

34. Hughesdon PE, Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis", *Obstet Gynecol Survey* 37:59, 1982. http://www.ncbi.nlm.nih.gov/pubmed/7033852

35. Hull MGR, Epidemiology of infertility and polycystic ovar ian disease: endocrinological and demographic studies, *Gynaecol Endocrinol* 1:235, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3140583

36. Chang RJ, Ovarian steroid secretion in polycystic ovarian disease, *Seminars Reprod Endocrinol* 2:244, 1984.

37. Dunaif A, Green G, Futterweit W, Dobrjansky A, Suppression of hyperandrogenism does not improve peripheral or hepatic insulin resistance in the polycystic ovary syndrome, *J Clin Endocrinol Metab* 70:699, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2106527

38. Gonzalez F, Hatala DA, Speroff L, Basal and dynamic hormonal responses to gonadotropin releasing hormone agonist treatment in women with polycystic ovaries with high and low dehydroepiandrosterone sulfate levels, *Am J Obstet Gynecol* 165:535, 1991.

39. Gonzalez F, Chang L, Horab T, Lobo RA, Evidence for heterogeneous etiologies of adrenal dysfunction in polycystic ovary syndrome, *Fertil Steril* 66:354, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8751729

40. Venturoli S, Porcu E, Fabbri R, Magrini O, Gammi L, Paradisi R, Forcacci M, Bolzani R, Flamigni C, Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome, *Clin Endocrinol* 28: 93, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3139335

41. Wajchenberg BL, Achando SS, Mathor MM, Czeresnia CE, Neto DG, Kirschner MA, The source (s) of estrogen production in hirsute women with polycystic ovarian disease as determined by simultaneous adrenal and ovarian venous catheterization, *Fertil Steril* 49:56, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3121397

42. Fauser BC, Observations in favor of normal early follicle development and disturbed dominant follicle selection in polycystic ovary syndrome, *Gynecol Endocrinol* 8:75, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7942082

43. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF, The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report, *Fertil Steril* 91:456, 2009. http://www.ncbi.nlm.nih.gov/pubmed/18950759

44. Geist SH, Reaction of the mature human ovary to antuitrin-S., *Am J Obstet Gynecol* 26:588, 1933.

45. Keettel WC, Bradbury JT, Stoddard FJ, Observations on the polycystic ovary syndrome., *Am J Obstet Gynecol* 73:954, 1957. http://www.ncbi.nlm.nih.gov/pubmed/13411068

46. McArthur JW, Ingersoll FM, Worcester J, The urinary excretion of interstitial-cell and follicle-stimulating hormone activity by women with diseases of the reproductive system., *J Clin Endocrinol Metab* 18:1202, 1958. http://www.ncbi.nlm.nih.gov/pubmed/13587638

47. Ingersoll FM, McArthur JW, Longitudinal studies of gonadotropin excretion in the Stein-Leventhal syndrome., *Am J Obstet Gynecol* 77, 1959. http://www.ncbi.nlm.nih.gov/pubmed/13637142

48. Taymor ML, Barnard R, Luteinizing hormone excretion in the polycystic ovary syndrome., *Fertil Steril* 13:501, 1962. http://www.ncbi.nlm.nih.gov/pubmed/13980388

49. Yen SS, Vela P, Rankin J, Inappropriate secretion of folliclestimulating hormone and luteinizing hormone in polycystic ovarian disease, *J Clin Endocrinol Metab* 30:435, 1970. http://www.ncbi.nlm.nih.gov/pubmed/5435284

50. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F, Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome, *J Clin Invest* 57:1320, 1976. http://www.ncbi.nlm.nih.gov/pubmed/770505

51. Balen AH, Hypersecretion of luteinizing hormone and the polycystic ovary syndrome, *Hum Reprod* 8 Suppl 2:123, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8276945

52. Kazer RR, Kessel B, Yen SS, Circulating luteinizing hormone pulse frequency in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 65:233, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3597703

53. Imse V, Holzapfel G, Hinney B, Kuhn W, Wuttke W, Comparison of luteinizing hormone pulsatility in the serum of women suffering from polycystic ovarian disease using a bioassay and five different immunoassays, *J Clin Endocrinol Metab* 74:1053, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1569153

54. Hayes FJ, Taylor AE, Martin KA, Hall JE, Use of a gonadotropinreleasing hormone antagonist as a physiologic probe in polycystic ovary syndrome: assessment of neuroendocrine and androgen dynamics, *J Clin Endocrinol Metab* 83:2343, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9661606

55. Lockwood GM, Muttukrishna S, Groome NP, Matthews DR, Ledger WL, Mid-follicular phase pulses of inhibin B are absent in polycystic ovarian syndrome and are initiated by successful laparoscopic ovarian diathermy: a possible mechanism regulating emergence of the dominant follicle, *J Clin Endocrinol Metab* 83: 1730, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9589683

56. Laven JS, Imani B, Eijkemans MJ, de Jong FH, Fauser BC, Absent biologically relevant associations between serum inhibin concentrations and characteristics of polycystic ovary syndrome normogonadotrophic anovulatory infertility, *Hum Reprod* 16:1359, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11425813

57. Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E, Frequency and amplitude of gonadotropinreleasing hormone stimulation and gonadotropin secretion in the rhesus monkey, *Endocrinology* 109:376, 1981. http://www.ncbi.nlm.nih.gov/pubmed/6788538

58. Gross KM, Matsumoto AM, Bremner WJ, Differential control of luteinizing hormone and follicle-stimulating hormone secretion by luteinizing hormone-releasing hormone pulse frequency in man, *J Clin Endocrinol Metab* 64:675, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3102545

59. Spratt DI, Finkelstein JS, Butler JP, Badger TM, Crowley WF, Jr., Effects of increasing the frequency of low doses of gonadotro pinreleasing hormone (GnRH) on gonadotropin secretion in GnRHde ficient men, *J Clin Endocrinol Metab* 64:1179, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3106396

60. Arroyo A, Laughlin GA, Morales AJ, Yen SSC, Inappropriate gonadotropin secretion in polycystic ovary syndrome: influence of adiposity, *J Clin Endocrinol Metab* 82:3728, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9360532

61. Lobo RA, Kletzky OA, Campeau JD, diZerega GS, Elevated bioactive luteinizing hormone in women with the polycystic ovary syndrome, *Fertil Steril* 39:674, 1983. http://www.ncbi.nlm.nih.gov/pubmed/9360532

62. Mavroudis K, Evans A, Mamtora H, Anderson DC, Robertson WR, Bioactive LH in women with polycystic ovaries and the effect of gonadotrophin suppression, *Clin Endocrinol (Oxf)* 29:633, 1988. http://www.ncbi.nlm.nih.gov/pubmed/2978466

63. Fauser BC, Pache TD, Lamberts SW, Hop WC, de Jong FH, Dahl KD, Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease, *J Clin Endocrinol Metab* 73:811, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1909705

64. Blank SK, McCartney CR, Marshall JC, The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome, *Hum Reprod Update* 12:351, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16670102

65. Buvat J, Buvat-Herbaut M, Marcolin G, Racadot A, Fourlinnie JC, Beuscart R, Fossati P, A double blind controlled study of the hormonal and clinical effects of bromocriptine in the polycystic ovary syndrome, *J Clin Endocrinol Metab* 63:119, 1986.
http://www.ncbi.nlm.nih.gov/pubmed/3519644

66. Murdoch AP, McClean KG, Watson MJ, Dunlop W, Kendall Taylor P, Treatment of hirsutism in polycystic ovary syndrome with bromocriptine, *Br J Obstet Gynaecol* 94:358, 1987.
http://www.ncbi.nlm.nih.gov/pubmed/3555604

67. Quigley ME, Yen SS, The role of endogenous opiates in LH secretion during the menstrual cycle, *J Clin Endocrinol Metab* 51: 179, 1980. http://www.ncbi.nlm.nih.gov/pubmed/7380991

68. Wardlaw SL, Wehrenberg WB, Ferin M, Antunes JL, Frantz AG, Effect of sex steroids on beta-endorphin in hypophyseal portal blood, *J Clin Endocrinol Metab* 55:877, 1982.
http://www.ncbi.nlm.nih.gov/pubmed/6288755

69. Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ, Progesterone modulation of pulsatile luteinizing hormone secretion in normal women, *J Clin Endocrinol Metab* 58:378, 1984.
http://www.ncbi.nlm.nih.gov/pubmed/6420438

70. Dunaif A, Graf M, Insulin administration alters gonadal steroid metabolism independent of changes in gonadotropin secretion in insulin-resistant women with the polycystic ovary syndrome, *J Clin Invest* 83:23, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2642919

71. Patel K, Coffier MS, Dahan MH, Yoo RY, Lawson MA, Malcom PJ, Chang RJ, Increased luteinizing hormone secretion in women with polycystic ovary syndrome is unaltered by prolonged insulin infusion, *J Clin Endocrinol Metab* 88:5456, 2003.
http://www.ncbi.nlm.nih.gov/pubmed/14602789

72. Mehta RV, Patel KS, Coffier MS, Dahan MH, Yoo RY, Archer JS, Malcom PJ, Chang RJ, Luteinizing hormone secretion is not influenced by insulin infusion in women with polycystic ovary syndrome despite improved insulin sensitivity during pioglitazone treatment, *J Clin Endocrinol Metab* 90:2136, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15644405

73. Eagleson CA, Bellows AB, Hu K, Gingrich MB, Marshall JC, Obese patients with polycystic ovary syndrome: evidence that metformin does not restore sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by ovarian steroids, *J Clin Endocrinol Metab* 88:5158, 2003.
http://www.ncbi.nlm.nih.gov/pubmed/14602743

74. Chang RJ, Mandel FP, Lu JK, Judd HL, Enhanced disparity of gonadotropin secretion by estrone in women with polycystic ovarian disease, *J Clin Endocrinol Metab* 54:490, 1982.
http://www.ncbi.nlm.nih.gov/pubmed/6799536

75. Dunaif A, Longcope C, Canick J, Badger T, Crowley WF, Jr., The effects of the aromatase inhibitor delta 1-testolactone on gonadotropin release and steroid metabolism in polycystic ovarian disease, *J Clin Endocrinol Metab* 60:773, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3919053

76. Daniels TL, Berga SL, Resistance of gonadotropin releasing hormone drive to sex steroid-induced suppression in hyperandrogenic anovulation, *J Clin Endocrinol Metab* 82:4179, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9398736

77. Pastor CL, Griffin-Korf ML, Aloi JA, Evans WS, Marshall JC, Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone, *J Clin Endocrinol Metab* 83:582, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9467578

78. Chhabra S, McCartney CR, Yoo RY, Eagleson CA, Chang RJ, Marshall JC, Progesterone inhibition of the hypothalamic gonadotropin-releasing hormone pulse generator: evidence for varied effects in hyperandrogenemic adolescent girls, *J Clin Endocrinol Metab* 90:2810, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15728200

79. Dumesic DA, Abbott DH, Eisner JR, Goy RW, Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood, *Fertil Steril* 67:155, 1997. http://www.ncbi.nlm.nih.gov/pubmed/8986701

80. Robinson JE, Forsdike RA, Taylor JA, In utero exposure of female lambs to testosterone reduces the sensitivity of the gonadotropinreleasing hormone neuronal network to inhibition by progesterone, *Endocrinology* 140:5797, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10579346

81. Foecking EM, Szabo M, Schwartz NB, Levine JE, Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropinreleasing hormone pulse generator, *Biol Reprod* 72:1475, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15744016

82. Abbott DH, Barnett DK, Bruns CM, Dumesic DA, Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome?, *Hum Reprod Update* 11:357, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15941725

83. Chang RJ, Laufer LR, Meldrum DR, DeFazio J, Lu JK, Vale WW, Rivier JE, Judd HL, Steroid secretion in polycystic ovarian disease after ovarian suppression by a long-acting gonadotropin-releasing hormone agonist, *J Clin Endocrinol Metab* 56:897, 1983. http://www.ncbi.nlm.nih.gov/pubmed/6403570

84. Steingold K, De Ziegler D, Cedars M, Meldrum DR, Lu JK, Judd HL, Chang RJ, Clinical and hormonal effects of chronic gonadotropin-releasing hormone agonist treatment in polycystic ovarian disease, *J Clin Endocrinol Metab* 65:773, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3116031

85. Adams JM, Taylor AE, Crowley WF, Jr., Hall JE, Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome, *J Clin Endocrinol Metab* 89:4343, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15356031

86. Archard C, Thiers J, Le virilisme pilaire et son association a l'insuffisance glycolytique (diabete des femmes a barbe), *Bull Acad Natl Med* 86:51, 1921.

87. Kahn CR, Rosenthal AS, Immunologic reactions to insulin: insulin allergy, insulin resistance, and the autoimmune insulin syndrome, *Diabetes Care* 2:283, 1979. http://www.ncbi.nlm.nih.gov/pubmed/510122

88. 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 Engl J Med* 294: 739, 1976. http://www.ncbi.nlm.nih.gov/pubmed/176581

89. Tsiodras S, Mantzoros C, Hammer S, Samore M, Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study, *Arch Intern Med* 160: 2050, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10888979

90. Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S, Gorden P, Clinical course of genetic diseases of the insulin receptor (type A and Rabson-Mendenhall syndromes): a 30-year prospective, *Medicine (Baltimore)* 83:209, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15232309

91. Burghen GA, Givens JR, Kitabchi AE, Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease, *J Clin Endocrinol Metab* 50:113, 1980. http://www.ncbi.nlm.nih.gov/pubmed/7350174

92. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA, Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome?, *Am J Obstet Gynecol* 167:1807, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1471702

93. Dunaif A, Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis, *Endocr Rev* 18:774, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9408743

94. Legro RS, Finegood D, Dunaif A, A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 83:2694, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9709933

95. DeUgarte CM, Bartolucci AA, Azziz R, Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment, *Fertil Steril* 83:1454, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15866584

96. Book CB, Dunaif A, Selective insulin resistance in the polycystic ovary syndrome, *J Clin Endocrinol Metab* 84:3110, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10487672

97. Ciaraldi TP, El-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SSC, Cellular mechanisms of insulin resistance in polycystic ovarian syndrome, *J Clin Endocrinol Metab* 75:577, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1322430

98. Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E, Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS), *Am J Physiol Endocrinol Metab* 281:E392, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11440917

99. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J, Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome, *Diabetes Care* 22: 141, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10333916

100. Legro RS, Kunselman AR, Dodson WC, Dunaif A, Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women, *J Clin Endocrinol Metab* 84:165, 1999. http://www.ncbi.nlm.nih.gov/pubmed/9920077

101. Tok EC, Ertunc D, Evruke C, Dilek S, The androgenic profile of women with non-insulin-dependent diabetes mellitus, *J Reprod Med* 49:746, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15493567

102. Moller DE, Flier JS, Insulin resistance-mechanisms, syndromes, and implications, *New Engl J Med* 325:938, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1881419

103. Baillargeon JP, Insulin action in polycystic ovary syndrome: in vivo and in vitro., in *The Polycystic Ovary Syndrome: Current Concepts on Pathogenesis and Clinical Care*, R. Azziz, Editor. 2007, Springer: New York. p. 43.

