SHORT REVIEW

Industrial endocrine disruptors and polycystic ovary syndrome

E. Palioura and E. Diamanti-Kandarakis

Endocrine Unit, Third Department of Internal Medicine, Medical School University of Athens, Sotiria Hospital, Athens, Greece

ABSTRACT. Polycystic ovary syndrome (PCOS) is a complex and enigmatic syndrome of unknown origin and etiology enclosing a broad spectrum of phenotypic manifestations. PCOS pathophysiology combines reproductive and metabolic abnormalities into a heterogeneous disorder that has pervasive and devastating health consequences. Inquiring the generative roots of the syndrome, it has become increasingly apparent the role of the environment as a determinant factor. Experimental exposure to industrial endocrine disruptors has been related with the impairment of normal reproductive function and metabolic regulation possibly favoring the development of or aggravating PCOS-resembling clinical disorders. Industrial chemicals may reflect the contributing role of an unfavorable environment to unveil PCOS characteristics in genetically predisposed individuals or further deteriorate the hormonal and fertility imbalances of PCOS-affected females. (J. Endocrinol. Invest. 36: 1105-1111, 2013)

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder that represents the most common endocrinopathy among women of reproductive age with a prevalence of 6.5-6.8% (1, 2). Cardinal features of the syndrome are reproductive (chronic anovulation, hyperandrogenism, polycystic sonographic appearance of the ovaries) and metabolic (insulin resistance, metabolic syndrome, obesity) aberrations that synergistically lead to the clinical phenotype of PCOS (3). The recognition of the multiple, potential, long-term health hazards (increased risk for cardiovascular disease, Type 2 diabetes and presumably increased cancer risk) (4) has warranted medical interest on the mechanisms implicated on syndrome's pathogenesis. Though the etiologic roots of PCOS remain largely enigmatic, they appear to involve environmental/nutritional and genetic components.

One of the well-recognized environmental determinants of PCOS is diet with overnutrition-related obesity known to exacerbate many of the PCOS symptoms. In a more analytic perspective, not only the quantity but the quality of diet has been implicated as the high content of food in advanced glycated end-products has an aggravating effect on the clinical course of the syndrome (5).

Though nutrition represents a key factor in the environmental dimension of the syndrome, it is highly likely that other modifiers in the environment perhaps unrecognized or little recognized may also play a role, for instance exposure to industrial endocrine disruptors (ED). The modern industrialized environment in developed countries is a constant source of a variety of chemicals that can be detected in high levels at top of food chain due to their persistence and bioaccumulation potency. Exposure in people is typically a result of contamination of the food chain, inhalation of contaminated house dust or occupational exposure and rarely a result of accidental exposure.

The list of industrial disrupting chemicals is highly heterogeneous and includes synthetic chemicals used as industrial solvents/lubricants and their byproducts [polychlorinated biphenyls (PCB), polybrominated biphenyls (PBB), dioxins], pesticides and herbicides (such as dichlorodiphenyl trichloroethane (DDT) or its metabolites, methoxychlor (MXC)], fungicides (vinclozolin), heat stabilisers and chemical catalysts (such as tributyltin), plastic contaminants [e.g. bisphenol A (BPA)] and plasticizers (e.g.phthalates) or pharmaceuticals components (i.e. diethylstilbestrol; 17α-ethinylestradiol) (6). Humans and animals are typically exposed to a mixture of ED which may act additively, increasing the effect exerted on a single physiological process, or may initiate many, separate, disruptive effects that may, when combined, have more severe health consequences.

Hypothetically, the by-definition ability of ED to "interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis, reproduction, development, and/or behavior" (7) may aggravate or contribute to the hormonal imbalance characterizing PCOS. Such interplay could involve both the reproductive and metabolic feature of the syndrome favoring its clinical appearance in predisposed individuals.

Whether this hypothesis can be transformed from a biologically plausible interaction to a human health effect is very difficult to establish. However, as analyzed in the following sections of the review, there is a plethora of data documenting examples of adverse effects of industrial endocrine disruptors in reproductive system and metabolism.

This review attempts to summarize the experimental evidence linking exposure to most common industrial endocrine disrupting chemicals and PCOS features, the pathophysiological mechanisms underlying such interaction and the potential relevance to human population.

Key-words: Endocrine disruptors, PCOS.