104. Nelson-Degrave VL, Wickenheisser JK, Hendricks KL, Asano T, Fujishiro M, Legro RS, Kimball SR, Strauss JF, 3rd, McAllister JM, Alterations in mitogen-activated protein kinase kinase and extracellular regulated kinase signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome, *Mol Endocrinol* 19:379, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15514033

105. 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 the signal transduction system, *J Clin Endocrinol Metab* 83: 2001, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9626131

106. Franks S, Gilling-Smith C, Watson H, Willis D, Insulin action in the normal and polycystic ovary, *Endocrinol Metab Clin North Am* 28:361, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10352923

107. Willis D, Mason H, Gilling-Smith C, Franks S, Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries, *J Clin Endocrinol Metab* 81:302, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8550768

108. Smith S, Ravnikar VA, Barbieri RL, Androgen and insulin response to an oral glucose challenge in hyperandrogenic women, *Fertil Steril* 48:72, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3297813

109. Nestler JE, Barlascini CO, Matt DW, Steingold KA, Plymate SR, Clore JN, Blackard WG, Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 68:1027, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2498378

110. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R, The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome, *J Clin Endocrinol Metab* 81:3299, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8784087

111. Plymate SR, Matej LA, Jones RE, Friedl KE, Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin, *J Clin Endocrinol Metab* 67:460, 1988. http://www.ncbi.nlm.nih.gov/pubmed/2842359

112. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG, A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome, *J Clin Endocrinol Metab* 72:83, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1898744

113. Cohen JC, Hickman R, Insulin resistance and diminished glu cose tolerance in powerlifters ingesting anabolic steroids, *J Clin Endocrinol Metab* 64:960, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3549761

114. Barbieri RL, Makris A, Ryan KJ, Insulin stimulates androgen accumulation in incubations of human ovarian stroma and theca, *Obstet Gynecol* 64:73S, 1984. http://www.ncbi.nlm.nih.gov/pubmed/6382082

115. Willis D, Franks S, Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor, *J Clin Endocrinol Metab* 80:3788, 1995. http://www.ncbi.nlm.nih.gov/pubmed/8530637

116. Nestler JE, Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications, *Seminars Reprod Endocrinol* 15:111, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9165656

117. Nestler JE, Jakubowicz DJ, Iuorno MJ, Role of inositolphosphoglycan mediators of insulin action in the polycystic ovary syndrome, *J Pediatr Endocrinol Metab* 13 Suppl 5:1295, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11117673

118. Baptiste CG, Battista MC, Trottier A, Baillargeon JP, Insulin and hyperandrogenism in women with polycystic ovary syndrome, *J Steroid Biochem Mol Biol*, 2009. http://www.ncbi.nlm.nih.gov/pubmed/20036327

119. Czech MP, Corvera S, Signaling mechanisms that regulate glucose transport, *J Biol Chem* 274:1865, 1999. http://www.ncbi.nlm.nih.gov/pubmed/9890935

120. Bergman RN, Ader M, Free fatty acids and pathogenesis of type 2 diabetes mellitus, *Trends Endocrinol Metab* 11:351, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11042464

121. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR, Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction, *Mol Cell* 6:87, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10949030

122. Anthonsen MW, Ronnstrand L, Wernstedt C, Degerman E, Holm C, Identification of novel phosphorylation sites in hormonesensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro, *J Biol Chem* 273:215, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9417067

123. Berggreen C, Gormand A, Omar B, Degerman E, Goransson O, Protein kinase B activity is required for the effects of insulin on lipid metabolism in adipocytes, *Am J Physiol Endocrinol Metab* 296:E635, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19158325

124. Wu XK, Zhou SY, Liu JX, Pollanen P, Sallinen K, Makinen M, Erkkola R, Selective ovary resistance to insulin signaling in women with polycystic ovary syndrome, *Fertil Steril* 80:954, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14556818

125. Diamanti-Kandarakis E, Papavassiliou AG, Molecular mechanisms of insulin resistance in polycystic ovary syndrome, *Trends Mol Med* 12:324, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16769248

126. Dunaif A, Xia J, Book CB, Schenker E, Tang Z, Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome, *J Clin Invest* 96:801, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7635975

127. Li M, Youngren JF, Dunaif A, Gold.ne ID, Maddux BA, Zhang BB, Evans JL, Decreased insulin receptor (IR) autophosphorylation in fibroblasts from patients with PCOS: effects of serine kinase inhibitors and IR activators, *J Clin Endocrinol Metab* 87:4088, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12213853

128. Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Olefsky JM, Yen SS, Cellular insulin resistance in adipocytes from obese polycystic ovary syndrome subjects involves adenosine modulation of insulin sensitivity, *J Clin Endocrinol Metab* 82:1421, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9141527

129. Ek I, Arner P, Bergqvist A, Carlstrom K, Wahrenberg H, Impaired adipocyte lipolysis in nonobese women with the polycystic ovary syndrome: a possible link to insulin resistance?, *J Clin Endocrinol Metab* 82:1147, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9100587

130. Tanti JF, Gual P, Gremeaux T, Gonzalez T, Barres R, Le Marchand-Brustel Y, Alteration in insulin action: role of IRS-1 serine phosphorylation in the retroregulation of insulin signalling, *Ann Endocrinol (Paris)* 65:43, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15122091

131. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Tapanainen JS, Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome, *Hum Reprod* 15:1266, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10831553

132. Holte J, Bergh T, Berne C, Wide L, Lithell H, Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 80:2586, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7673399

133. Mai K, Bobbert T, Reinecke F, Andres J, Maser-Gluth C, Wudy SA, Mohlig M, Weickert MO, Hartmann MF, Schulte HM, Diederich S, Pfeiffer AF, Spranger J, Intravenous lipid and heparin infusion-induced elevation in free fatty acids and triglycerides modifies circulating androgen levels in women: a randomized, controlled trial, *J Clin Endocrinol Metab* 93:3900, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18664538

134. Pandey AV, Miller WL, Regulation of 17,20 lyase activity by cytochrome b5 and by serine phosphorylation of P450c17, *J Biol Chem* 280:13265, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15687493

135. Zhang LH, Rodriguez H, Ohno S, Miller WL, Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome, *Proc Natl Acad Sci U S A* 92:10619, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7479852

136. Dale PO, Tanbo T, Djoseland O, Jervell J, Abyholm T, Persistence of hyperinsulinemia in polycystic ovary syndrome after ovarian suppression by gonadotropin-releasing hormone agonist, *Acta Endocrinol* 126:132, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1543018

137. Elkind-Hirsch KE, Valdes CT, Malinak LR, Insulin resistance improves in hyperandrogenic women treated with Lupron, *Fertil Steril* 60:634, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8405516

138. Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, Furiani L, Caputo M, Muggeo M, The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women, *J Clin Endocrinol Metab* 81:952, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8772557

139. Lemieux S, Lewis GF, Ben-Chetrit A, Steiner G, Greenblatt EM, Correction of hyperandrogenmia by laparoscopic ovarian cautery in women with polycystic ovarian syndrome is not accompanied by improved insulin sensitivity or lipid-lipoprotein levels, *J Clin Endocrinol Metab* 84:4278, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10566685

140. Iuorno MJ, Jakubowicz DJ, Baillargeon JP, Dillon P, Gunn RD, Allan G, Nestler JE, Effects of d-chiro-inositol in lean women with the polycystic ovary syndrome, *Endocr Pract* 8:417, 2002. http://www.ncbi.nlm.nih.gov/pubmed/15251831

141. Baillargeon JP, Diamanti-Kandarakis E, Ostlund RE, Jr., Apridonidze T, Iuorno MJ, Nestler JE, Altered D-chiro-inositol urinary clearance in women with polycystic ovary syndrome, *Diabetes Care* 29:300, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16443877

142. Baillargeon JP, Nestler JE, Ostlund RE, Apridonidze T, Diamanti-Kandarakis E, Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism, *Hum Reprod* 23:1439, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18375940

143. Cheang KI, Baillargeon JP, Essah PA, Ostlund RE, Jr., Apridonize T, Islam L, Nestler JE, Insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator correlates with insulin sensitivity in women with polycystic ovary syndrome, *Metabolism* 57:1390, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18803944

144. Korhonen S, Hippelainen M, Niskanen L, Vanhala M, Saarikoski S, Relationship of the metabolic syndrome and obesity to polycystic ovary syndrome: a controlled, population-based study, *Am J Obstet Gynecol* 184:289, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11228476

145. Alvarez-Blasco F, Botella-Carretero JI, San Millan JL, Escobar-Morreale HF, Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women, *Arch Intern Med* 166:2081, 2006. http://www.ncbi.nlm.nih.gov/pubmed/17060537

146. Yildiz BO, Knochenhauer ES, Azziz R, Impact of obesity on the risk for polycystic ovary syndrome, *J Clin Endocrinol Metab* 93: 162, 2008. http://www.ncbi.nlm.nih.gov/pubmed/17925334

147. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN, Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 91:48, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16249284

148. Dokras A, Jagasia DH, Maifeld M, Sinkey CA, VanVoorhis BJ, Haynes WG, Obesity and insulin resistance but not hyperandrogenism mediates vascular dysfunction in women with polycystic ovary syndrome, *Fertil Steril* 86:1702, 2006. http://www.ncbi.nlm.nih.gov/pubmed/17067587

149. Barber TM, McCarthy MI, Wass JA, Franks S, Obesity and polycystic ovary syndrome, *Clin Endocrinol (Oxf)* 65:137, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16886951

150. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS, A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome, *Hum Reprod Update* 12:673, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16891296

151. Carpentier AC, Postprandial fatty acid metabolism in the development of lipotoxicity and type 2 diabetes, *Diabetes Metab* 34:97, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18353699

152. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM, IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance, *Science* 271:665, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8571133

153. Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC, The insulinrelated ovarian regulatory system in health and disease, *Endocr Rev* 20:535, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10453357

154. Svendsen PF, Nilas L, Norgaard K, Jensen JE, Madsbad S, Obesity, body composition and metabolic disturbances in polycystic ovary syndrome, *Hum Reprod* 23:2113, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18556679

155. Yildirim B, Sabir N, Kaleli B, Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome, *Fertil Steril* 79:1358, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12798883

156. Michelmore K, Ong K, Mason S, Bennett S, Perry L, Vessey M, Balen A, Dunger D, Clinical features in women with polycystic ovaries: relationships to insulin sensitivity, insulin gene VNTR and birth weight, *Clin Endocrinol (Oxf)* 55:439, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11678825

157. Rebuffe-Scrive M, Cullberg G, Lundberg PA, Lindstedt G, Bjorntorp P, Anthropometric variables and metabolism in polycys tic ovarian disease, *Horm Metab Res* 21:391, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2777199

158. Carmina E, Rosato F, Janni A, Rizzo M, Longo RA, Extensive clinical experience: relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism, *J Clin Endocrinol Metab* 91:2, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16263820

159. Azziz R, Sanchez LA, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR, Androgen excess in women: experience with over 1000 consecutive patients, *J Clin Endocrinol Metab* 89:453, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14764747

160. Flegal KM, Carroll MD, Ogden CL, Curtin LR, Prevalence and trends in obesity among US adults, 1999-2008, *JAMA* 303:235, 2010. http://www.ncbi.nlm.nih.gov/pubmed/20071471

161. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO, The prevalence and features of the polycystic ovary syndrome in an unselected population, *J Clin Endocrinol Metab* 89:2745, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15181052

162. 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, *J Clin Endocrinol Metab* 84:4006, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10566641

163. Michelmore KF, Balen AH, Dunger DB, Vessey MP, Polycystic ovaries and associated clinical and biochemical features in young women, *Clin Endocrinol (Oxf)* 51:779, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10619984

164. Baillargeon JP, Nestler JE, Commentary: polycystic ovary syndrome: a syndrome of ovarian hypersensitivity to insulin?, *J Clin Endocrinol Metab* 91:22, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16263814

165. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R, Obesity and the polycystic ovary syndrome, *Int J Obes Relat Metab Disord* 26:883, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12080440

166. Pasquali R, Gambineri A, Pagotto U, The impact of obesity on reproduction in women with polycystic ovary syndrome, *Br J Obstet Gynaecol* 113:1148, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16827825

167. Hirschberg AL, Polycystic ovary syndrome, obesity and reproductive implications, *Womens Health (Lond Engl)* 5:529, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19702452

168. Plymate SR, Fariss BL, Bassett ML, Matej L, Obesity and its role in polycystic ovary syndrome, *J Clin Endocrinol Metab* 52:1246, 1981. http://www.ncbi.nlm.nih.gov/pubmed/6785298

169. Linne Y, Effects of obesity on women's reproduction and complications during pregnancy, *Obes Rev* 5:137, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15245382

170. Kumar A, Woods KS, Bartolucci AA, Azziz R, Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS), *Clin Endocrinol (Oxf)* 62:644, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15943823

171. Cedars MI, Steingold KA, de Ziegler D, Lapolt PS, Chang RJ, Judd HL, Long-term administration of gonadotropin-releasing hormone agonist and dexamethasone: assessment of the adrenal role in ovarian dysfunction, *Fertil Steril* 57:495, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1531463

172. Barnes RB, Rosenfield RL, Burstein S, Ehrmann DA, Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome, *New Engl J Med* 320:559, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2521688

173. Nelson VL, Qin KN, Rosenfield RL, Wood JR, Penning TM, Legro RS, Strauss JF, 3rd, McAllister JM, The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome, *J Clin Endocrinol Metab* 86:5925, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11739466

174. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA, Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries, *J Clin Endocrinol Metab* 86:1318, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11238527

175. Nelson VL, Legro RS, Strauss JF, 3rd, McAllister JM, Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries, *Mol Endocrinol* 13: 946, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10379893

176. Gilling-Smith C, Story H, Rogers V, Franks S, Evidence for a primary abnormality of thecal cell steroidogenesis in the polycystic ovary syndrome, *Clin Endocrinol (Oxf)* 47:93, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9302378

177. Ehrmann DA, Barnes RB, Rosenfield RL, Polycystic ovary syndrome as a form of functional ovarian hyperandrogensim due to dysregulation of androgen secretion, *Endocr Rev* 16:322, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7671850

178. Wickenheisser JK, Nelson-Degrave VL, McAllister JM, Dysregulation of cytochrome P450 17alpha-hydroxylase messenger ribonucleic acid stability in theca cells isolated from women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:1720, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15598676

179. Doi SA, Neuroendocrine dysfunction in PCOS: a critique of recent reviews, *Clin Med Res* 6:47, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18952851