Correspondence: E. Diamanti-Kandarakis, Professor of Medicine – Endocrinology and Metabolism, Head of the Third Department of Medicine, Endocrine Unit, Medical School University of Athens – Greece; SOTIRIA Hospital, Mesogion Avenue, Holargos.

E-mail: e.diamanti.kandarakis@gmail.com

Accepted July 12, 2013.

INDUSTRIAL ENDOCRINE DISRUPTORS AND PCOS: THE BIOLOGICAL PLAUSIBILITY

Industrial chemical effects on the ovary – A link to PCOS

Intrinsic abnormalities in ovarian steroidogenesis are fundamental in PCOS pathogenesis with the ovary remaining the primary source of hyperandrogenism. There is clear evidence for increased testosterone production in PCOS theca cells attributed to increased synthesis of testosterone precursors (8) with CYP17 (coding for P450c17, and the associated P450 reductase) and CYP11a (coding for P450scc) being incriminated as plausible candidate genes in hyperandrogenemia's genesis (9).

Endocrine disruptors' interference with this PCOS-related aberration is plausible given that substantial experimental data converge to the biological plausibility of ED to interfere with several aspects of ovarian physiology. In particular, the dynamic process of steroidogenesis appears to be a particularly sensitive target of disruption (10).

According to experimental studies, increased testosterone synthesis has been observed in rat ovarian theca-interstitial cells in response to exposure to an abundant environmental endocrine disruptor, BPA (11). Interestingly, key ovarian pathway enzymes controlling theca cell steroidogenesis and more specifically 17β-hydroxylase (P450c17), cholesterol side chain cleavage enzyme (P450scc) and steroidogenic acute regulatory protein (StAR) have been implicated as the underlying mechanism (11).

The stimulatory effect of BPA on enzymatic activity of P450c17 may interestingly point to a link between BPA exposure and the PCOS given that deregulation of this steroidogenic enzyme has been described as one of the key etiologies of ovarian hyperandrogenism in the syndrome.

On the other hand, an *in vitro* study using mouse ovarian antral follicles has shown that both StAR and P450scc mRNA expression are inhibited in theca and granulosa cells following exposure to BPA. Furthermore, the granulosa-theca cell communication including hormone diffusion from the theca into the granulosa cells is disrupted (12). The apparent differences between the above studies may reflect the different animal models and/or the different experimental design.

Although theca cell dysfunction seems to be the main defect of intraovarian PCOS hyperandrogenism, granulosa cell deregulation may also play a role, *via* secretion of intra-ovarian regulatory factors, such as anti-Müllerian hormone (AMH), that are believed to modulate follicular steroidogenesis through a paracrine inhibitory effect (13). Interestingly, methoxychlor (an organochlorine pesticide) was shown to stimulate AMH production in the rat ovary (10) and may hypothetically contribute to the elevated serum AMH levels that are believed to disturb follicle selection in PCOS women.

Another potential site of interference between ED and granulosa cells is matrix metalloproteinase 9 (MMP-9), a proteolytic enzyme found with raised concentrations in serum (14) and in the follicular fluid (15) of women with PCOS compared to normal counterparts. Granulosa-lutein cell incubated with BPA at doses compatible with human exposure levels showed a dose-dependent increase in MMP-9 output (16). Though MMP-9 role in PCOS pathogenesis is speculative, it is believed to interfere with abnormalities of extracellular matrix remodeling that are associated with follicular development, thus, possibly contributing to inappropriate atresia in PCOS women (17).

Another common feature in PCOS pathogenesis is the abnormalities in ovarian folliculogenesis; the follicles are present in large numbers but they are arrested at an early to mid developmental state and fail to mature. As a result, the PCOS ovary is characterized by arrested growth of antral follicles (typically arrested at a diameter of 4-8 mm) with recruitment and growth of follicles to the small antral stage, without selection of a dominant, preovulatory follicle (18). The aberrant follicle development is not only confined to the gonadotropin-dependent antral stages given that significant abnormalities (increased density of small pre-antral follicles and an increased proportion of early growing follicles) have been observed during the very earliest stages of folliculogenesis (19).