180. Strauss JF, 3rd, Some new thoughts on the pathophysiology and genetics of polycystic ovary syndrome, *Ann N Y Acad Sci* 997:42, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14644808

181. Azziz R, Black V, Hines GA, Fox LM, Boots LR, Adrenal andro gen excess in the polycystic ovary syndrome: sensitivity and respon sivity of the hypothalamic-pituitary-adrenal axis, *J Clin Endocrinol Metab* 83:2317, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9661602

182. Ehrmann DA, Rosenfield RL, Barnes RB, Brigell DF, Sheikh Z, Detection of functional ovarian hyperandrogenism in women with androgen excess, *New Engl J Med* 327:157, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1319000

183. Rosenfield RL, Famg IS, The effects of prolonged physiologic estradiol therapy on the maturation of hypogonadal teenagers, *J Pediatr* 85:830, 1974. http://www.ncbi.nlm.nih.gov/pubmed/4370903

184. Anderson D, Yen SSC, Effects of estrogens on adrenal 3ß-hydrox-ysteroid dehydrogenase in ovariectomized women, *J Clin Endocrinol Metab* 43:561, 1976. http://www.ncbi.nlm.nih.gov/pubmed/182711

185. Rose DP, Fern M, Liskowski L, Milbrath JR, Effect of treatment with estrogen conjugates on endogenous plasma steroids, *Obstet Gynecol* 49:80, 1977. http://www.ncbi.nlm.nih.gov/pubmed/187991

186. Tazuke S, Khaw K-T, Barrett-Connor E, Exogenous estrogen and endogenous sex hormones, *Medicine* 71:44, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1549058

187. Chang RJ, Mandel FP, Wolfren AR, Judd HL, Circulating lev els of plasma adrenocorticotropin in polycystic ovary disease, *J Clin Endocrinol Metab* 54:1265, 1982. http://www.ncbi.nlm.nih.gov/pubmed/6281296

188. Stewart PM, Penn R, Holder R, Parton A, Ratcliffe JG, London DR, The hypothalamo-pituitary-adrenal axis across the normal menstrual cycle and in polycystic ovary syndrome, *Clin Endocrinol* 38:387, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8391404

189. Escobar-Morreale HF, Serrano-Gotarredona J, García-Robles R, Sancho JM, Varela C, Lack of an ovarian function influence on the increased adrenal androgen secretion present in women with functional ovarian hyperandrogenism, *Fertil Steril* 67:654, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9093190

190. Azziz R, Bradley Jr EL, Potter HD, Boots LR, Adrenal androgen excess in women: lack of a role for 17-hydroxylase and 17,20-lyase dysregulation, *J Clin Endocrinol Metab* 80:400, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7852496

191. Techatraisak K, Conway GS, Rumsby G, Frequency of a polymorphism in the regulatory region of the 17a-hydroxylase-17,20-lyase (CYP17) gene in hyperandrogenic states, *Clin Endocrinol* 46: 131, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9135692

192. Moghetti P, Castello R, Negri C, Tosi F, Spiazzi GG, Brun E, balducci R, Toscano V, Muggeo M, Insulin infusion amplifies 17a-hydroxycorticosteroid intermediates response to ACTH in hyperandrogenic women: apparent relative impairment of 17, 20-lyase activity, *J Clin Endocrinol Metab* 81:881, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8772544

193. Nestler JE, Clore JN, Strauss III JF, Blackard WG, Effects of hyperinsulinemia on serum testosterone, progesterone, dehydroepiandrosterone sulfate, and cortisol levels in normal women and in a woman with hyperandrogenism, insulin resistance and acanthosis nigricans, *J Clin Endocrinol Metab* 64:180, 1987. http://www.ncbi.nlm.nih.gov/pubmed/2946716

194. Nestler JE, McClanahan MA, Clore JN, Blackard WG, Insulin inhibits adrenal 17,20-lyase activity in man, *J Clin Endocrinol Metab* 74:362, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1730815

195. l'Allemand D, Penhoat A, Lebrethon MC, Ardevol R, Baehr V, Oelkers W, Saez JM, Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid levels and corticotropin steroidogenic responsiveness in cultured human adrenocortical cells, *J Clin Endocrinol Metab* 81: 3892, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8923834

196. Kristiansen SB, Endoh A, Casson PR, Buster JE, Hornsby PJ, Induction of steroidogenic enzyme genes by insulin and IGF-I in cultured adult human adrenocortical cells, *Steroids* 62:258, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9055386

197. Erickson GF, Hsueh AJN, Quigley ME, Rebar R, Yen SSC, Functional studies of aromatase activity in human granulosa cells from normal and polycystic ovaries, *J Clin Endocrinol Metab* 49:514, 1979. http://www.ncbi.nlm.nih.gov/pubmed/479344

198. Erickson GF, Magoffin DA, Garzo VG, Cheung AP, Chang RJ, Granulosa cells of polycystic ovaries: are they normal or abnormal?, *Hum Reprod* 7:293, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1587932

199. Mason HD, Margara R, Winston RL, Seppala M, Koistinen R, Franks S, Insulin-like growth factor-I (IGF-I) inhibits production of IGF-binding protein-1 while stimulating estradiol secretion in granulosa cells from normal and polycystic human ovaries, *J Clin Endocrinol Metab* 76:1275, 1993. http://www.ncbi.nlm.nih.gov/pubmed/7684393

200. Mason HD, Willis DS, Beard RW, Winston RML, Margara R, Franks S, Estradiol production by granulosa cells of normal and polycystic ovaries: relationship to menstrual cycle history and concentrations of gonadotropins and sex steroids in follicular fluid, *J Clin Endocrinol Metab* 79:1355, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7962330

201. Almahbobi G, Anderiesz C, Hutchinson P, McFarlane JR, Wood C, Trounson AO, Functional integrity of granulosa cells from polycystic ovaries, *Clin Endocrinol (Oxf)* 44:571, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8762734

202. Judd HL, Rigg LA, Anderson DC, Yen SSC, The effect of ovarian wedge resection on circulating gonadotropin and ovarian steroid levels in patients with polycystic ovary syndrome, *J Clin Endocrinol Metab* 43:347, 1976. http://www.ncbi.nlm.nih.gov/pubmed/950366

203. Mahesh VB, Bratlid D, Lindabeck T, Hormone levels follow ing wedge resection in polycystic ovary syndrome, *Obstet Gynecol* 51:64, 1978. http://www.ncbi.nlm.nih.gov/pubmed/144874

204. Katz M, Carr PJ, Cohen BM, Milhin RP, Hormonal effects of wedge resection of polycystic ovaries, *Obstet Gynecol* 51: 437, 1978. http://www.ncbi.nlm.nih.gov/pubmed/662227

205. Casper RF, Greenblatt EM, Laparoscopic ovarian cautery for induction of ovulation in women with polycystic ovarian disease, *Seminars Reprod Endocrinol* 8:2080, 1990. http://www.ncbi.nlm.nih.gov/pubmed/8316557

206. Kaaijk EM, Hamerlynck JVTH, Beek JF, van der Veen F, Clinical outcome after unilateral oophorectomy in patients with polycystic ovary syndrome, *Hum Reprod* 14:889, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10221214

207. Kase N, Kowal J, Perloff W, Soffer LJ, In vitro production of androgens by a virilizing adenoma and associated polycystic ovaries, *Acta Endocrinol* 44:15, 1963. http://www.ncbi.nlm.nih.gov/pubmed/14059886

208. Zourlas PA, Jones Jr HW, Stein-Leventhal syndrome with masculinizing ovarian tumors, *Obstet Gynecol* 34:861, 1969. http://www.ncbi.nlm.nih.gov/pubmed/4312488

209. Dunaif A, Scully RE, Andersen RN, Chapin DS, Crowley Jr WF, The effects of continuous androgen secretion on the hypothalamicpituitary axis in women: evidence from a luteinized thecoma of the ovary, *J Clin Endocrinol Metab* 59:389, 1984. http://www.ncbi.nlm.nih.gov/pubmed/6086692

210. Amerikia H, Savoy-Moore RT, Sundareson AS, Moghissi KS, The effects of long-term androgen treatment on the ovary, *Fertil Steril* 45:202, 1986. http://www.ncbi.nlm.nih.gov/pubmed/3949022

211. Pache TD, Fauser BCJM, Polycystic ovaries in female-to-male transsexuals, *Clin Endocrinol* 39:702, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8123169

212. Givens JR, Familial polycystic ovarian disease, *Endocrinol Metab Clin North Am* 17:1, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3058473

213. Carey AH, Chan KL, Short F, White DM, Williamson R, Franks S, Evidence for a single gene effect in polycystic ovaries and male pattern baldness, *Clin Endocrinol* 38:653, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8334753

214. Govind A, Obhrai MS, Clayton RN, Polycystic ovaries are inherited as an autosomal domiant trait: analysis of 29 polycystic ovary syndrome and 10 control families, *J Clin Endocrinol Metab* 84:38, 1999. http://www.ncbi.nlm.nih.gov/pubmed/9920059

215. Norman RJ, Masters S, Hague W, Hyperinsulinemia is common in family members of women with polycystic ovary syndrome, *Fertil Steril* 66:942, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8941059

216. Hague WM, Adams J, Reeders ST, Peto TE, Jacobs HS, Familial polycystic ovaries: a genetic disease?, *Clin Endocrinol (Oxf)* 29: 593, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3076848

217. Legro RS, Driscoll D, Strauss JF, III., Fox J, Dunaif A, Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome, *Proc Natl Acad Sci USA* 95:14956, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9843997

218. Kashar-Miller M, Azziz R, Heritability and the risk of developing androgen excess, *J Steroid Biochem Mol Biol* 69:261, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10419000

219. Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R, Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS, *Fertil Steril* 75:53, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11163816

220. Yildiz BO, Yarali H, Oguz H, Bayraktar M, Glucose intolerance, insulin resistance, and hyperandrogenemia in first degree relatives of women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 88:2031, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12727950

221. Kaushal R, Parchure N, Bano G, Kaski JC, Nussey SS, Insulin resistance and endothelial dysfunction in the brothers of Indian subcontinent Asian women with polycystic ovaries, *Clin Endocrinol (Oxf)* 60:322, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15008997

222. Leibel NI, Baumann EE, Kocherginsky M, Rosenfield RL, Relationship of adolescent polycystic ovary syndrome to parental metabolic syndrome, *J Clin Endocrinol Metab* 91:1275, 2006. *J Clin Endocrinol Metab* 91:1275, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16449341

223. Sam S, Legro RS, Essah PA, Apridonidze T, Dunaif A, Evidence for metabolic and reproductive phenotypes in mothers of women with polycystic ovary syndrome, *Proc Natl Acad Sci U S A* 103:7030, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16632599

224. El Mkadem SA, Lautier C, Macari F, Molinari N, Lefebrvre P, Renard E, Gris JC, Cros G, Daures JP, Bringer J, White MF, Grigorescu F, Role of allelic variants $Gly^{972}Arg$ of IRS-1 and $Gly^{1057}Asp$ of IRS-2 in moderate-to-severe insulin resistance of women with polycystic ovary syndrome, *Diabetes* 50:2164, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11522686

225. Sir-Petermann T, Perez-Bravo F, Angel B, Maliqueo M, Calvillan M, Palomino A, $G^{972}R$ polymorphism of IRS-1 in women with polycystic ovary syndrome, *Diabetologia* 44:1200, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11596679

226. Ehrmann DA, Tang X, Yoshiuchi I, Cox NJ, Bell GI, Relationship of insulin receptor substrate-1 and -2 genotypes to phenotypic features of polycystic ovary syndrome, *J Clin Endocrinol Metab* 87:4287, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12213887

227. Franks S, Gharani N, Waterworth D, Batty S, White D, Williamson R, McCarthy M, The genetic basis of polycystic ovary syndrome, *Hum Reprod* 12:2641, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9455828

228. Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, Batty S, Conway GS, White D, Todd JA, Franks S, Williamson R, Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome, *Lancet* 349:986, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9100625

229. Witchel SF, Lee PA, Suda-Hartmann M, Smith R, Hoffman EP, 17 alpha-hydroxylase/17,20-lyase dysregulation is not caused by mutations in the coding regions of cyp17, *J Pediatr Adolesc Gynecol* 11:133, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9704303

230. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss III JF, Spielman RS, Dunaif A, Thirtyseven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin, *Proc Natl Acad Sci USA* 96: 8573, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10411917

231. Diamanti-Kandarakis E, Bartzis MI, Bergiele AT, Tsianateli TC, Kouli CR, Microsatellite polymorphism (tttta)(n) at -528 base pairs of gene CYP11alpha influences hyperandrogenemia in patients with polycystic ovary syndrome, *Fertil Steril* 73:735, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10731534

232. Diamanti-Kandarakis E, Piperi C, Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth, *Hum Reprod Update* 11:631, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15994846

233. Zawadski JK, Dunaif A, Diagnostic criteria for polycystic ovary syndrome: towards a rational approach., in *Polycystic ovary syndrome*, J.R. Givens, F.P. Haseltine, and G.R. Merriam, Editors. 1992, Blackwell Scientific Publications: Boston. p. 377.

234. Raj SG, Thompson IE, Berger MJ, Talert LM, Taymor ML, Diagnostic value of androgen measurements in polycystic ovary syndrome, *Obstet Gynecol* 52:169, 1978. http://www.ncbi.nlm.nih.gov/pubmed/567313

235. Adams J, Polson DW, Franks S, Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism, *Br Med J (Clin Res Ed)* 293:355, 1986. http://www.ncbi.nlm.nih.gov/pubmed/3089520

236. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, *Fertil Steril* 81:19, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14711538

237. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS), *Hum Reprod* 19:41, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14688154

238. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, Neubauer G, Klibanski A, Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods, *J Clin Endocrinol Metab* 89:525, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14764757

239. Vermeulen A, Verdonck L, Kaufman JM, A critical evaluation of simple methods for the estimation of free testosterone in serum, *J Clin Endocrinol Metab* 84:3666, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10523012

240. Van Uytfanghe K, Stockl D, Kaufman JM, Fiers T, De Leenheer A, Thienpont LM, Validation of 5 routine assays for serum free testosterone with a candidate reference measurement procedure based on ultrafiltration and isotope dilution-gas chromatographymass spectrometry, *Clin Biochem* 38:253, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15708548

241. Boots LR, Potter S, Potter D, Azziz R, Measurement of total serum testosterone levels using commercially available kits: high degree of between-kit variability, *Fertil Steril* 69:286, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9496343

242. Van Uytfanghe K, Stockl D, Kaufman JM, Fiers T, Ross HA, De Leenheer AP, Thienpont LM, Evaluation of a candidate reference measurement procedure for serum free testosterone based on ultrafiltration and isotope dilution-gas chromatography-mass spectrometry, *Clin Chem* 50:2101, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15375014

243. Sodergard R, Backstrom T, Shanbhag V, Carstensen H, Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature, *J Steroid Biochem* 16:801, 1982. http://www.ncbi.nlm.nih.gov/pubmed/7202083

244. Martin KA, Chang RJ, Ehrmann DA, Ibanez L, Lobo RA, Rosenfield RL, Shapiro J, Montori VM, Swiglo BA, Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline, *J Clin Endocrinol Metab* 93: 1105, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18252793

245. Azziz R, Fox LM, Zacur HA, Parker CR, Jr., Boots LR, Adrenocortical secretion of dehydroepiandrosterone in healthy women: highly variable response to adrenocorticotropin, *J Clin Endocrinol Metab* 86:2513, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11397848

246. Lobo RA, Paul WL, Goebelsmann U, Dehydroepiandrosterone sulfate as an indicator of adrenal androgen function, *Obstet Gynecol* 57:69, 1981. http://www.ncbi.nlm.nih.gov/pubmed/6450345

247. Korth-Schutz S, Levine LS, New MI, Dehydroepiandrosterone sulfate (DS) levels, a rapid test for abnormal adrenal androgen secretion, *J Clin Endocrinol Metab* 42:1005, 1976. http://www.ncbi.nlm.nih.gov/pubmed/180041

248. Feher T, Poteczin E, Bodrogi L, Relationship between serum dehydroepiandrosterone sulphate and urinary 17-ketosteroid values, *Exp Clin Endocrinol* 85:209, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3160599

249. Abraham GE, Chakmakjian ZH, Serum steroid levels during the menstrual cycle in a bilaterally adrenalectomized woman, *J Clin Endocrinol Metab* 37:581, 1973. http://www.ncbi.nlm.nih.gov/pubmed/4270264

250. Abraham GE, Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle, *J Clin Endocrinol Metab* 39: 340, 1974. http://www.ncbi.nlm.nih.gov/pubmed/4278727

251. Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB, The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man, J Endocrinol 57:123, 1973. http://www.ncbi.nlm.nih.gov/pubmed/4349596

252. Rosenfeld RS, Rosenberg BJ, Fukushima DK, Hellman L, 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate, *J Clin Endocrinol Metab* 40:850, 1975. http://www.ncbi.nlm.nih.gov/pubmed/123927

253. Mikulecky M, Kreze A, Putz Z, Moravcik M, Daily variation of serum cortisol, 17-hydroxyprogesterone and five androgens in healthy women, *Braz J Med Biol Res* 28:485, 1995. http://www.ncbi.nlm.nih.gov/pubmed/8520547

254. Zerah M, Rheaume E, Mani P, Schram P, Simard J, Labrie F, New MI, No evidence of mutations in the genes for type I and type II 3 beta-hydroxysteroid dehydrogenase (3 beta HSD) in nonclassical 3 beta HSD deficiency, *J Clin Endocrinol Metab* 79:1811, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7989489

255. Carbunaru G, Prasad P, Scoccia B, Shea P, Hopwood N, Ziai F, Chang YT, Myers SE, Mason JI, Pang S, The hormonal phenotype of Nonclassic 3 beta-hydroxysteroid dehydrogenase (HSD3B) deficiency in hyperandrogenic females is associated with insulinresistant polycystic ovary syndrome and is not a variant of inherited HSD3B2 deficiency, *J Clin Endocrinol Metab* 89:783, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14764797

256. Huerta R, Dewailly D, Decanter C, Knochenhauer ES, Boots LR, Azziz R, 11beta-hydroxyandrostenedione and delta5-andros tenediol as markers of adrenal androgen production in patients with 21-hydroxylase-deficient nonclassic adrenal hyperplasia, *Fertil Steril* 72:996, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10593370

257. Kamilaris TC, DeBold CR, Manolas KJ, Hoursanidis A, Panageas S, Yiannatos J, Testosterone-secreting adrenal adenoma in a peripubertal girl, *JAMA* 258:2558, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3312660

258. Rosenfield RL, Polycystic ovary syndrome and insulin-resistant hyperinsulinemia, *J Am Acad Dermatol* 45:S95, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11511859

259. Deplewski D, Rosenfield RL, Role of hormones in pilosebaceous unit development, *Endocr Rev* 21:363, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10950157

260. DeUgarte CM, Woods KS, Bartolucci AA, Azziz R, Degree of facial and body terminal hair growth in unselected black and white women: toward a populational definition of hirsutism, *J Clin Endocrinol Metab* 91:1345, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16449347

261. Williamson K, Gunn AJ, Johnson N, Milsom SR, The impact of ethnicity on the presentation of polycystic ovarian syndrome, *Aust N Z J Obstet Gynaecol* 41:202, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11453273

262. Wijeyaratne CN, Balen A, Barth JH, et al, Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: is there a difference?, *Clin Endocrinol* 57:343, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12201826

263. Ferriman D, Gallwey JD, Clinical assessment of body hair growth in women, *J Clin Endocrinol Metab* 21:1440, 1961. http://www.ncbi.nlm.nih.gov/pubmed/13892577

264. Hatch R, Rosenfield RL, Kim MH, Tredway D, Hirsutism: implications, etiology, and management, *Am J Obstet Gynecol* 140: 815, 1981. http://www.ncbi.nlm.nih.gov/pubmed/7258262

265. Falsetti L, Gambera A, Andrico S, Sartori E, Acne and hirsut ism in polycystic ovary syndrome: clinical, endocrine-metabolic and ultrasonographic differences, *Gynecol Endocrinol* 16:275, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12396556

266. Rea JN, Newhouse ML, Halil T, Skin disease in Lambeth. A community study of prevalence and use of medical care, *Br J Prev Soc Med* 30:107, 1976. http://www.ncbi.nlm.nih.gov/pubmed/133742

267. Johnson MT, Roberts J, Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974, *Vital Health Stat 11*:i, 1978. http://www.ncbi.nlm.nih.gov/pubmed/741665

268. Cunliffe WJ, Gould DJ, Prevalence of facial acne vulgaris in late adolescence and in adults, *Br Med J* 1:1109, 1979. http://www.ncbi.nlm.nih.gov/pubmed/156054

269. Dalgard F, Svensson A, Holm JO, Sundby J, Self-reported skin morbidity in Oslo. Associations with sociodemographic factors among adults in a cross-sectional study, *Br J Dermatol* 151: 452, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15327554

270. Galobardes B, Davey Smith G, Jefferys M, McCarron P, Has acne increased? Prevalence of acne history among university students between 1948 and 1968. The Glasgow Alumni Cohort Study, *Br J Dermatol* 152:824, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15840138

271. Cela E, Robertson C, Rush K, Kousta E, White DM, Wilson H, Lyons G, Kingsley P, McCarthy MI, Franks S, Prevalence of polycystic ovaries in women with androgenic alopecia, *Eur J Endocrinol* 149:439, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14585091

272. Futterweit W, Dunaif A, Yeh HC, Kingsley P, The prevalence of hyperandrogenism in 109 consecutive female patients with diffuse alopecia, *J Am Acad Dermatol* 19:831, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3192772

273. Ludwig E, Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex, *Br J Dermatol* 97:247, 1977. http://www.ncbi.nlm.nih.gov/pubmed/921894

274. O'Driscoll JB, Mamtora H, Higginson J, Pollock A, Kane J, Anderson DC, Aprospective study of the prevalence of clear-cut endocrine disorders and polycystic ovaries in 350 patients presenting with hirsutism or androgenic alopecia, *Clin Endocrinol (Oxf)* 41:231, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7923828

275. Malcolm CE, Cumming DC, Does anovulation exist in eumenorrheic women?, *Obstet Gynecol* 102:317, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12907106

276. Petsos P, Mamtora H, Ratcliffe WA, Anderson DC, Inadequate luteal phase usually indicates ovulatory dysfunction: observations from serial hormone and ultrasound monitoring of 115 cycles, *Gynecol Endocrinol* 1:37, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3140578

277. Chang WY, Knochenhauer ES, Bartolucci AA, Azziz R, Phenotypic spectrum of polycystic ovary syndrome: clinical and biochemical characterization of the three major clinical subgroups, *Fertil Steril* 83:1717, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15950641

278. Carmina E, Lobo RA, Do hyperandrogenic women with nor mal menses have polycystic ovary syndrome?, *Fertil Steril* 71:319, 1999. http://www.ncbi.nlm.nih.gov/pubmed/9988405

279. Carmina E, Prevalence of idiopathic hirsutism, *Eur J Endocrinol* 139:421, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9820619

280. Azziz R, Waggoner WT, Ochoa T, Knochenhauer ES, Boots LR, Idiopathic hirsutism: an uncommon cause of hirsutism in Alabama, *Fertil Steril* 70:274, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9696220

281. Koivunen R, Laatikainen T, Tomas C, Huhtaniemi I, Tapanainen J, Martikainen H, The prevalence of polycystic ovaries in healthy women, *Acta Obstet Gynecol Scand* 78:137, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10023877

282. Norman RJ, Hague WM, Masters SC, Wang XJ, Subjects with polycystic ovaries without hyperandrogenaemia exhibit similar disturbances in insulin and lipid profiles as those with polycystic ovary syndrome, *Hum Reprod* 10:2258, 1995. http://www.ncbi.nlm.nih.gov/pubmed/8530647

283. Chang PL, Lindheim SR, Lowre C, Ferin M, Gonzalez F, Berglund L, Carmina E, Sauer MV, Lobo RA, Normal ovulatory women with polycystic ovaries have hyperandrogenic pituitary-ovar ian responses to gonadotropin-releasing hormone-agonist testing, *J Clin Endocrinol Metab* 85:995, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10720029

284. Carmina E, Wong L, Chang L, Paulson RJ, Sauer MV, Stanczyk FZ, Lobo RA, Endocrine abnormalities in ovulatory women with polycystic ovaries on ultrasound, *Hum Reprod* 12:905, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9194637

285. Pache TD, Wladimiroff JW, Hop WC, Fauser BC, How to discriminate between normal and polycystic ovaries: transvaginal US study, *Radiology* 183:421, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1561343

286. van Santbrink EJ, Hop WC, Fauser BC, Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome, *Fertil Steril* 67:452, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9091329

287. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D, Ultrasound examination of polycystic ovaries: is it worth counting the follicles?, *Hum Reprod* 18:598, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12615832

288. Jonard S, Robert Y, Dewailly D, Revisiting the ovarian volume as a diagnostic criterion for polycystic ovaries, *Hum Reprod* 20: 2893, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16006474

289. Carmina E, Orio F, Palomba S, Longo RA, Lombardi G, Lobo RA, Ovarian size and blood flow in women with polycystic ovary syndrome and their correlations with endocrine parameters, *Fertil Steril* 84:413, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16084883

290. Falsetti L, Eleftheriou G, Hyperinsulinemia in the polycystic ovary syndrome: a clinical, endocrine and echographic study in 240 patients, *Gynecol Endocrinol* 10:319, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8915661

291. Khoury MY, Baracat EC, Pardini DP, Haidar MA, da Motta EL, de Lima GR, Polycystic ovary syndrome: clinical and laboratory evaluation, *Sao Paulo Med J* 114:1222, 1996. http://www.ncbi.nlm.nih.gov/pubmed/9197039

292. Alborzi S, Khodaee R, Parsanejad ME, Ovarian size and response to laparoscopic ovarian electro-cauterization in polycystic ovarian disease, *Int J Gynaecol Obstet* 74:269, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11543751

293. Amer SA, Li TC, Bygrave C, Sprigg A, Saravelos H, Cooke ID, An evaluation of the inter-observer and intra-observer variability of the ultrasound diagnosis of polycystic ovaries, *Hum Reprod* 17: 1616, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12042287

294. Hahn S, Tan S, Elsenbruch S, Quadbeck B, Herrmann BL, Mann K, Janssen OE, Clinical and biochemical characterization of women with polycystic ovary syndrome in North Rhine-Westphalia, *Horm Metab Res* 37:438, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16034717

295. Legro RS, Myers ER, Barnhart HX, Carson SA, Diamond MP, Carr BR, Schlaff WD, Coutifaris C, McGovern PG, Cataldo NA, Steinkampf MP, Nestler JE, Gosman G, Guidice LC, Leppert PC, The Pregnancy in Polycystic Ovary Syndrome study: baseline characteristics of the randomized cohort including racial effects, *Fertil Steril* 86:914, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16963034

296. Polson DW, Adams J, Wadsworth J, Franks S, Polycystic ovaries—a common finding in normal women, *Lancet* 1:870, 1988. http://www.ncbi.nlm.nih.gov/pubmed/2895373

297. Clayton RN, Ogden V, Hodgkinson J, Worswick L, Rodin DA, Dyer S, Meade TW, How common are polycystic ovaries in nor mal women and what is their significance for the fertility of the population?, *Clin Endocrinol* 37:127, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1395063

298. Farquhar CM, Birdsall M, Manning P, Mitchell JM, France JT, The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women, *Aust NZ Obstet Gynaecol* 34:67, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8053879

299. van Santbrink EJP, Hop WC, Fauser BCJM, Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultra sound vesus endocrine characteristics of polycystic ovary syndrome, *Fertil Steril* 67:452, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9091329

300. Lowe P, Kovacs G, Howlett D, Incidence of polycystic ovaries and polycystic ovary syndrome amongst women in Melbourne, Australia, *Aust N Z J Obstet Gynaecol* 45:17, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15730359

301. Futterweit W, Yeh HC, Mechanick JI, Ultrasonographic study of ovaries of 19 women with weight loss-related hypothalamic oligoamenorrhea, *Biomed Pharmacother* 42:279, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3056534

302. Ardaens Y, Robert Y, Lemaitre L, Fossati P, Dewailly D, Polycystic ovarian disease: contribution of vaginal endosonography and reas sessment of ultrasonic diagnosis, *Fertil Steril* 55:1062, 1991. http://www.ncbi.nlm.nih.gov/pubmed/2037103

303. Broekmans FJ, Knauff EA, Valkenburg O, Laven JS, Eijkemans MJ, Fauser BC, PCOS according to the Rotterdam consensus criteria: Change in prevalence among WHO-II anovulation and association with metabolic factors, *Br J Obstet Gynaecol* 13: 1210, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16972863

304. Hassan MA, Killick SR, Ultrasound diagnosis of polycystic ovaries in women who have no symptoms of polycystic ovary syndrome is not associated with subfecundity or subfertility, *Fertil Steril* 80: 966, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14556819

305. Wong IL, Morris RS, Lobo RA, Paulson RJ, Sauer MV, Isolated polycystic morphology in ovum donors predicts response to ovarian stimulation, *Hum Reprod* 10:524, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7782426

306. Joseph-Horne R, Mason H, Batty S, White D, Hillier S, Urquhart M, Franks S, Luteal phase progesterone excretion in ovulatory women with polycystic ovaries, *Hum Reprod* 17:1459, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12042261

307. Morales AJ, Laughlin GA, Bützow T, Maheshwari H, Baumann G, Yen SSC, Insulin, somatotropic, and luteinizing hormone axes in lean and obese women with polycystic ovary syndrome: common and distinct features, *J Clin Endocrinol Metab* 81:2854, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8768842