The underlying basis for the abnormalities in anovulatory PCOS remains uncertain. Theoretically, endocrine disruptors could favor the existence of an abnormal intraovarian endocrine environment affecting normal follicle growth. Indeed, experimental data point to the ability of environmental ED to affect every stage of folliculogenesis from primordial follicular assembly to ovulation and luteal phase (10). The underlying mechanisms are complex and include (but not limited to) interplay with pituitary gonadotropins, predominant ovarian steroid hormones (i.e., progesterone, estrogens, and androgens) and local regulatory factors affecting follicle growth (i.e., AMH) (10).

BPA is a characteristic example of such interference as antral follicles isolated from 32-day-old mice cultured with vehicle control and BPA exhibited inadequate follicle growth that may be the result of the impairing hormone production, analyzed previously (12).

Industrial chemical effects on metabolic regulation – A link to PCOS

Fundamental characteristic of PCOS physiology is metabolic derangement, central to which is peripheral insulin resistance and compensatory hyperinsulinemia, resulting from a post-receptor-binding defect in insulin action (20). Furthermore, the metabolic profile of PCOS women consists of multiple risk factors for cardiovascular disease, including obesity, dyslipidemia, hypertension, impaired glucose tolerance/Type 2 diabetes, metabolic syndrome, subclinical vascular disease all of which synergistically predispose a long-term health risk (21).

The impact of environment on these metabolic disorders seems plausible with diet recognized as a determinant factor. Apart from nutrition, other environmental factors may contribute to the metabolic characteristics of the syndrome or may exacerbate their clinical course. This issue is very timely as a significant portion of recent research is focused on illuminating the impact of industrial chemicals on several aspects of metabolic disorders providing a further explanation for the increasing global

trends of diseases such as diabetes mellitus and obesity. Starting from obesity, encountered in 30-70% of PCOS-affected women, the role of industrial chemicals, as the one of the potential causes of obesity and associated metabolic syndrome, has become very suspicious. This is reflected by the so-called "obesogens" hypothesis, a term applied to describe molecules that inappropriately regulate lipid metabolism and adipogenesis and provoke metabolic alterations leading to obesity (22). "Obesogens" are believed to derail the homeostatic mechanisms important for weight control, such that exposed individuals are predisposed to weight gain despite normal diet and exercise (23).

The list of chemicals studied as possible candidate "obesogens" is dramatically expanding and includes among others: BPA, phthalates, organotins, polybrominated diphenyl ethers, polyfluoroalkyl chemicals, organochlorine pesticides, PCB (24). The potential pathways through which industrial chemicals might act to alter adipocyte differentiation and favor obesity development are complex and not totally understood.

To begin with, they are believed to include actions at a molecular level by modulating the activity of genes involved at multiple stages of adipocyte differentiation. To be more specific, organotics may act as retinoid X receptor (RXR) and peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists to directly modulate the activity of genes involved at multiple stages of adipocyte differentiation [early mitogenic genes such as c-*jun*, transcription factors responsible for clonal expansion and differentiation such as C/EBP and sterol regulatory element-binding protein 1 (Srebp-1c), as well as direct targets of PPAR signaling such as adipocyte P2 (aP2), fatty acid synthase, and fatty acid transport protein] which, in turn, promote adipogenesis both *in vivo* and *in vitro* (23, 25). Supportive of such interplay are studies showing that *in utero* administration of this organotic is followed by increased adipose mass in adult mice (25).

Another chemical with widespread use and ubiquitous presence in the environment that has been incriminated for its effects on metabolism is BPA. The issue of potential BPA interference with metabolic imbalances is very timely especially in light of a recent cross-sectional study in the general adult population of the United States that reported an association between higher urinary BPA concentrations with diabetes, cardiovascular diagnoses and clinically abnormal concentrations of the liver enzymes γ-glutamyltransferase (26). An analysis of the posterior data from the US National Health and Nutrition Survey (NHANES) conducted by Melzer et al. confirmed the association between urinary BPA levels with coronary heart disease, rising serious concern regarding the biological safety of the environmental chemical (27).

The proposed mechanisms by which BPA is believed to alter lipid homeostasis and body weight control mechanisms include up-regulation of lipogenic enzymes and proadipogenic transcription factors in animal adipocytes (28) while in human adipocytes BPA was shown to inhibit adiponectin (a key adipokine that protects humans from metabolic syndrome) and stimulate the release of inflammatory adipokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) (29).