308. Manieri C, Pastorino R, Marolda AR, Musso MC, Gellona A, Barberis A, Boggio G, Messina M, Gonadotropin evaluation in the diagnosis of polycystic ovary syndrome using either a monoclonal or a polyclonal antibody radioimmunoassay, *J Endocrinol Invest* 15:219, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1624683

309. Homburg R, What is polycystic ovarian syndrome? A proposal for a consensus on the definition and diagnosis of polycystic ovarian syndrome, *Hum Reprod* 17:2495, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12351518

310. Dunaif A, Segal K, Futterweit W, Dobrjansky A, Profound peripheral resistance independent of obesity in polycystic ovary syndrome, *Diabetes* 38:1165, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2670645

311. Dunaif A, Finegood DT, Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome, *J Clin Endocrinol Metab* 81:942, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8772555

312. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS, Insulin secretory defects in polycystic ovary syndrome. Relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus, *J Clin Invest* 96:520, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7615824

313. Ehrmann DA, Breda E, Corcoran MC, Cavaghan MK, Imperial J, Toffolo G, Cobelli C, Polonsky KS, Impaired beta-cell compensation to dexamethasone-induced hyperglycemia in women with polycystic ovary syndrome, *Am J Physiol Endocrinol Metab* 287:E241, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14665448

314. Ehrmann DA, Kasza K, Azziz R, Legro RS, Ghazzi MN, Effects of race and family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:66, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15507516

315. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, Duncan AW, Diagnosing insulin resistance in the general population, *Diabetes Care* 24:460, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11289468

316. Ferrannini E, Mari A, How to measure insulin sensitivity, *J Hypertens* 16:895, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9794728

317. Laakso M, How good a marker is insulin level for insulin resistance?, *Am J Epidemiol* 137:959, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8317453

318. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia* 28:412, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3899825

319. Wallace TM, Matthews DR, The assessment of insulin resistance in man, *Diabet Med* 19:527, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12099954

320. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M, Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity, *Diabetes Care* 23:57, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10857969

321. Hanson RL, Pratley RE, Bogardus C, Narayan KM, Roumain JM, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC, Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies, *Am J Epidemiol* 151:190, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10645822

322. Skrha J, Haas T, Sindelka G, Prazny M, Widimsky J, Cibula D, Svacina S, Comparison of the insulin action parameters from hyperinsulinemic clamps with homeostasis model assessment and QUICKI indexes in subjects with different endocrine disorders, *J Clin Endocrinol Metab* 89:135, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14715840

323. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ, Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans, *J Clin Endocrinol Metab* 85:2402, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10902785

324. Carmina E, Lobo RA, Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome, *Fertil Steril* 82:661, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15374711

325. American Association of Clinical Endocrinologists, American Association of Clinical Endocrinologists position statement on metabolic and cardiovascular consequences of polycystic ovary syndrome, *Endocr Pract* 11:126, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15915567

326. Salley KE, Wickham EP, Cheang KI, Essah PA, Karjane NW, Nestler JE, Glucose intolerance in polycystic ovary syndrome-a position statement of the Androgen Excess Society, *J Clin Endocrinol Metab* 92:4546, 2007. http://www.ncbi.nlm.nih.gov/pubmed/18056778

327. Ibáñez L, Potau N, Georgopoulos N, Prat N, Gussinye M, Carrascosa A, Growth hormone, insulin-like growth factor-I axis, and insulin secretion in hyperandrogenic adolescents, *Fertil Steril* 64:1113, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7589662

328. Ibáñez L, de Zegher F, Potau N, Anovulation after precocious pubarche: early markers and time course in adolescence, *J Clin Endocrinol Metab* 84:2691, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10443661

329. Ibáñez L, Valls C, Potau N, Marcos MV, de Zegher F, Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism after precocious pubarche, *J Clin Endocrinol Metab* 85:3526, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11061495

330. Ibanez L, Valls C, Potau N, Marcos MV, de Zegher F, Polycystic ovary syndrome after precocious pubarche: ontogeny of the lowbirthweight effect, *Clin Endocrinol (Oxf)* 55:667, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11894979

331. Ibanez L, Valls C, Ferrer A, Marcos MV, Rodriguez-Hierro F, de Zegher F, Sensitization to insulin induces ovulation in nonobese adolescents with anovulatory hyperandrogenism, *J Clin Endocrinol Metab* 86:3595, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11502783

332. Ibanez L, De Zegher F, Flutamide-metformin plus an oral contraceptive (OC) for young women with polycystic ovary syndrome: switch from third- to fourth-generation OC reduces body adiposity, *Hum Reprod* 19:1725, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15229206

333. Legro RS, Kunselman AR, Dunaif A, Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome, *Am J Med* 111:607, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11755503

334. Graf MJ, Richards CJ, Brown V, Meissner L, Dunaif A, The independent effects of hyperandrogenaemia, hyperinsulinaemia, and obesity on lipid and lipoprotein profiles in women, *Clin Endocrinol* 33:119, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2205423

335. Talbott E, Clerici A, Berga SL, Kuller L, Guzick D, Detre K, Daniels T, Engberg RA, Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study, *J Clin Epidemiol* 51:415, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9619969

336. Legro RS, Azziz R, Ehrmann D, Fereshetian AG, O'Keefe M, Ghazzi MN, Minimal response of circulating lipids in women with polycystic ovary syndrome to improvement in insulin sensitivity with troglitazone, *J Clin Endocrinol Metab* 88:5137, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14602740

337. Valkenburg O, Steegers-Theunissen RP, Smedts HP, Dallinga-Thie GM, Fauser BC, Westerveld EH, Laven JS, A more atherogenic serum lipoprotein profile is present in women with polycystic ovary syndrome: a case-control study, *J Clin Endocrinol Metab* 93:470, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18056772

338. Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB, Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 61: 946, 1985. http://www.ncbi.nlm.nih.gov/pubmed/4044782

339. Robinson S, Henderson AD, Gelding SV, Kiddy D, Niththyananthan R, Bush A, Richmond W, Johnston DG, Franks S, Dyslipidaemia is associated with insulin resistance in women with polycystic ovaries, *Clin Endocrinol (Oxf)* 44:277, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8729522

340. Recabarren SE, Smith R, Rios R, Maliqueo M, Echiburu B, Codner E, Cassorla F, Rojas P, Sir-Petermann T, Metabolic profile in sons of women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 93:1820, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18230657

341. Sam S, Legro RS, Bentley-Lewis R, Dunaif A, Dyslipidemia and metabolic syndrome in the sisters of women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:4797, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15899949

342. 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, *Hum Reprod* 10:2107, 1995. http://www.ncbi.nlm.nih.gov/pubmed/8567849

343. Taponen S, Martikainen H, Jarvelin MR, Sovio U, Laitinen J, Pouta A, Hartikainen AL, McCarthy MI, Franks S, Paldanius M, Ruokonen A, Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study, *J Clin Endocrinol Metab* 89:2114, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15126528

344. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN, PCOS/Troglitazone Study Group, Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebocontrolled trial, *J Clin Endocrinol Metab* 86:1626, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11297595

345. Laitinen J, Taponen S, Martikainen H, Pouta A, Millwood I, Hartikainen AL, Ruokonen A, Sovio U, McCarthy MI, Franks S, Jarvelin MR, Body size from birth to adulthood as a predictor of self-reported polycystic ovary syndrome symptoms, *Int J Obes Relat Metab Disord* 27:710, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12833115

346. Moran C, Tapia MC, Hernandez E, Vazquez G, Garcia-Hernandez E, Bermudez JA, Etiological review of hirsutism in 250 patients, *Arch Med Res* 25:311, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7803980

347. Redmond GP, Thyroid dysfunction and women's reproductive health, *Thyroid* 14 Suppl 1:S5, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15142372

348. Lazarus JH, Thyroid disorders associated with pregnancy: etiology, diagnosis, and management, *Treat Endocrinol* 4:31, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15649099

349. Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ, Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study, *Clin Endocrinol (Oxf)* 59:282, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12919150

350. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ, Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child, *New Engl J Med* 341:549, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10451459

351. Krassas GE, Pontikides N, Kaltsas T, Papadopoulou P, Batrinos M, Menstrual disturbances in thyrotoxicosis, *Clin Endocrinol (Oxf)* 40:641, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8013145

352. Krassas GE, Pontikides N, Kaltsas T, Papadopoulou P, Paunkovic J, Paunkovic N, Duntas LH, Disturbances of menstruation in hypothyroidism, *Clin Endocrinol (Oxf)* 50:655, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10468932

353. Schiebinger RJ, Chrousos GP, Cutler Jr GB, Loriaux DL, The effect of serum prolactin on plasma adrenal androgens and the production and metabolic clearance rate of dehdroepiandrosterone sulfate in normal and hyperprolactinemic subjects, *J Clin Endocrinol Metab* 62:202, 1986. http://www.ncbi.nlm.nih.gov/pubmed/2999177

354. Higuchi K, Nawata H, Maki T, Higashizima M, Kato K, Ibayashi H, Prolactin has a direct effect on adrenal androgen secretion, *J Clin Endocrinol Metab* 59:714, 1984. http://www.ncbi.nlm.nih.gov/pubmed/6090494

355. Glintborg D, Henriksen JE, Andersen M, Hagen C, Hangaard J, Rasmussen PE, Schousboe K, Hermann AP, Prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian premenopausal women with hirsutism as the referral diagnosis, *Fertil Steril* 82:1570, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15589862

356. Derksen J, Nagesser SK, Meinders AE, Haak HR, van de Velde CJ, Identification of virilizing adrenal tumors in hirsute women., *New Eng J Med* 331:968, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8084355

357. Barbieri RL, Hyperandrogenic disorders, *Clin Obstet Gynecol* 33:640, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2225591

358. Zargar AH, Wani AI, Masoodi SR, Laway BA, Bashir MI, Salahuddin M, Epidemiologic and etiologic aspects of hirsutism in Kashmiri women in the Indian subcontinent, *Fertil Steril* 77: 674, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11937114

359. White PC, New MI, Dupont B, Congenital adrenal hyperplasia. (1), *New Engl J Med* 316:1519, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3295543

360. White PC, New MI, Dupont B, Congenital adrenal hyperplasia (2), *New Engl J Med* 316:1580, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3295546

361. New MI, Female pseudohermaphroditism, *Seminars Perinatol* 16:289, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1485187

362. Moran C, Azziz R, 21-hydroxylase-deficient nonclassic adrenal hyperplasia: the great pretender, *Seminars Reprod Med* 21: 295, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14593552

363. White PC, Speiser PW, Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, *Endocr Rev* 21:245, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10857554

364. Speiser PW, White PC, Congenital adrenal hyperplasia, *New Engl J Med* 349:776, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12930931

365. Azziz R, Dewailly D, Owerbach D, Clinical review 56: Nonclassic adrenal hyperplasia: current concepts, *J Clin Endocrinol Metab* 78:810, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8157702

366. Speiser PW, Dupont B, Rubenstein P, Piazza A, Kastelan A, New MI, High frequency of non-classical steroid 21-hydroxylase deficiency, *Am J Hum Genet* 37:650, 1985. http://www.ncbi.nlm.nih.gov/pubmed/9556656

367. Spritzer P, Billaud L, Thalabard JC, Birman P, Mowszowicz I, Raux-Demay MC, Clair F, Kuttenn F, Mauvais-Jarvis P, Cyproterone acetate versus hydrocortisone treatment in late-onset adrenal hyperplasia, *J Clin Endocrinol Metab* 70:642, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2137832

368. Frank-Raue K, Junga G, Raue F, Vecsei P, Ziegler R, [Therapy of hirsutism in females with adrenal enzyme defects of steroid hormone biosynthesis: comparison of dexamethasone with cyproterone acetate], *Klin Wochenschr* 68:597, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2142968

369. Lee HH, Kuo JM, Chao HT, Lee YJ, Chang JG, Tsai CH, Chung BC, Carrier analysis and prenatal diagnosis of congenital adrenal hyperplasia caused by 21-hydroxylase deficiency in Chinese, *J Clin Endocrinol Metab* 85:597, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10690861

370. New MI, White PC, Genetic disorders of steroid hormone synthesis and metabolism, *Baillieres Clin Endocrinol Metab* 9:525, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7575331

371. Cutfield WS, Webster D, Newborn screening for congenital adrenal hyperplasia in New Zealand, *J Pediatr* 126:118, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7815200

372. Pang S, Murphey W, Levine LS, Spence DA, Leon A, LaFranchi S, Surve AS, New MI, A pilot newborn screening for congenital adrenal hyperplasia in Alaska, *J Clin Endocrinol Metab* 55: 413, 1982. http://www.ncbi.nlm.nih.gov/pubmed/7096533

373. Therrell BL, Jr., Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, Gonzalez J, Gunn S, Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia, *Pediatrics* 101:583, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9521938

374. Azziz R, Zacur H, 21-Hydroxylase deficiency in female hyperandrogenism: screening and diagnosis, *J Clin Endocrinol Metab* 69: 577, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2547827

375. Azziz R, Hincapie LA, Knochenhauer ES, Dewailly D, Fox L, Boots LR, Screening for 21-hydroxylase deficient nonclassic adrenal hyperplasia among hyperandrogenic women: a prospective study, *Fertil Steril* 72:915, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10561000

376. Moran C, Azziz R, Carmina E, Dewailly D, Fruzzetti F, Ibañez L, Knochenhauer ES, Marcondes JA, Mendonca BB, Pignatelli D, Pugeat M, Rohmer V, Speiser PW, Witchel SF, 21-

Hydroxylasede ficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study, *Am J Obstet Gynecol* 183:1468, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11120512

377. New MI, Lorenzen F, Lerner AJ, Kohn B, Oberfield SE, Pollack MS, Dupont B, Stoner E, Levy DJ, Pang S, Levine LS, Genotyping steroid 21-hydroxylase deficiency: hormonal reference data, *J Clin Endocrinol Metab* 57:320, 1983. http://www.ncbi.nlm.nih.gov/pubmed/6306039

378. Schweikert HU, Wilson JD, Regulation of human hair growth by steroid hormones. I. Testerone metabolism in isolated hairs, *J Clin Endocrinol Metab* 38:811, 1974. http://www.ncbi.nlm.nih.gov/pubmed/4823922

379. Moller DE, Vidal-Puig A, Azziz R, *S*evere insulin-resistant hyperandrogenic syndromes, in *Androgen excess disorders in women.*, R. Azziz, J.E. Nestler, and D. Dewailly, Editors. 2006, Humana Press: Totowa, NJ. p. 129.