Another mechanism recently proposed to mediate BPA adverse effect on lipid metabolism is through the induction of oxidative stress in liver mitochondria (30). When human HepG2 cells were exposed to decreasing and non-toxic BPA concentrations a severe mitochondrial dysfunction was observed with significant increase in mitochondrial reactive oxygen species production and subsequent lipoperoxidation. These effects on hepatic physiology, namely the induction of lipid droplet accumulation, the oxidative stress-associated lipoperoxidation and the pro-inflammatory state with IL8 and TNF-α secretion (analyzed previously), may contribute to steatosis and subsequent non-alcoholic fatty liver disease, a hepatic manifestation of metabolic syndrome.

Even with a direct effect on hepatic hemostasis (30) or with an indirect effect on adiponectin production by adipose tissue (28), BPA seems to favor the development of a key component of metabolic syndrome, liver steatosis. Most intriguingly, all the above effects are noticed at nanomolar BPA concentrations that are in a range relevant to human exposure.

Interestingly, overweight/obese PCOS women exhibit an increased prevalence of hypertransaminasemia and of nonalcoholic fatty liver disease (NAFLD) (31), thus, an aggravating impact of BPA on this population could be implied. Theoretically, BPA exposure could be responsible for inducing alterations similar to those encountered in NAFLD *via* a direct hepatotoxic effect.

Besides BPA and organotins, polybrominated diphenyl ethers represent another class of chemicals who have been incriminated as ED of adipocyte metabolism. Hoppe and Carey demonstrated that daily exposure of rats to pentabrominated diphenyl ether *in vivo* for at least 4 weeks resulted in a significant increase in lipolysis and a significant decrease in insulin-stimulated glucose oxidation, both of which are characteristics associated with obesity, insulin resistance, and Type 2 diabetes (32). Finally, phthalates is another class of chemical with "obesogen" properties given that male and female rats intrauterine exposed to diisobutyl phthalates demonstrated reduced plasma leptin levels (33), a key hormone of metabolic regulation.

With regard to human population, evidence is even more limited leading to inconclusive findings. There is data suggestive of a potential association between phthalates metabolites (34) and persistent organic pollutants (35) with somatometric characteristics such as increased body mass index and waist circumference. Overall, the epidemiological link between specific obesogen exposure and obesity is highly suggestive, causality and overall significance currently remain ambiguous.

As already mentioned, there is a higher prevalence of carbohydrate metabolism disorders, such as impaired glucose tolerance or frank diabetes mellitus in women with PCOS compared to normal controls. The possible association of industrial chemicals with such disorders represents a new fascinating area of research after the discovery that the pancreatic cells – both β- and α- are also a target of disruption. Long-term treatment of male mice with BPA alters the pancreatic content and secre-

tion of insulin and results in post-prandial insulinemia and insulin resistance (36). Pancreatic α-cells have also been implied as potential targets for endocrine disruption. Low doses of BPA were shown to impair the molecular signaling that leads to secretion of glucagon by suppressing intracellular calcium ion oscillations in α-cells in response to low blood glucose levels, through a non-genomic mechanism (37).

Industrial chemical effects on the hypothalamic-pituitary axis – A link to PCOS

Except for the major role of gonadal activity over reproductive function, primary control of reproduction is exerted *via* regulation of GnRH secretion from the hypothalamus and subsequent gonadotropin release from the pituitary gland.

In PCOS-affected women, neuroendocrine defects are implicated in syndrome's pathogenesis. In particular, neuroendocrine abnormalities include increased GnRH pulse frequency, elevated LH levels and normal or even suppressed FSH levels in comparison with levels in regularly ovulating normal women, leading to an increased LH to FSH ratio in most patients. The raised plasma LH concentrations, that are associated with increased LH pulse frequency and pulse amplitude, promote ovarian theca cells steroidogenesis (13). On the other hand, lower FSH levels during the early follicular phase are considered to pose a negative impact to normal follicle maturation.

Of the neuroendocrine systems, the hypothalamus-pituitary-gonad axis is of the best studied in the arena of endocrine disruption. Accumulative evidence illustrate the interference of endocrine disrupting chemicals at different, sometimes multiple, levels of the axis leading to alteration of GnRH signaling in the pituitary, which, in turn, may perturb, gonadotropin release and action at the level of the gonad (38). The potential mechanisms of disruption of GnRH signaling include direct affect on expression of steroid sensitive GnRH afferent systems such as kisspeptin and galanin as well as a direct effect on GnRH neurons expression in the hypothalamus or an indirect affect by impaired steroid feedback on GnRH neurons. At level of the pituitary gland, industrial chemicals *per se* have been shown to disturb gonadotropins release (38).