380. Barbieri RL, Ryan KJ, Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with dis tinct pathophysiologic features, *Am J Obstet Gynecol* 147:90, 1983. http://www.ncbi.nlm.nih.gov/pubmed/6351620

381. Vidal-Puig A, Moller DE, *Inherited causes of androgen excess: classi fication, prevalence, clinical manifestations, and diagnosis.*, in *Androgen excess disorders in women.*, R. Azziz, J.E. Nestler, and D. Dewailly, Editors. 1997, Lippincott-Raven: Philadelphia, PA. p. 227.

382. Geist SH, Gains JA, Diffuse luteinization of the ovaries associated with masculinization syndrome., *Am J Obstet Gynecol* 43: 975, 1942. http://www.ncbi.nlm.nih.gov/pubmed/13326341

383. Nagamani M, Van Dinh T, Kelver ME, Hyperinsulinemia in hyperthecosis of the ovaries, *Am J Obstet Gynecol* 154:384, 1986. http://www.ncbi.nlm.nih.gov/pubmed/3511712

384. Carmina E, Chu MC, Longo RA, Rini GB, Lobo RA, Phenotypic variation in hyperandrogenic women influences the findings of abnormal metabolic and cardiovascular risk parameters, *J Clin Endocrinol Metab* 90:2545, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15728203

385. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM, The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline, *J Clin Endocrinol Metab* 93:1526, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18334580

386. Unluhizarci K, Gokce C, Atmaca H, Bayram F, Kelestimur F, A detailed investigation of hirsutism in a Turkish population: idiopathic hyperandrogenemia as a perplexing issue, *Exp Clin Endocrinol Diabetes* 112:504, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15505757

387. Moran LJ, Brinkworth G, Noakes M, Norman RJ, Effects of lifestyle modification in polycystic ovarian syndrome, *Reprod Biomed Online* 12:569, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16790100

388. Huber-Bucholz M-M, Carey DGP, Norman RJ, Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone, *J Clin Endocrinol Metab* 84:1470, 1999.

389. Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ, Franks S, Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome, *Clin Endocrinol* 36:105, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1559293

390. Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori M, Morselli-Labate AM, Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome, *J Clin Endocrinol Metab* 85: 2767, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10946879

391. 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, *Hum Reprod* 18:1928, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12923151

392. Moran LJ, Noakes M, Clifton PM, Tomlinson L, Galletly C, Norman RJ, Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 88:812, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12574218

393. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin, *New Engl J Med* 346:393, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11832527

394. Stamets K, Taylor DS, Kunselman A, Demers LM, Pelkman CL, Legro RS, A randomized trial of the effects of two types of shortterm hypocaloric diets on weight loss in women with polycystic ovary syndrome, *Fertil Steril* 81:630, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15037413

395. Moran LJ, Noakes M, Clifton PM, Wittert GA, Williams G, Norman RJ, Short-term meal replacements followed by dietary macronutrient restriction enhance weight loss in polycystic ovary syndrome, *Am J Clin Nutr* 84:77, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16825684

396. Ehrmann DA, Polycystic ovary syndrome, *New Engl J Med* 352:1223, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15788499

397. Gokcel A, Gumurdulu Y, Karakose H, Melek Ertorer E, Tanaci N, BascilTutuncu N, Guvener N, Evaluation of the safety and efficacy of sibutramine, orlistat and metformin in the treatment of obesity, *Diabetes Obes Metab* 4:49, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11874442

398. Kay JP, Alemzadeh R, Langley G, D'Angelo L, Smith P, Holshouser S, Beneficial effects of metformin in normoglycemic morbidly obese adolescents, *Metabolism* 50:1457, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11735093

399. Freemark M, Bursey D, The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes, *Pediatrics* 107:E55, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11335776

400. Yki-Jarvinen H, Nikkila K, Makimattila S, Metformin prevents weight gain by reducing dietary intake during insulin therapy in patients with type 2 diabetes mellitus, *Drugs* 58 (Suppl 1):53, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10576526

401. Lord JM, Flight IH, Norman RJ, Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiroinositol) for polycystic ovary syndrome, *Cochrane Database Syst Rev*:CD003053, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12917943

402. Sahin I, Serter R, Karakurt F, Demirbas B, Culha C, Taskapan C, Kosar F, Aral Y, Metformin versus flutamide in the treatment of metabolic consequences of non-obese young women with polycystic ovary syndrome: a randomized prospective study, *Gynecol Endocrinol* 19:115, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15697072

403. Tang T, Glanville J, Orsi N, Barth JH, Balen AH, The use of metformin for women with PCOS undergoing IVF treatment, *Hum Reprod* 21:1416, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16501038

404. Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Martikainen HK, Tapanainen JS, Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study, *J Clin Endocrinol Metab* 85:3161, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10999803

405. Harborne L, Fleming R, Lyall H, Sattar N, Norman J, Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome, *J Clin Endocrinol Metab* 88:4116, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12970273

406. Harborne LR, Sattar N, Norman JE, Fleming R, Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses, *J Clin Endocrinol Metab* 90:4593, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15886247

407. Morin-Papunen L, Vauhkonen I, Koivunen R, Ruokonen A, Martikainen H, Tapanainen JS, Metformin versus ethinyl estradiol-cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome: a randomized study, *J Clin Endocrinol Metab* 88:148, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12519844

408. Costello MF, Shrestha B, Eden J, Johnson NP, Sjoblom P, Metformin versus oral contraceptive pill in polycystic ovary syndrome: a Cochrane review, *Hum Reprod* 22:1200, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17261574

409. Elter K, Imir G, Durmusoglu F, Clinical, endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovarian syndrome: a randomized controlled study, *Hum Reprod* 17:1729, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12093831

410. Tan S, Hahn S, Benson S, Dietz T, Lahner H, Moeller LC, Schmidt M, Elsenbruch S, Kimmig R, Mann K, Janssen OE, Metformin improves polycystic ovary syndrome symptoms irrespective of pretreatment insulin resistance, *Eur J Endocrinol* 157:669, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17984248

411. Hoeger KM, Exercise therapy in polycystic ovary syndrome, *Seminars Reprod Med* 26:93, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18181087

412. Coulam CB, Annegers JF, Krans JS, Chronic anovulation syndrome and associated neoplasia, *Obstet Gynecol* 61:403, 1983. http://www.ncbi.nlm.nih.gov/pubmed/6828267

413. Ron E, Lunenfeld B, Menczer J, Blumstein T, Katz L, Oelsner G, Serr D, Cancer incidence in a cohort of infertile women, *Am J Epidemiol* 125:780, 1987. http://www.ncbi.nlm.nih.gov/pubmed/3565353

414. Escobedo LG, Lee NC, Peterson HB, Wingo PA, Infertility-associated endometrial cancer risk may be limited to specific sub groups of infertile women, *Obstet Gynecol* 77:124, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1984211

415. Wild S, Pierpoint T, Jacobs H, McKeigue P, Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study, *Hum Fertil (Camb)* 3:101, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11844363

416. Hardiman P, Pillay OC, Atiomo W, Polycystic ovary syndrome and endometrial carcinoma, *Lancet* 361:1810, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12781553

417. Farhi DC, Nosanchuk J, Silverberg SG, Endometrial adenocarcinoma in women under 25 years of age, *Obstet Gynecol* 68:741, 1986. http://www.ncbi.nlm.nih.gov/pubmed/3785784

418. Dockerty MB, Lovelady SB, Faust GT, Carcinoma of the corpus uteri in young women, *Am J Obstet Gynecol* 61:966, 1991. http://www.ncbi.nlm.nih.gov/pubmed/14837988

419. Gitsch G, Hanzal E, Jensen D, Hacker NF, Endometrial cancer in premenopausal women 45 years and younger, *Obstet Gynecol* 85:504, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7898824

420. Paraskevaidis E, Kalantaridou SN, Papadimitriou D, Pappa L, Malamou-Mitsi V, Zikopoulos K, Kazantzis E, Lolis ED, Agnantis NJ, Transvaginal uterine ultrasonography compared with endometrial biopsy for the detection of endometrial disease in perimenopausal women with uterine bleeding, *Anticancer Res* 22: 1829, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12168877

421. Cheung AP, Ultrasound and menstrual history in predicting endometrial hyperplasia in polycystic ovary syndrome, *Obstet Gynecol* 98:325, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11506853

422. Dueholm M, Jensen ML, Laursen H, Kracht P, Can the endometrial thickness as measured by trans-vaginal sonograpy be used to exclude polyps or hyperplasia in pre-menopausal patients with abnormal bleeding?, *Acta Obstet Gynecol Scand* 80:645, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11437723

423. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M, Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by oopen, long-term clinical evaluation, *J Clin Endocrinol Metab* 85:139, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10634377

424. Unluhizarci K, Kelestimur F, Bayram F, Sahin Y, Tutus A, The effects of metformin on insulin resistance and ovarian steroidogene sis in women with polycystic ovary syndrome, *Clin Endocrinol (Oxf)* 51:231, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10468995

425. Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N, Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebocontrolled trial, *J Clin Endocrinol Metab* 87:569, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11836287

426. Velazquez E, Acosta A, Mendoza SG, Menstrual cyclicity after metformin therapy in polycystic ovary syndrome, *Obstet Gynecol* 90:392, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9277650

427. Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L, Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome, *Metabolism* 48:511, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10206447

428. Genazzani AD, Battaglia C, Malavasi B, Strucchi C, Tortolani F, Gamba O, Metformin administration modulates and restores luteinizing hormone spontaneous episodic secretion and ovarian function in nonobese patients with polycystic ovary syndrome, *Fertil Steril* 81:114, 2004. http://www.ncbi.nlm.nih.gov/pubmed/14711553

429. Eisenhardt S, Schwarzmann N, Henschel V, Germeyer A, von Wolff M, Hamann A, Strowitzki T, Early effects of metformin in women with polycystic ovary syndrome: a prospective randomized, double-blind, placebo-controlled trial, *J Clin Endocrinol Metab* 91:946, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16352680

430. Essah PA, Apridonidze T, Iuorno MJ, Nestler JE, Effects of shortterm and long-term metformin treatment on menstrual cyclicity in women with polycystic ovary syndrome, *Fertil Steril* 86:230, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16716324

431. Givens JR, Andersen RN, Wiser WL, Fish SA, Dynamics of suppression and recovery of plasma FSH, LH, androstenedione and testosterone in polycystic ovarian disease using an oral contraceptive, *J Clin Endocrinol Metab* 38:727, 1974. http://www.ncbi.nlm.nih.gov/pubmed/4823918

432. Givens JR, Andersen RN, Wiser WL, Umstot ES, Fish SA, The effectiveness of two oral contraceptives in suppressing plasma androstenedione, testosterone, LH, and FSH, and in stimulating plasma testosterone-binding capacity in hirsute women, *Am J Obstet Gynecol* 124:333, 1976. http://www.ncbi.nlm.nih.gov/pubmed/1251854

433. Raj SG, Raj MH, Talbert LM, Sloan CS, Hicks B, Normalization of testosterone levels using a low estrogen-containing oral contraceptive in women with polycystic ovary syndrome, *Obstet Gynecol* 60:15, 1982. http://www.ncbi.nlm.nih.gov/pubmed/7201126

434. Dewis P, Petsos P, Newman M, Anderson DC, The treatment of hirsutism with a combination of desogestrel and ethinyl oestradiol, *Clin Endocrinol (Oxf)* 22:29, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3156694

435. Granger LR, Roy S, Mishell DR, Jr., Changes in unbound sex steroids and sex hormone binding globulin-binding capacity during oral and vaginal progestogen administration, *Am J Obstet Gynecol* 144:578, 1982. http://www.ncbi.nlm.nih.gov/pubmed/6890312

436. Cullberg G, Hamberger L, Mattsson LA, Mobacken H, Samsioe G, Effects of a low-dose desogestrel-ethinylestradiol combination on hirsutism, androgens and sex hormone binding globulin in women with a polycystic ovary syndrome, *Acta Obstet Gynecol Scand* 64:195, 1985. http://www.ncbi.nlm.nih.gov/pubmed/3160211

437. Norman RJ, Dewailly D, Legro RS, Hickey TE, Polycystic ovary syndrome, *Lancet* 370:685, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17720020

438. Vrbikova J, Cibula D, Combined oral contraceptives in the treatment of polycystic ovary syndrome, *Hum Reprod Update* 11: 277, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15790599

439. Kojima T, Lindheim SR, Duffy DM, Vijod MA, Stanczyk FZ, Lobo RA, Insulin sensitivity is decreased in normal women by doses of ethinyl estradiol used in oral contraceptives, *Am J Obstet Gynecol* 169:1540, 1993. http://www.ncbi.nlm.nih.gov/pubmed/8267059

440. Korytkowski MT, Mokan M, Horwitz MJ, Berga SL, Metabolic effects of oral contraceptives in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 80:3327, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7593446

441. Armstrong VL, Wiggam MI, Ennis CN, Sheridan B, Traub AI, Atkinson AB, Bell PM, Insulin action and insulin secretion in polycystic ovary syndrome treated with ethinyl oestradiol/cyproterone acetate, *QJM* 94:31, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11161134

442. Nader S, Riad-Gabriel MG, Saad MF, The effect of a desogestrelcontaining oral contraceptive on glucose tolerance and leptin concentrations in hyperandrogenic women, *J Clin Endocrinol Metab* 82:3074, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9284746

443. Cibula D, Sindelka G, Hill M, Fanta M, Skrha J, Zivny J, Insulin sensitivity in non-obese women with polycystic ovary syndrome during treatment with oral contraceptives containing low-androgenic progestin, *Hum Reprod* 17:76, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11756365

444. Cibula D, Fanta M, Vrbikova J, Stanicka S, Dvorakova K, Hill M, Skrha J, Zivny J, Skrenkova J, The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients, *Hum Reprod* 20:180, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15576394

445. Escobar-Morreale HF, Lasuncion MA, Sancho J, Treatment of hirsutism with ethinyl estradiol-desogestrel contraceptive pills has beneficial effects on the lipid profile and improves insulin sensitivity, *Fertil Steril* 74:816, 2000. http://www.ncbi.nlm.nih.gov/pubmed/11020530

446. Cagnacci A, Paoletti AM, Renzi A, Orru M, Pilloni M, Melis GB, Volpe A, Glucose metabolism and insulin resistance in women with polycystic ovary syndrome during therapy with oral contraceptives containing cyproterone acetate or desogestrel, *J Clin Endocrinol Metab* 88:3621, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12915645

447. Mastorakos G, Koliopoulos C, Deligeoroglou E, Diamanti-Kandarakis E, Creatsas G, Effects of two forms of combined oral contraceptives on carbohydrate metabolism in adolescents with polycystic ovary syndrome, *Fertil Steril* 85:420, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16595221

448. Kjos SL, Shoupe D, Douyan S, Friedman RL, Bernstein GS, Mestman JH, Mishell Jr DR, Effect of low-dose oral contraceptives on carbohydrate and lipid metabolism in women with recent gestational diabetes: results of a controlled, randomized, prospective study, *Am J Obstet Gynecol* 163:1822, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2256489