Since hormone-sensitive neuroendocrine system seems to be another possible target of disruption, it is rational to consider that PCOS neuroendocrine status is also impaired by specific industrial chemicals.

For instance, in the case of widespread BPA, laboratory studies indicate its biological ability to interrupt both GnRH and gonadotropin secretion. Laboratory rodents exposed neonatally to BPA presented altered pituitary function as reflected by increased GnRH pulsatility and lowering basal and GnRH-induced LH levels at post-natal day 13 (39). Accelerated GnRH pulse frequency has also been reported in hypothalamic explants from adult rats exposed to BPA in neonate life (40). Interestingly, as mentioned above, alterations in GnRH pulsatility is a neuroendocrine aberration found in adult PCOS women, thus, a contributory role of BPA in this abnormality cannot be excluded.

EXPERIMENTAL AND HUMAN DATA LINKING INDUSTRIAL ENDOCRINE DISRUPTORS TO PCOS

What could be conducted from the above is that mounting experimental data converge to the biological ability of industrial endocrine disruptors to affect the reproductive system and metabolic tissues leading to or aggravating the clinical manifestation of several disorders affecting PCOS individuals. Solitary features of the syndrome have been observed in several animal models exposed to the chemicals, however, the coexistence of most of the phenotypic traits of PCOS seems to be missing from literature.

A rational question would be whether exposure of female reproductive system to endocrine disrupting chemicals during sensitive "windows" of development (*in utero*, neonate life) could result in PCOS in adulthood or whether exposure of PCOS-affected females during adulthood could exacerbate syndrome' clinical course.

A recent study by Fernandez et al. has shed some light on this hypothetical interplay using a rat model. Authors presented experimental data showing the development of a PCOS resembling syndrome in adult female rats exposed to high BPA levels (500 μg) during neonate life, a period crucial for the development of the hypothalamic-pituitary-gonadal axis (40). In particular, researchers reported, during adulthood, the coexistence of biochemical hyperandrogenemia, anovulation, infertility, polycystic ovarian morphology, and increased GnRH pulse frequency in rats subcutaneously exposed to this disruptor early in life. As expected, the severity of the observed disorders appeared to be dose-dependent with the highest dose (500 μg BPA) provoking irreversible alterations in the hypothalamic–pituitary–gonadal axis. However, even the lowest dose (50 μg BPA), that is more relevant to human exposure, was associated with subtle alterations at the hypothalamic level that could lead to subfertility (40). As all these reproductive aberrations are well-matched with those found in PCOS women, a potentially causal relation between BPA exposure and the syndrome could be hypothesized.

Analogous effects have been observed in sheep and rhesus monkeys following pre-natal exposure to high levels of testosterone (41, 42). Analytically, rhesus monkey *in utero* exposed to high testosterone levels developed PCOS like characteristics in adult life; namely, anovulatory infertility, hypersecretion of LH, elevated circulating levels of testosterone, neuroendocrine feedback defects, central adiposity and compensatory insulin resistance, and polycystic ovaries with ovarian hyperandrogenism and follicular arrest in adulthood (41).

In the sheep model, a similar PCOS phenotype derives from pre-natal exposure to exogenous testosterone (42). In both animal models, pre-natal exposure to high levels of testosterone results in fetal programming of PCOS traits. The results presented here reinforce the notion that *in utero* exposure of human female fetuses to industrial endocrine disruptors could result in PCOS in adulthood, along with associated metabolic disorders.

Although several studies suggest a potential link between endocrine disruptors' exposure and adverse reproductive and metabolic outcomes resembling the PCOS phenotype, relatively few studies have investi-