449. Kjos SL, Peters RK, Xiang A, Thomas D, Schaefer U, Buchanan TA, Contraception and the risk of type 2 diabetes in Latino women with prior gestational diabetes, *JAMA* 280:533, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9707143

450. Garg SK, Chase HP, Marshall G, Hoops SL, Holmes DL, Jackson WE, Oral contraceptives and renal and retinal complications in young women with insulin-dependent diabetes mellitus, *JAMA* 271:1099, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8151852

451. Petersen KR, Skouby SO, Sidelmann J, Mølsted-Petersen L, Jespersen J, Effects of contraceptive steroids on cardiovascular risk factors in women with insulin-dependent diabetes mellitus, *Am J Obstet Gynecol* 171:400, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8059818

452. Pasquali R, Gambineri A, Anconetani B, Vicennati V, Colitta D, Caramelli E, Casimirri F, Morselli-Labate AM, The natural history of the metabolic syndrome in young women with the polycystic ovary syndrome and the effect of long-term oestrogen-progestagen treatment, *Clin Endocrinol (Oxf)* 50:517, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10468913

453. McLellan AR, Rentoul J, MacKie R, McInnes GT, Lack of effect of spironolactone on hair shaft diameter in hirsute females, *Postgrad Med J* 65:459, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2690044

454. Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M, Castello R, Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial, *J Clin Endocrinol Metab* 85:89, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10634370

455. Van der Spuy ZM, le Roux PA, Cyproterone acetate for hirsutism, *Cochrane Database Syst Rev*:CD001125, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14583927

456. Ibanez L, de Zegher F, Low-dose flutamide-metformin therapy for hyperinsulinemic hyperandrogenism in non-obese adolescents and women, *Hum Reprod Update* 12:243, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16407452

457. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M, Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation, *J Clin Endocrinol Metab* 85:139, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10634377

458. Ehrmann DA, Schneider DJ, Sobel BE, Cavaghan MK, Imperial J, Rosenfield RL, Polonsky KS, Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 82:2108, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9215280

459. Ehrmann DA, Cavaghan MK, Imperial J, Sturis J, Rosenfield RL, Polonsky KS, Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 82:524, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9024248

460. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R, Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome, *New Engl J Med* 338:1876, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9637806

461. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ, Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy, *Metabolism* 43:647, 1994. http://www.ncbi.nlm.nih.gov/pubmed/8177055

462. Nestler JE, Jakubowicz DJ, Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome, *New Engl J Med* 335:617, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8687515

463. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G, Ovulatory and metabolic effects of D-*chiro*-inositol in the polycystic ovary syndrome, *New Eng J Med* 340:1314, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10219066

464. Cosma M, Swiglo BA, Flynn DN, Kurtz DM, Labella ML, Mullan RJ, Elamin MB, Erwin PJ, Montori VM, Clinical review: Insulin sensitizers for the treatment of hirsutism: a systematic review and metaanalyses of randomized controlled trials, *J Clin Endocrinol Metab* 93:1135, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18252787

465. Legro RS, Pregnancy considerations in women with polycystic ovary syndrome, *Clin Obstet Gynecol* 50:295, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17304043

466. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC, Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility, *J Clin Endocrinol Metab* 84:1617, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10323389

467. Imani B, Eijkemans MJ, Faessen GH, Bouchard P, Giudice LC, Fauser BC, Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in

normogonadotropic anovulatory infertility: an approach to increase safety and efficiency, *Fertil Steril* 77:83, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11779595

468. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN, Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial, *J Clin Endocrinol Metab* 86:1626, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11297595

469. Creanga AA, Bradley HM, McCormick C, Witkop CT, Use of metformin in polycystic ovary syndrome: a meta-analysis, *Obstet Gynecol* 111:959, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18378757

470. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Ewens KG, Spielman RS, Leppert PC, Myers ER, Ovulatory response to treatment of polycystic ovary syndrome is associated with a polymorphism in the STK11 gene, *J Clin Endocrinol Metab* 93:792, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18000088

471. Lord JM, Flight IH, Norman RJ, Metformin in polycystic ovary syndrome: systematic review and meta-analysis, *Br Med J* 327: 951, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14576245

472. Mathur R, Alexander CJ, Yano J, Trivax B, Azziz R, Use of metformin in polycystic ovary syndrome, *Am J Obstet Gynecol* 199:596, 2008. http://www.ncbi.nlm.nih.gov/pubmed/19084097

473. Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, van der Veen F, Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial, *Bmj* 332:1485, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16769748

474. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Leppert PC, Myers ER, Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome, *New Engl J Med* 356:551, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17287476

475. Zain MM, Jamaluddin R, Ibrahim A, Norman RJ, Comparison of clomiphene citrate, metformin, or the combination of both for firstline ovulation induction, achievement of pregnancy, and live birth in Asian women with polycystic ovary syndrome: a randomized controlled trial, *Fertil Steril* 91:514, 2009. http://www.ncbi.nlm.nih.gov/pubmed/18321486

476. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE, Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone, *Fertil Steril* 75:310, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11172832

477. Kocak M, Caliskan E, Simsir C, Haberal A, Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome, *Fertil Steril* 77:101, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11779598

478. Malkawi HY, Qublan HS, The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycystic ovary syndrome, *Saudi Med J* 23:663, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12070543

479. Hwu YM, Lin SY, Huang WY, Lin MH, Lee RK, Ultra-short metformin pretreatment for clomiphene citrate-resistant polycystic ovary syndrome, *Int J Gynaecol Obstet* 90:39, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15921686

480. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH, Insulinsensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiroinositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility, *Cochrane Database Syst Rev*:CD003053, 2010. http://www.ncbi.nlm.nih.gov/pubmed/20091537

481. Baillargeon JP, Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Nestler JE, Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity, *Fertil Steril* 82:893, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15482765

482. Rouzi AA, Ardawi MS, A randomized controlled trial of the efficacy of rosiglitazone and clomiphene citrate versus metformin and clomiphene citrate in women with clomiphene citrate-resistant polycystic ovary syndrome, *Fertil Steril* 85:428, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16595222

483. Gilbert C, Valois M, Koren G, Pregnancy outcome after first-trimester exposure to metformin: a meta-analysis, *Fertil Steril* 86: 658, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16879826

484. Homburg R, Pregnancy complications in PCOS, *Best Pract Res Clin Endocrinol Metab* 20:281, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16772158

485. Palomba S, Falbo A, Orio F, Jr., Manguso F, Russo T, Tolino A, Annamaria C, Dale B, Zullo F, A randomized controlled trial evaluating metformin pre-treatment and co-administration in non-obese insulin-resistant women with polycystic ovary syndrome treated with controlled ovarian stimulation plus timed intercourse or intrauterine insemination, *Hum Reprod* 20:2879, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15958399

486. Palomba S, Orio F, Jr., Nardo LG, Falbo A, Russo T, Corea D, Doldo P, Lombardi G, Tolino A, Colao A, Zullo F, Metformin administration versus laparoscopic ovarian diathermy in clomiphene citrate-resistant women with polycystic ovary syndrome: a prospective parallel randomized double-

blind placebo-controlled trial, *J Clin Endocrinol Metab* 89:4801, 2004.
http://www.ncbi.nlm.nih.gov/pubmed/15472166

487. Glueck CJ, Goldenberg N, Wang P, Loftspring M, Sherman A, Metformin during pregnancy reduces insulin, insulin resistance, insulin secretion, weight, testosterone and development of gestational diabetes: prospective longitudinal assessment of women with polycystic ovary syndrome from preconception throughout pregnancy, *Hum Reprod* 19:510, 2004.
http://www.ncbi.nlm.nih.gov/pubmed/14998944

488. Hellmuth E, Damm P, Molsted-Pedersen L, Oral hypoglycaemic agents in 118 diabetic pregnancies, *Diabet Med* 17:507, 2000. http://www.ncbi.nlm.nih.gov/pubmed/10972579

489. Hughes RC, Rowan JA, Pregnancy in women with Type 2 diabetes: who takes metformin and what is the outcome?, *Diabet Med* 23: 318, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16492217

490. van Santbrink EJ, Fauser BC, Ovulation induction in normogonadotropic anovulation (PCOS), *Best Pract Res Clin Endocrinol Metab* 20:261, 2006.
http://www.ncbi.nlm.nih.gov/pubmed/16772156

491. Lopez E, Gunby J, Daya S, Parrilla JJ, Abad L, Balasch J, Ovulation induction in women with polycystic ovary syndrome: randomized trial of clomiphene citrate versus low-dose recombinant FSH as firstline therapy, *Reprod Biomed Online* 9:382, 2004.
http://www.ncbi.nlm.nih.gov/pubmed/15511336

492. Christin-Maitre S, Hugues JN, A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome, *Hum Reprod* 18:1626, 2003.
http://www.ncbi.nlm.nih.gov/pubmed/12871872

493. Mulders AG, Eijkemans MJ, Imani B, Fauser BC, Prediction of chances for success or complications in gonadotrophin ovulation induction in normogonadotrophic anovulatory infertility, *Reprod Biomed Online* 7:170, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14567885

494. Tummon I, Gavrilova-Jordan L, Allemand MC, Session D, Polycystic ovaries and ovarian hyperstimulation syndrome: a sys tematic review, *Acta Obstet Gynecol Scand* 84:611, 2005.
http://www.ncbi.nlm.nih.gov/pubmed/15954867

495. Navot D, Goldstein N, Mor-Josef S, Simon A, Relou A, Birkenfeld A, Multiple pregnancies: risk factors and prognostic variables during induction of ovulation with human menopausal gonadotrophins, *Hum Reprod* 6:1152, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1806576

496. van Santbrink EJ, Hohmann FP, Eijkemans MJ, Laven JS, Fauser BC, Does metformin modify ovarian responsiveness during exogenous FSH ovulation induction in normogonadotrophic

anovulation? A placebo-controlled double-blind assessment, *Eur J Endocrinol* 152:611, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15817918

497. Yarali H, Yildiz BO, Demirol A, Zeyneloglu HB, Yigit N, Bukulmez O, Koray Z, Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial, *Hum Reprod* 17:289, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11821265

498. Costello MF, Chapman M, Conway U, A systematic review and meta-analysis of randomized controlled trials on metformin coadministration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome, *Hum Reprod* 21: 1387, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16449310

499. Kjotrod SB, von During V, Carlsen SM, Metformin treatment before IVF/ICSI in women with polycystic ovary syndrome; a prospective, randomized, double blind study, *Hum Reprod* 19:1315, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15117902

500. Moll E, van der Veen F, van Wely M, The role of metformin in polycystic ovary syndrome: a systematic review, *Hum Reprod Update* 13:527, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17767003

501. Farquhar C, Lilford RJ, Marjoribanks J, Vandekerckhove P, Laparoscopic “drilling” by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome, *Cochrane Database Syst Rev*:CD001122, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16034856

502. Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ, Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome, *Hum Reprod* 16:1995, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11527911

503. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A, Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study, *J Clin Endocrinol Metab* 90: 3236, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15797965

504. Orio F, Palomba S, Colao A, Cardiovascular risk in women with polycystic ovary syndrome, *Fertil Steril* 86 (Suppl 1):S20, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16798279

505. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N, Low grade chronic inflammation in women with polycystic ovarian syndrome, *J Clin Endocrinol Metab* 86:2453, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11397838

506. Morin-Papunen L, Rautio K, Ruokonen A, Hedberg P, Puukka M, Tapanainen JS, Metformin reduces serum C-reactive protein levels in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 88:4649, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14557435

507. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, Blumenfeld Z, Increased C-reactive protein levels in the poly cystic ovary syndrome: a marker of cardiovascular disease, *J Clin Endocrinol Metab* 89:2160, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15126536

508. Ibanez L, de Zegher F, Flutamide-metformin plus ethinylestradioldrospirenone for lipolysis and antiatherogenesis in young women with ovarian hyperandrogenism: the key role of metformin at the start and after more than one year of therapy, *J Clin Endocrinol Metab* 90:39, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15483105

509. Orio F, Jr., Palomba S, Cascella T, Di Biase S, Manguso F, Tauchmanova L, Nardo LG, Labella D, Savastano S, Russo T, Zullo F, Colao A, Lombardi G, The increase of leukocytes as a new putative marker of low-grade chronic inflammation and early cardiovascular risk in polycystic ovary syndrome, *J Clin Endocrinol Metab* 90: 2, 2005.

510. Tarkun I, Arslan BC, Canturk Z, Turemen E, Sahin T, Duman C, Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation, *J Clin Endocrinol Metab* 89:5592, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15531516

511. Gonzalez F, Minium J, Rote NS, Kirwan JP, Hyperglycemia alters tumor necrosis factor-alpha release from mononuclear cells in women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:5336, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15985479

512. Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS, Evidence for an association between metabolic cardiovascular syndrome and coronary and aortic calcification among women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 89:5454, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15531497

513. Talbott EO, Zborowski JV, Boudreaux MY, McHugh-Pemu KP, Sutton-Tyrrell K, Guzick DS, The relationship between C-reactive protein and carotid intima-media wall thickness in middle-aged women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 89:6061, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15579759

514. Sampson M, Kong C, Patel A, Unwin R, Jacobs H, Ambulatory blood pressure profiles and plasminogen activator inhibitor (PAI-1) activity in lean women with and without polycystic ovary syndrome, *Clin Endocrinol* 45:623, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8977761

515. Schneider D, Sobel B, Synergistic augmentation of expression of PAI-1 induced by insulin, VLDL, and fatty acids, *Coronary Artery Dis* 7:813, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8993938

516. Lakhani K, Prelevic GM, Seifalian AM, Atiomo WU, Hardiman P, Polycystic ovary syndrome, diabetes and cardiovascular disease: risks and risk factors, *J Obstet Gynaecol* 24:613, 2004. http://www.ncbi.nlm.nih.gov/pubmed/16147598

517. Essah PA, Nestler JE, The metabolic syndrome in polycystic ovary syndrome, *J Endocrinol Invest* 29:270, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16682845

518. Dahlgren E, Janson PO, Johansson S, Lapidus L, Oden A, Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women, *Acta Obstet Gynecol Scand* 71:599, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1336918

519. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS, Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome, *J Clin Endocrinol Metab* 91: 1357, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16434451

520. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, Kuller L, Coronary heart disease risk factors in women with polycystic ovary syndrome, *Arterioscler Thromb Vasc Biol* 15:821, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7600112

521. Reaven GM, Banting lecture 1988. Role of insulin resistance in human disease, *Diabetes* 37:1595, 1988. http://www.ncbi.nlm.nih.gov/pubmed/3056758

522. Carmina E, Napoli N, Longo RA, Rini GB, Lobo RA, Metabolic syndrome in polycystic ovary syndrome (PCOS): lower prevalence in southern Italy than in the USA and the influence of criteria for the diagnosis of PCOS, *Eur J Endocrinol* 154:141, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16382003

523. Alberti KG, Zimmet PZ, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation, *Diabet Med* 15:539, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9686693

524. Balkau B, Charles MA, Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR), *Diabet Med* 16:442, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10342346

525. Expert Panel on Detection E, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III), Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) *JAMA* 285:2486, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11368702

526. Alberti KG, Zimmet P, Shaw J, The metabolic syndrome-a new worldwide definition, *Lancet* 366:1059, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16182882

527. Cameron AJ, Shaw JE, Zimmet PZ, The metabolic syndrome: prevalence in worldwide populations, *Endocrinol Metab Clin North Am* 33:351, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15158523

528. Tan CE, Ma S, Wai D, Chew SK, Tai ES, Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians?, *Diabetes Care* 27:1182, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15111542

529. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr., Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity, *Circulation* 120:1640, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19805654

530. Bailey CJ, Turner RC, Metformin, *New Engl J Med* 334:574, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8569826

531. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK, Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome, *Fertil Steril* 69:691, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9548159

532. Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ, Metformin increases AMPactivated protein kinase activity in skeletal muscle of subjects with type 2 diabetes, *Diabetes* 51:2074, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12086935

533. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE, Role of AMP-activated protein kinase in mechanism of metformin action, *J Clin Invest* 108:1167, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11602624

534. Zou MH, Kirkpatrick SS, Davis BJ, Nelson JS, Wiles WGt, Schlattner U, Neumann D, Brownlee M, Freeman MB, Goldman MH, Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species, *J Biol Chem* 279:43940, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15265871

535. Fryer LG, Parbu-Patel A, Carling D, The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways, *J Biol Chem* 277:25226, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11994296

536. Hawley SA, Gadalla AE, Olsen GS, Hardie DG, The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism, *Diabetes* 51:2420, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12145153

537. Nestler JE, Metformin for the treatment of the polycystic ovary syndrome, *New Engl J Med* 358:47, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18172174

538. Marcondes JA, Yamashita SA, Maciel GA, Baracat EC, Halpern A, Metformin in normal-weight hirsute women with polycystic ovary syndrome with normal insulin sensitivity, *Gynecol Endocrinol* 23:273, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17558685

539. Yilmaz M, Biri A, Karakoc A, Toruner F, Bingol B, Cakir N, Tiras B, Ayvaz G, Arslan M, The effects of rosiglitazone and metformin on insulin resistance and serum androgen levels in obese and lean patients with polycystic ovary syndrome, *J Endocrinol Invest* 28:1003, 2005. http://www.ncbi.nlm.nih.gov/pubmed/16483179

540. Ortega-Gonzalez C, Luna S, Hernandez L, Crespo G, Aguayo P, Arteaga-Troncoso G, Parra A, Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulinresistant women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 90:1360, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15598674

541. Palomba S, Falbo A, Russo T, Manguso F, Tolino A, Zullo F, De Feo P, Orio F, Jr., Insulin sensitivity after metformin suspension in normal-weight women with polycystic ovary syndrome, *J Clin Endocrinol Metab* 92:3128, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17519312

542. Nawrocka J, Starczewski A, Effects of metformin treatment in women with polycystic ovary syndrome depends on insulin resistance, *Gynecol Endocrinol* 23:231, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17505944

543. Salpeter SR, Buckley NS, Kahn JA, Salpeter EE, Meta-analysis: metformin treatment in persons at risk for diabetes mellitus, *Am J Med* 121:149, 2008. http://www.ncbi.nlm.nih.gov/pubmed/18261504

544. Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, Katsilambros N, Kreatsas G, Panidis D, Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin, *Hum Reprod* 21:1426, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16497699

545. Samy N, Hashim M, Sayed M, Said M, Clinical significance of inflammatory markers in polycystic ovary syndrome: their relationship to insulin resistance and body mass index, *Dis Markers* 26: 163, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19729797

546. Tarkun I, Cetinarslan B, Turemen E, Sahin T, Canturk Z, Komsuoglu B, Effect of rosiglitazone on insulin resistance, C-reactive protein and endothelial function in non-obese young women with polycystic ovary syndrome, *Eur J Endocrinol* 153:115, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15994753

547. Topcu S, Tok D, Caliskan M, Ozcimen EE, Gullu H, Uckuyu A, Erdogan D, Zeyneloglu H, Muderrisoglu H, Metformin therapy improves coronary microvascular function in patients with polycystic ovary syndrome and insulin resistance, *Clin Endocrinol (Oxf)* 65: 75, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16817823

548. Agarwal N, Rice SP, Bolusani H, Luzio SD, Dunseath G, Ludgate M, Rees DA, Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: a randomized, placebo-controlled, crossover trial, *J Clin Endocrinol Metab* 95:722, 2010. http://www.ncbi.nlm.nih.gov/pubmed/19996308

549. Teede HJ, Meyer C, Hutchison SK, Zoungas S, McGrath BP, Moran LJ, Endothelial function and insulin resistance in polycystic ovary syndrome: the effects of medical therapy, *Fertil Steril* 93: 184, 2010. http://www.ncbi.nlm.nih.gov/pubmed/19019358

550. Legro RS, Zaino RJ, Demers LM, Kunselman AR, Gnatuk CL, Williams NI, Dodson WC, The effects of metformin and rosiglitazone, alone and in combination, on the ovary and endometrium in polycystic ovary syndrome, *Am J Obstet Gynecol* 196:402 e1, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17403436

551. Glintborg D, Hermann AP, Andersen M, Hagen C, Beck-Nielsen H, Veldhuis JD, Henriksen JE, Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome, *Fertil Steril* 86:385, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16782094

552. Koo YA, Shin SY, Yoon BK, Choi D, Pioglitazone for treating polycystic ovary syndrome in non-obese women of reproductive age with different clinical presentations, *Gynecol Endocrinol* 23:461, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17852414

553. Aroda VR, Ciaraldi TP, Burke P, Mudaliar S, Clopton P, Phillips S, Chang RJ, Henry RR, Metabolic and hormonal changes induced by pioglitazone in polycystic ovary syndrome: a randomized, placebo-controlled clinical trial, *J Clin Endocrinol Metab* 94:469, 2009. http://www.ncbi.nlm.nih.gov/pubmed/18984667

554. Mastorakos G, Koliopoulos C, Creatsas G, Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives, *Fertil Steril* 77:919, 2002. http://www.ncbi.nlm.nih.gov/pubmed/12009344

555. Diamanti-Kandarakis E, Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Nestler JE, A modern medical quandary: polycystic ovary syndrome, insulin resistance, and oral contraceptive pills, *J Clin Endocrinol Metab* 88:1927, 2003. http://www.ncbi.nlm.nih.gov/pubmed/12727935

556. Ibanez L, De Zegher F, Flutamide-metformin therapy to reduce fat mass in hyperinsulinemic ovarian hyperandrogenism: effects in adolescents and in women on third-generation oral contraception, *J Clin Endocrinol Metab* 88:4720, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14557446

557. Ibanez L, de Zegher F, Ethinylestradiol-drospirenone, flutamidemetformin, or both for adolescents and women with hyperinsulinemic hyperandrogenism: opposite effects on adipocytokines and body adiposity, *J Clin Endocrinol Metab* 89:1592, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15070917

558. Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC, Effects of metformin and ethinyl estradiol-cyproterone acetate on lipid levels in obese and non-obese women with polycystic ovary syndrome, *Eur J Endocrinol* 152:269, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15745936

559. Mitkov M, Pehlivanov B, Terzieva D, Combined use of metformin and ethinyl estradiol-cyproterone acetate in polycystic ovary syndrome, *Eur J Obstet Gynecol Reprod Biol* 118:209, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15653205

560. Duleba AJ, Banaszewska B, Spaczynski RZ, Pawelczyk L, Simvastatin improves biochemical parameters in women with polycystic ovary syndrome: results of a prospective, randomized trial, *Fertil Steril* 85:996, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16580386

561. Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ, Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial, *J Clin Endocrinol Metab* 92:456, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17105841

562. Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL, The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double-blind placebo-controlled study, *J Clin Endocrinol Metab* 94:103, 2009. http://www.ncbi.nlm.nih.gov/pubmed/18940877

563. Banaszewska B, Pawelczyk L, Spaczynski RZ, Duleba AJ, Comparison of simvastatin and metformin in treatment of polycystic ovary syndrome: prospective randomized trial, *J Clin Endocrinol Metab* 94:4938, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19890022

564. Goldstein JL, Brown MS, Regulation of the mevalonate pathway, *Nature* 343:425, 1990. http://www.ncbi.nlm.nih.gov/pubmed/1967820

565. Izquierdo D, Foyouzi N, Kwintkiewicz J, Duleba AJ, Mevastatin inhibits ovarian theca-interstitial cell proliferation and steroidogenesis, *Fertil Steril* 82 Suppl 3:1193, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15474095

566. Edison RJ, Muenke M, Mechanistic and epidemiologic considerations in the evaluation of adverse birth outcomes following gestational exposure to statins, *Am J Med Genet A* 131:287, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15546153

567. Chang RJ, Nakamura RM, Judd HL, Kaplan SA, Insulin resistance in nonobese patients with polycystic ovarian disease, *J Clin Endocrinol Metab* 57:356, 1983. http://www.ncbi.nlm.nih.gov/pubmed/6223044

568. Dunaif A, Segal KR, Futterweit W, Dobrjansky A, Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome, *Diabetes* 38:1165, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2670645

569. Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T, Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome, *Diabetes* 41:1257, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1397698

570. Jahanfar S, Eden JA, Warren P, Seppälä M, Nguyen TV, A twin study of polycystic ovary syndrome, *Fertil Steril* 63:478, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7531655

571. Campbell PJ, Gerich JE, Impact of obesity on insulin action in vol unteers with normal glucose tolerance: demonstration of a threshold for the adverse effect of obesity, *J Clin Endocrinol Metab* 70:1114, 1990. http://www.ncbi.nlm.nih.gov/pubmed/2180975

572. Bjorntorp P, The android woman-a risky condition, *J Intern Med* 239:105, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8568477

573. Holte J, Gennarelli G, Wide L, Lithell H, Berne C, High prevalence of polycystic ovaries and associated clinical, endocrine, and metabolic features in women with previous gestational diabetes mellitus, *J Clin Endocrinol Metab* 83:1143, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9543131

574. Wild RA, Alaupovic P, Parker IJ, Lipid and apolipoprotein abnormalities in hirsute women. I. The association with insulin resistance, *Am J Obstet Gynecol* 166:1191, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1566769

575. Wild RA, Obesity, lipids, cardiovascular risk, and androgen excess, *Am J Med* 98:27S, 1995. http://www.ncbi.nlm.nih.gov/pubmed/7825638

576. Reaven GM, Relationship between insulin resistance and hypertension, *Diabetes Care* 14 Suppl 4:33, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1748055

577. Velazquez EM, Mendoza SG, Wang P, Glueck CJ, Metformin therapy is associated with a decrease in plasma plasminogen activator inhibitor-1, lipoprotein(a), and immunoreactive insulin levels in patients with the polycystic ovary syndrome, *Metabolism* 46: 454, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9109854

578. Diamanti-Kandarakis E, Spina G, Kouli C, Migdalis I, Increased endothelin-1 levels in women with polycystic ovary syndrome and the beneficial effect of metformin therapy, *J Clin Endocrinol Metab* 86:4666, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11600523

579. Dahlgren E, Landin K, Krotkiewski M, Holm G, Janson PO, Effects of two antiandrogen treatments on hirsutism and insulin sensitivity in women with polycystic ovary syndrome, *Hum Reprod* 13:2706, 1998. http://www.ncbi.nlm.nih.gov/pubmed/9804218

580. Godsland IF, Walton C, Felton C, Proudler A, Patel A, Wynn V, Insulin resistance, secretion, and metabolism in users of oral contraceptives, *J Clin Endocrinol Metab* 74:64, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1530790

581. Chasan-Taber L, Colditz GA, Willett WC, Stampfer MJ, Hunter DJ, Colditz GA, Spiegelman D, Manson JE, A prospective study of oral contraceptives and NIDDM among U.S. women, *Diabetes Care* 20:330, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9051382

582. Rimm EB, Manson JE, Stampfer MJ, Colditz GA, Willett WC, Rosner B, Hennekens CH, Speizer FE, Oral contraceptive use and the risk of type 2 (non-insulin-dependent) diabetes mellitus in a large prospective study of women, *Diabetologia* 35:967, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1451955

583. Solomon CG, Hu FB, Dunaif A, Rich-Edwards J, Willett WC, Hunter DJ, Colditz GA, Speizer FE, Manson JE, Long or highly irregular menstrual cycles as a marker for risk of type 2 diabetes mel litus, *JAMA* 286:2421, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11712937

584. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, Acute myocardial infarction and combined oral contraceptives: results of an international multicentre case-control study, *Lancet* 349:1202, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9130941

585. Croft P, Hannaford PC, Risk factors for acute myocardial infarction in women: evidence from the Royal College of General Practitioners' oral contraception study, *Br Med J* 298:165, 1989. http://www.ncbi.nlm.nih.gov/pubmed/2493841

586. Sidney S, Petitti DB, Quesenberry CP, Klatsky AL, Ziel HK, Wolf S, Myocardial infarction in users of low-dose oral contraceptives, *Obstet Gynecol* 88:939, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8942831

587. Lewis MA, Heinemann LAJ, Spitzer WO, MacRae KD, Bruppacher R, for the Transnational Research Group on Oral Contraceptives and the Health of Young Women, The use of oral contraceptives and the occurrence of acute myocardial infarction in young women. Results from the Transnational Study on Oral Contraceptives and the Health of Young Women, *Contraception* 56: 129, 1997. http://www.ncbi.nlm.nih.gov/pubmed/9347202

588. Tanis BC, van den Bosch MA, Kemmeren JM, Cats VM, Helmerhorst FM, Algra A, van der Graaf Y, Rosendaal FR, Oral contraceptives and the risk of myocardial infarction, *New Engl J Med* 345:1787, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11752354

589. Dunaif A, Green G, Phelps RG, Lebwohl M, Futterweit W, Lewy L, Acanthosis nigricans, insulin action, and hyperandrogenism: clinical, histological, and biochemical findings, *J Clin Endocrinol Metab* 73:590, 1991. http://www.ncbi.nlm.nih.gov/pubmed/1874935

590. Sharma ST, Wickham EP, 3rd, Nestler JE, Changes in glucose tolerance with metformin treatment in polycystic ovary syndrome: a retrospective analysis, *Endocr Pract* 13:373, 2007. http://www.ncbi.nlm.nih.gov/pubmed/17669713

591. Lewy VD, Danadian K, Witchel SF, Arslanian S, Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome, *J Pediatr* 138:38, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11148510

592. Palmert MR, Gordon CM, Kartashov AI, Legro RS, Emans EJ, Dunaif A, Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome, *J Clin Endocrinol Metab* 87:1017, 2002. http://www.ncbi.nlm.nih.gov/pubmed/11889155