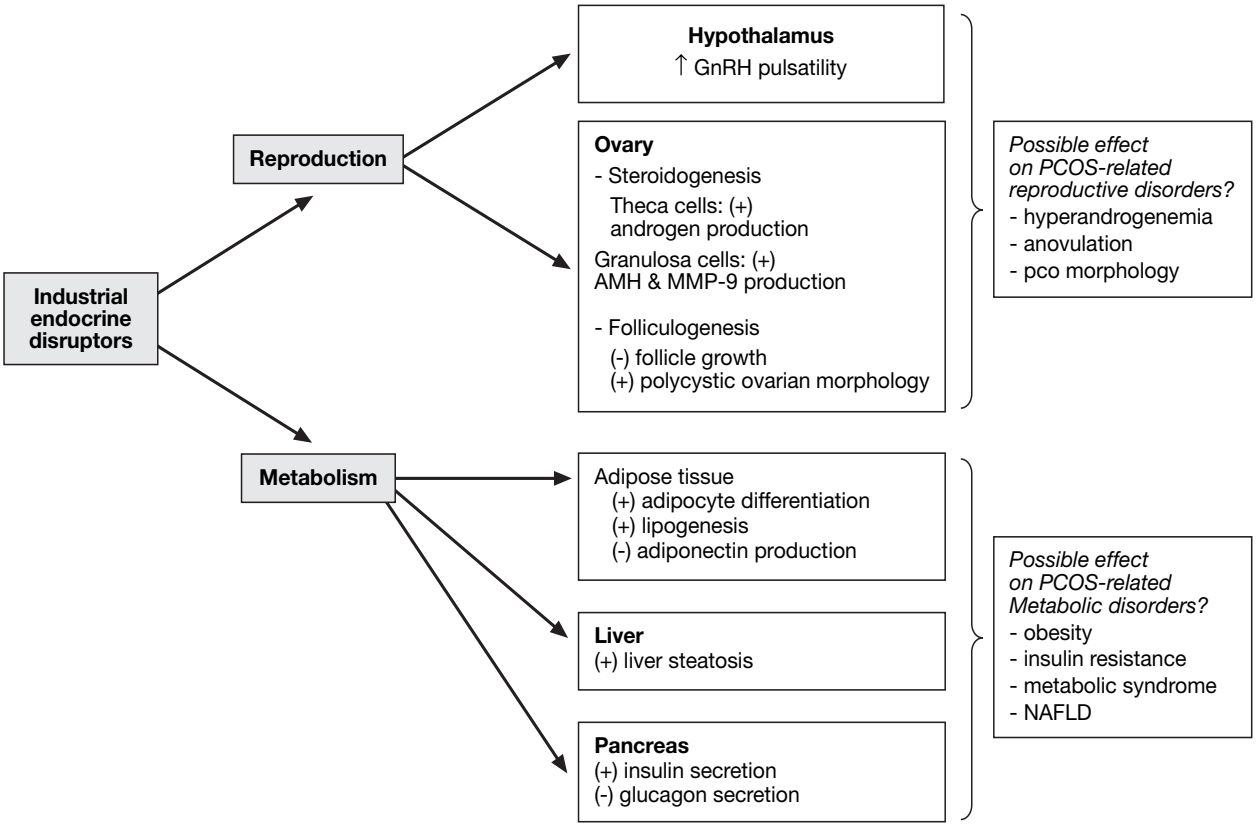

Fig. 1 - *The potential pathways through which industrial endocrine disruptors might act to interfere with the reproductive and metabolic aberrations characterizing polycystic ovary syndrome (PCOS). The stimulatory (+) and inhibitory (–) effects of the environmental endocrine disruptors are noted. AMH: anti-Müllerian hormone; MMP-9: matrix metalloproteinase-9; NAFLD: non-alcoholic fatty liver disease.*

gated consequences of such exposure in the human PCOS population.

What has been observed so far is that women with PCOS compared to non-obese regularly ovulating women have higher serum levels of the endocrine-disrupting chemical BPA (43) and both groups display lower BPA levels compared to males (44). Furthermore, a positive correlation between BPA and androgens has been reported (43).

What remains enigmatic is whether elevated BPA levels is a consequence, and not a cause, of PCOS, because women with PCOS have higher circulating testosterone levels than do healthy women, and androgens interfere with BPA clearance in the liver by decreasing uridine diphosphate-glucuronosyl transferase activity which, in turn, leads to increased serum levels of BPA (45). On the other hand, BPA *per se* has been shown to favor increased testosterone concentrations by two potential pathways. Firstly, BPA has been reported to significantly inhibit the activity of two different testosterone hydroxylases (2-and 6-hydroxylase), leading to decreased testosterone catabolism and indirectly to increased testosterone concentrations (46). Secondly, BPA as a potent ligand of SHBG in the liver may displace androgens from SHBG binding sites and likely leads to increased circulating free androgen concentrations (47), creating a vicious circle between androgens and BPA.

Additional evidence concerning the possible interaction of BPA with hormonal and metabolic abnormalities characterizing PCOS women has been provided by a recent study in which researchers divided 71 women with PCOS (NIH Consensus criteria) and 100 healthy female controls into subgroups matched by age and body composition (48). Serum BPA levels were found to be significantly higher in women with PCOS in comparison to their normal ovulating, non-hyperandrogenemic peers, independently of the degree of obesity. Furthermore, BPA levels were strongly associated with androgens and insulin resistance indices, implying a potential role of this endocrine disruptor to the two major components of PCOS pathophysiology (48).

CONCLUSION

Plethora of experimental studies provides clear evidence for the adverse effects of environmental factors on reproductive function and metabolic regulation, the two major components of PCOS (Fig. 1). It remains to determine whether environmental exposure to industrial products, especially during sensitive stages of development,

could favor the clinical manifestation of PCOS later in life in genetically predisposed individuals or exacerbates its clinical course during adulthood.

Reflecting the harmful influence of the modern environment, BPA is currently emerging as a key player in PCOS pathogenesis presumably *via* bidirectional interplay with androgens. Once again in the history of endocrine diseases, environment, and in particular endocrine disruptors, may be the missing piece to solve the "puzzle" of PCOS pathogenesis, the commonest endocrinopathy among women.

REFERENCES

1. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 1999, 84: 4006-11.
2. 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 2000, 85: 2434-8.
3. Diamanti-Kandarakis E. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Rev Mol Med 2008, 10: e3.
4. Bates GW, Legro RS. Longterm management of Polycystic Ovarian Syndrome (PCOS). Mol Cell Endocrinol 2012, 7: e48963.
5. Diamanti-Kandarakis E, Christakou C, Marinakis E. Phenotypes and enviromental factors: their influence in PCOS. Curr Pharm Des 2012, 18: 270-82.
6. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev 2009, 30: 293-42.
7. U.S. EPA. Special report on Environmental Endocrine Disruption: An Effects Assessment and Analysis. Office of Research and Development, 1997, EPA/630/R-96/012, Washington D.C.
8. Nelson VL, Qin KN, Rosenfield RL, et al. The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2001, 86: 5925-33.
9. Pasquali R, Stener-Victorin E, Yildiz BO, et al. PCOS Forum: research in polycystic ovary syndrome today and tomorrow. Clin Endocrinol (Oxf) 2011, 74: 424-33.
10. Uzumcu M, Zachow R. Developmental exposure to environmental endocrine disruptors:Consequences within the ovary and on female reproductive function. Reprod Toxicol 2007, 23: 337-52.
11. Zhou W, Liu J, Liao L, Han S, Liu J. Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. Mol Cell Endocrinol 2008, 283: 12-8.
12. Peretz J, Gupta RK, Singh J, Hernandez-Ochoa I, Flaws JA. Bisphenol A impairs follicle growth, inhibits steroidogenesis, and downregulates rate-limiting enzymes in the estradiol biosynthesis pathway. Toxicological Sciences 2011, 119: 209-17.
13. Diamanti-Kandarakis E, Piperi C. Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth. Hum Reprod Update 2005, 11: 631-43.
14. Lewandowski KC, Komorowski J, O'Callaghan CJ, et al. Increased circulating levels of matrix metalloproteinase-2 and -9 in women with the polycystic ovary syndrome. J Clin Endocrinol Metab 2006, 91: 1173-7.
15. Diamanti-Kandarakis E, Livadas S, Kandarakis SA, Margeli A, Papassotiriou I. Serum concentrations of atherogenic proteins neutrophil gelatinase-associated lipocalin and its complex with matrix metalloproteinase-9 are significantly lower in women with polycystic ovary syndrome: hint of a protective mechanism? Eur J Endocrinol 2008, 158: 525-31.
16. Dominguez MA, Petrea MA, Neal MS, Fostera WG. Bisphenol A concentration-dependently increases human granulosa-lutein cell matrix metalloproteinase-9 (MMP-9) enzyme output. Reprod Toxicol 2008, 25: 420-5.
17. Goldman S, Shalev E. MMPS and TIMPS in ovarian physiology and pathophysiology. Front Biosci 2004, 9: 2474-83.
18. Franks S, Mason H, Willis D. Follicular dynamics in the polycystic ovary syndrome. Mol Cell Endocrinol 2000, 163: 49-52.
19. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update 2008, 14: 367-78.
20. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 2012, 33: 981-1030.
21. Bates GW, Legro RS. Long term management of Polycystic Ovarian Syndrome (PCOS). Mol Cell Endocrinol 2012, 373: 91-7.
22. Baillie-Hamilton PF. Chemical toxins: a hypothesis to explain the global obesity epidemic. J Altern Complement Med 2002, 8: 185-92.
23. Grün F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology. 2006, 147 (6 Suppl): S50-5.
24. Hatch EE, Nelson JW, Stahlhut RW, Webster TF. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl 2010, 33: 324-32.
25. Grün F, Watanabe H, Zamanian Z, et al. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. Mol Endocrinol 2006, 20: 2141-55.
26. Lang IA, Galloway TS, Scarlett A, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 2008, 300: 1303-10.
27. Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PLoS One 2010, 5: e8673.
28. Somm E, Schwitzgebel VM, Toulotte A, et al. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 2009, 117: 1549-55.
29. Ben-Jonathan N, Hugo ER, Brandebourg TD. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. Mol Cell Endocrinol 2009, 304: 49-54.
30. Huc L, Lemarié A, Guéraud F, Héliès-Toussaint C. Low concentrations of bisphenol A induce lipid accumulation mediated by the production of reactive oxygen species in the mitochondria of HepG2 cells. Toxicol In Vitro 2012, 26: 709-17.
31. Economou F, Xyrafis X, Livadas S, et al. In overweight/obese but not in normal-weight women, polycystic ovary syndrome is associated with elevated liver enzymes compared to controls. Hormones (Athens) 2009, 8: 199-206.
32. Hoppe AA, Carey GB. Polybrominated diphenyl ethers as endocrine disruptors of adipocyte metabolism. Obesity (Silver Spring) 2007, 15: 2942-50.
33. Boberg J, Metzdorff S, Wortzigera R, et al. Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. Toxicology 2008, 250: 75-81.
34. Hatch EE, Nelson JW, Qureshi MM, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environmental Health 2008, 7: 27.
35. Elobeid MA, Padilla MA, Brock DW, Ruden DM, Allison DB. Endocrine disruptors and obesity: an examination of selected persistent organic pollutants in the NHANES 1999-2002 data. Int J Environ Res Public Health 2010, 7: 2988-3005.
36. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol A disrupts pancreatic β-cell function in vivo and induces insulin resistance. Environ Health Perspect 2006, 114: 106-12.
37. Alonso-Magdalena P, Laribi O, Ropero AB, et al. Low doses of bisphenol A and diethylstilbestrol impair Ca2+ signals in pancreatic α-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. Environ Health Perspect 2005, 113: 969-77.
38. Fowler PA, Bellingham M, Sinclair KD, et al. Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. Mol Cell Endocrinol 2012, 355: 231-9.
39. Fernández M, Bianchi M, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a alters reproductive parameters and go-

nadotropin releasing hormone signaling in female rats. Environ Health Perspect 2009, 117: 757-62.
40. Fernandez MO, Bourguignon N, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environ Health Perspect 2010, 118: 1217-22.
41. 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 2005, 11: 357-74.
42. West C, Foster DL, Evans NP, Robinson J, Padmanabhan V. Intrafollicular activin availability is altered in prenatally-androgenized lambs. Mol Cell Endocrinol 2001, 185: 51-9.
43. Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr J 2004, 51: 165-9.
44. Takeuchi T, Tsutsumi O. Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels. Bioch Bioph Res Com 2002, 291: 76-8.
45. Takeuchi T, Tsutsumi O, Ikezuki Y, et al. Elevated serum bisphenol A levels under hyperandrogenic conditions may be caused by decreased UDP-glucuronosyltransferase activity. Endocr J 2006, 53: 485-91.
46. Hanioka N, Jinno H, Nishimura T, Ando M. Suppression of male-specific cytochrome P450 isoforms by bisphenol A in rat liver. Arch Toxicol 1998, 72: 387-94.
47. Dechaud H, Ravard C, Claustrat F, de la Perriere AB, Pugeat M. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 1999, 64: 328-34.
48. Kandaraki E, Chatzigeorgiou A, Livadas S, et al. Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. J Clin Endocrinol Metab 2011, 96: E480-4.